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# Synthetic Calanolides with Bactericidal Activity against Replicating and Nonreplicating *Mycobacterium tuberculosis*

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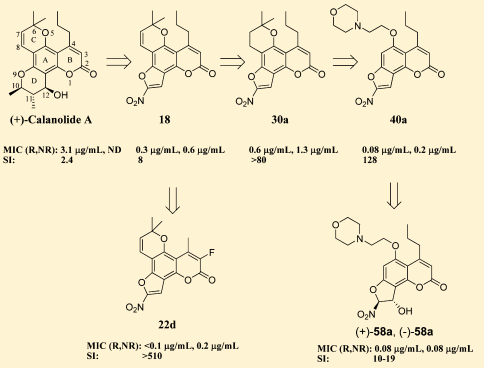
Supporting Information

**ABSTRACT:** It is urgent to introduce new drugs for tuberculosis to shorten the prolonged course of treatment and control drug-resistant *Mycobacterium tuberculosis* (*Mtb*). One strategy toward this goal is to develop antibiotics that eradicate both replicating (R) and nonreplicating (NR) *Mtb*. Naturally occurring (+)-calanolide A was active against R-*Mtb*. The present report details the design, synthesis, antimycobacterial activities, and structure−activity relationships of synthetic calanolides. We identified potent dual-active nitro-containing calanolides with minimal in vitro toxicity that were cidal to axenic *Mtb* and *Mtb* in human macrophages, while sparing Gram-positive and -negative bacteria and yeast. Two of the nitrobenzofuran-containing lead compounds were found to be genotoxic to mammalian cells. Although genotoxicity precluded clinical progression, the profound, selective mycobactericidal activity of these calanolides will be useful in identifying pathways for killing both R- and NR-*Mtb*, as well as in further structure-based design of more effective and drug-like antimycobacterial agents.




## INTRODUCTION

Tuberculosis (TB), resulting from *Mycobacterium tuberculosis* (*Mtb*), is the leading cause of death by a single bacterial agent. Fueled in part by pandemics of HIV infection and diabetes, TB remains prevalent. *Mtb* can persist for prolonged periods in a state that is non- or slowly replicating, here called non-replicating (NR) for simplicity. An estimated one-third of the world's population is latently infected with *Mtb*.[1,2] If *Mtb* does not contain genetic mutations associated with resistance to a given drug, the drug can generally kill *Mtb* rapidly in vitro under conditions where the bacterium is otherwise replicating (R). However, most TB drugs are far less effective against the same strains of *Mtb* under NR conditions.[3] In patients with active, uncomplicated TB, the presence of NR subpopulations of *Mtb* may account for the need to extend treatment for 6 months to achieve acceptable cure rates.[4] A variety of NR models, such as carbon starvation or hypoxia, have been employed to identify compounds that kill NR-*Mtb*.[5] An additional NR model uses a combination of four physiologic conditions: hypoxia, mild acidity, nitrosative stress, and restriction of the major carbon source to a fatty acid.[5b]

In a previous report, the HIV-1-active[6] natural product (+)-calanolide A was active against R-*Mtb* (MIC = 3.1 μg/mL), but the compound was almost equally toxic to Vero cells ($LD_{50}$ = 7.6 μg/mL), with an unacceptable selectivity index (SI) of 2.4.[7] Although a small number of pyranocoumarins have also been shown to possess antimycobacterial activity,[8] as a class, calanolides have not been thoroughly explored as antimycobacterial agents. To do this, we initiated a high-throughput screening of a chemical library enriched for calanolides synthesized in our laboratory and discovered several 12-oxo-calanolides[10a] with activity against both R- and NR-*Mtb*. A further SAR campaign identified more potent synthetic calanolides with decreased toxicity, including three compounds with activity against *Mtb* infecting human macrophages.

## CHEMISTRY

**Round 1 of Design and Synthesis: Structural Diversifications of Ring D.** The chiral ring D in (+)-calanolide A possesses three asymmetric carbons at the C10-, C11-, and C12-positions that complicate its synthesis.[9] Our previous work revealed that 10-chloromethyl-11-demethyl-12-oxo-calanolide had higher potency toward drug-susceptible and drug-resistant HIV-1 and afforded a superior safety profile compared to the parent compound, (+)-calanolide A.[10] For these reasons, we

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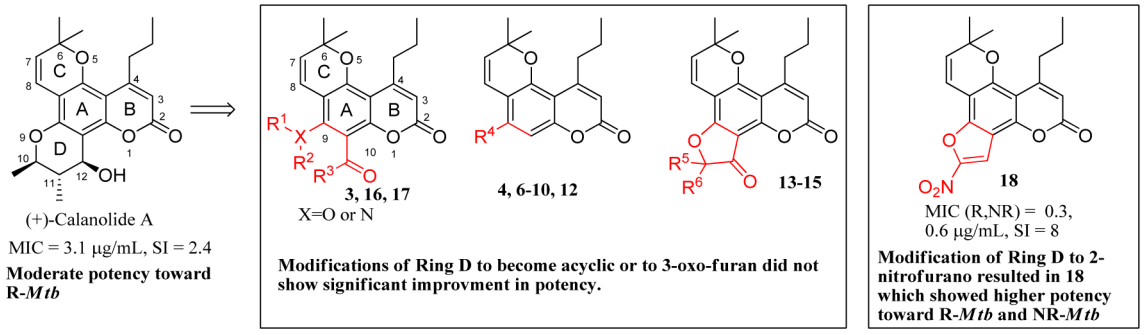
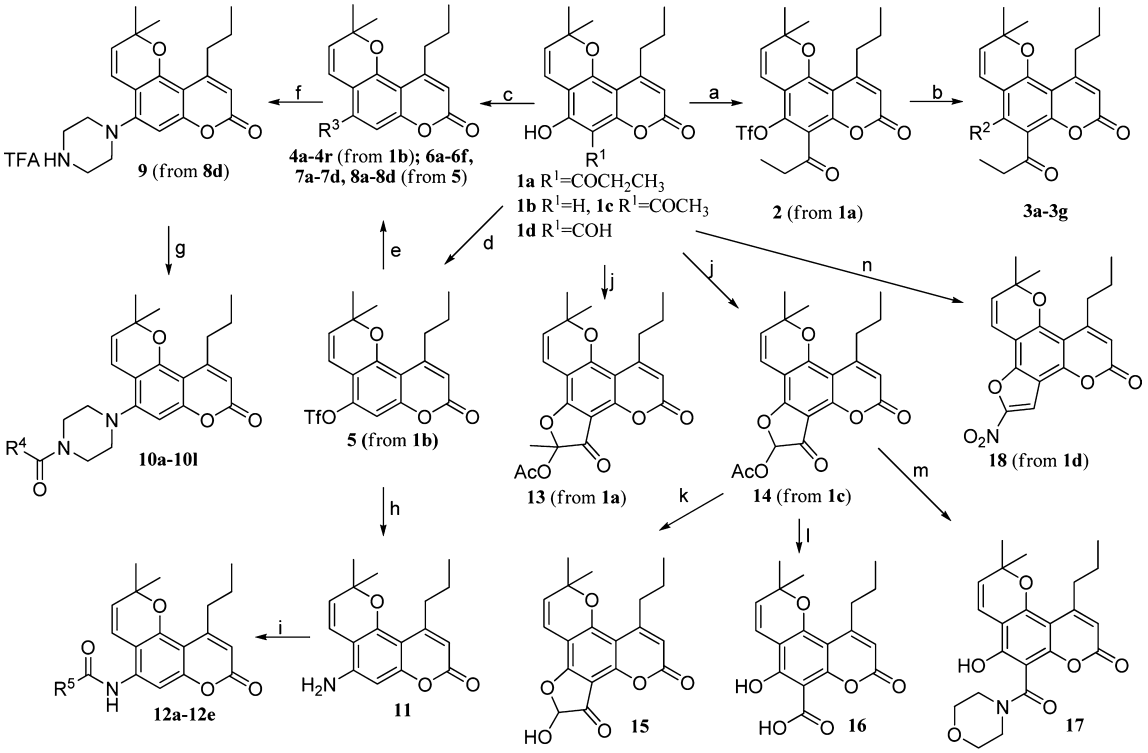



**Figure 1.** Summary of SARs from round 1 of design and synthesis.

**Scheme 1. Synthesis of Compounds from Round 1**[a]




[a]Reagents and conditions: (a) $Tf_2O$, lutidine, $CH_2Cl_2$, 0 °C, 30 min, 73%; (b) secondary amine, $Et_3N$, DMF, 90 °C, 12 h, 25−85%; (c) For **4a**: $Me_2SO_4$, $K_2CO_3$, acetone, rt, 12 h, 92%; For **4b**: acetic anhydride, pyridine, DMAP, $CH_2Cl_2$, rt, 12 h, 84%; For **4c**: benzoyl chloride, pyridine, DMAP, $CH_2Cl_2$, rt, 12 h, 93%; For **4d**: cinnamic acid, DCC, DMAP, $CH_2Cl_2$, rt, 12 h, 54%; For **4e−4q**: $R^3OH$, $PPh_3$, DEAD, THF, rt, 12 h, 38−95%; For **4r**: 2-chloro-*N*-phenethylacetamide, $K_2CO_3$, 100 °C, 24 h, DMF, 30%; (d) $Tf_2O$, pyridine, $CH_2Cl_2$, 0 °C, 30 min, 79%; (e) For **6a−6f**: acetylene, $PdCl_2(PPh_3)_2$, CuI, Ar, $Et_3N$, THF, reflux, 12 h, 48−92%; For **7a−7d**: Primary amine, $Pd(OAc)_2$, BINAP, $Cs_2CO_3$, Ar, dioxane, 100 °C 5 h, 68−87%; For **8a−8d**: secondary amine, $Et_3N$, DMF, 90 °C, 12 h, 37−76%; (f) TFA, $CH_2Cl_2$, rt, 30 min, 89%; (g) $R^4$-N═C═O or isopentanoyl chloride, $Et_3N$, THF, reflux, 60−93%; (h) i, diphenylmethanimine, $Pd(OAc)_2$, BINAP, $Cs_2CO_3$, Ar, dioxane, 100 °C, 5 h, 55%; ii, 2 M HCl, THF, rt, 12 h, 73%; (i) For **12a**: acetic anhydride, pyridine, DMAP, THF rt, 12 h, 64%; For **12b**: benzoyl chloride, pyridine, THF, rt, 12 h, 71%; For **12c**: 4-fluorophenyl-N═C═O, THF, reflux, 3 h, 69%, **12d**:1-naphthyl-N═C═O, THF, reflux, 3 h, 52%. For **12e**: pyrazine-2-carboxylic acid, HOBt, BOP, DMF, rt, 3 h, 78%; (j) iodobenzene diacetate, TBAI, AcONa, $CH_3CN$, rt, 5 h, 35−41%; (k) sat. aq $NaHCO_3$, THF, rt, 72h, 55%; (l) $H_2O$, DBU, THF, reflux, 5 h, 83%; (m) morpholine, DBU, THF, −15 °C to rt, 5 h, 91%; (n) i, $NO_2CH_2Br$, $K_2CO_3$, acetone, rt, 3 h; ii, acetic anhydride, 100 °C, 1 h, 92%.

explored ring D diversifications in our first round of compound design.

On the basis of the reported active calanolides,[8] we anticipated that the ABC scaffold with appropriate side chains at the C9 and/or C10 position might show improved activity against *Mtb* (Figure 1). To test this hypothesis, we synthesized compounds belonging to the ABC scaffold with diversified side chains at C9 and/or C10 positions (Scheme 1, Table 1).

Intermediates **1a−1d** were prepared as described.[11] Intermediate **1a** was reacted with triflic anhydride to prepare trifluoromethanesulfonate **2**. Employing $S_NAr$ reactions of **2**, compounds **3a−3g** (Table 1) were attained using nucleophilic secondary amines (Scheme 1). A propiophenone at the C10 position of these compounds was placed to mimic the chroman-4-one unit of the previously published compound **F18**.[10b]

To develop analogues exploring a wide range of substitutions at the C9 position of the ABC scaffold (Table 1), intermediate


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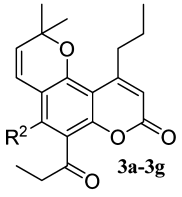
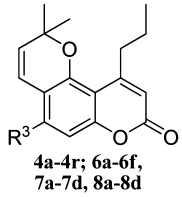
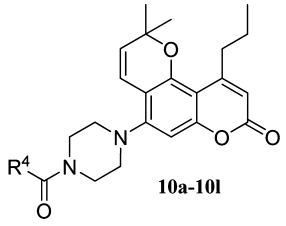
**Table 1. In Vitro Activity of the Analogues in Round 1 of Design and Synthesis**



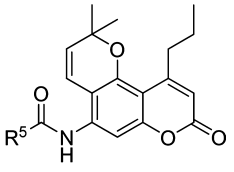

**3a-3g**




**4a-4r; 6a-6f, 7a-7d, 8a-8d**




**10a-10l**




**12a-12e**

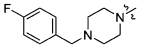
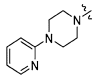
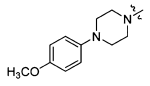
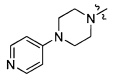
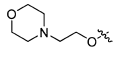
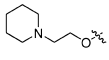
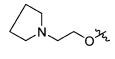
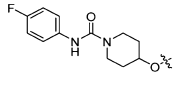
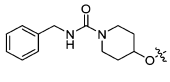
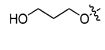
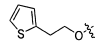
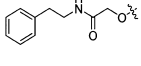
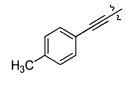
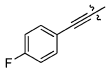
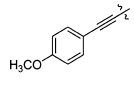
| Compds | Substituents of $R^2$ to $R^5$ | $MIC_{90}$ (μg/mL) R, NR (NR7d) | $LD_{50}$, SI | Compds | Substituents of $R^2$ to $R^5$ | $MIC_{90}$ (μg/mL) R, NR (NR7d) | $LD_{50}$, SI |
|---|---|---|---|---|---|---|---|
| **3a** |  | > 18, >18 | ND | **3b** |  | > 21, >21 | ND |
| **3c** |  | > 21, >21 | ND | **3d** |  | >26, 26 | ND |
| **3e** |  | > 24, >24 | ND | **3f** |  | > 26, >26 | ND |
| **3g** |  | > 24, >24 | ND | **4a** | $CH_3O$ | > 15, >15 | ND |
| **4b** | $CH_3COO$ | > 16, >16 | ND | **4c** | PhCOO | > 20, >20 | ND |
| **4d** | PhCH=CHCOO | > 21, >21 | ND | **4e** |  | 20, > 20 | 20, CN |
| **4f*** |  | > 20, >20 (7) | >20, CN | **4g** |  | 19, > 19 | 19, 1 |
| **4h** |  | > 23, >23 | >23, CN | **4i** |  | > 18, >18 | >18, CN |
| **4j** |  | > 17, >17 | ND | **4k*** |  | > 18, >18 (2) | >18, CN |
| **4l** |  | > 16, >16 | >16, CN | **4m** |  | > 24, >24 | ND |
| **4n** |  | > 25, >25 | >25, CN | **4o** |  | > 17, >17 | ND |
| **4p** |  | > 19, >19 | ND | **4q** |  | > 20, >20 | ND |
| **4r** |  | > 22, >22 | ND | **6a** |  | > 19, >19 | >19, CN |
| **6b** |  | > 19, >19 | >19, CN | **6c** |  | > 20, >20 | >20, CN |
| **6d** |  | > 16, >16 | >16, CN | **6e** |  | > 19, >19 | >19, CN |



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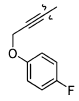
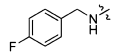
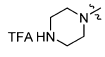
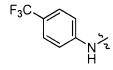
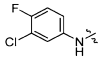
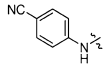
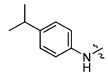
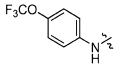
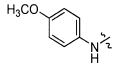
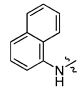
**Table 1. continued**

| Compds | Substituents of $R^2$ to $R^5$ | $MIC_{90}$ (μg/mL) R, NR (NR7d) | $LD_{50}$, SI | Compds | Substituents of $R^2$ to $R^5$ | $MIC_{90}$ (μg/mL) R, NR (NR7d) | $LD_{50}$, SI |
|---|---|---|---|---|---|---|---|
| **6f** |  | > 21, >21 | >21, CN | **7a** |  | 19, >19 | ND |
| **7b** |  | > 18, >18 | ND | **7c** |  | > 20, >20 | ND |
| **7d** |  | > 20, >20 | ND | **8a** |  | > 18, >18 | ND |
| **8b** |  | > 19, >19 | ND | **8c*** |  | >16, >16 (2) | >16, CN |
| **8d** |  | ND, ND | ND | **9** |  | 23, >23 | 12, 0.5 |
| **10a** |  | > 25, >25 | >25, CN | **10b** |  | > 27, >27 | >27, CN |
| **10c** |  | > 26, >26 | >26, CN | **10d** |  | > 25, >25 | >25, CN |
| **10e** |  | > 24, >24 | >24, CN | **10f** |  | > 26, >26 | >26, CN |
| **10g** |  | > 28, >28 | >28, CN | **10h** |  | > 28, >28 | >28, CN |
| **10i** |  | > 25, >25 | >25, CN | **10j** |  | > 24, >24 | >24, CN |
| **10k** | n-$C_7H_{15}$-NH | > 25, >25 | >25, CN | **10l** | sec-butyl | > 22, >22 | 22, <1 |
| **12a** | $CH_3$ | > 16, >16 | ND | **12b** |  | > 19, >19 | ND |
| **12c** |  | > 21, >21 | ND | **12d** |  | > 23, >23 | ND |
| **12e** |  | >20, >20 | ND | **13^** | - | >20, >20 | ND |
| **14^** | - | >100, 6 | ND | **15^** | - | >17, 17 | ND |
| **16^** | - | > 17, >17 | >17, CN | **17^** | - | > 20, >20 | >20, CN |
| **18**^** | - | 0.3, 0.6 | 3, 8 | | | | |

*These compounds gained activity when exposed to NR conditions for longer (7 days) with indicated MICs in parentheses. **The activity was confirmed in WT *Mtb* H37Rv, MICs in the table are indicative of WT *Mtb* H37Rv bacteria. ^See Scheme 1 for structures of compounds **13**–**18**. SI, selectivity index was determined by $LD_{50}$ for HepG2 cells/$MIC_{90}$ for R-*Mtb*. ND, not determined. CN, cannot be calculated.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**1b** was reacted alternatively with $Me_2SO_4$, acetic anhydride, benzoyl chloride, cinnamic acid, or 2-chloro-*N*-phenethylacetamide to produce compounds **4a**−**4d** and **4r**, respectively. Reaction of **1b** with a series of commercially available alcohols under Mitsunobu reaction conditions produced compounds **4e**−**4q**. Trifluoromethanesulfonate **5** was synthesized from **1b**. Intermediate **5** allowed us to construct a C−C bond as well as a C−N bond side chains at the C9 position by taking advantage of palladium-catalyzed cross-coupling reactions. For example, a Sonogashira cross-coupling reaction between **5** and commercially available acetylenes gave C9 alkynyl-substituted compounds **6a**−**6f**. When **5** underwent Buchwald−Hartwig cross-coupling with benzylamines and aniline, analogues **7a**−**7d** were obtained. Benefiting from $S_NAr$ reactions with secondary amines, **5** was also used to produce compounds **8a**−**8c** in moderate yield. Potentially resulting from the pyranone's lactone, the reaction between **5** and secondary amines such as dimethylamine led to multiple additional side reactions when the reaction temperature was increased or the reaction time was prolonged. When *tert*-butylpiperazine-1-carboxylate was reacted with **5**, **8d** was produced. The Boc group of **8d** was cleaved in TFA to generate compound **9**. Derivatization of **9** with commercially available isocyanates and acetyl chloride in the presence of triethylamine yielded compounds **10a**−**10l**. By using diphenylmethanimine as a coupling partner with **5** followed by hydrolysis, **5** was converted into compound **11**, which was treated with either acetic anhydride, benzoyl chloride, 4-fluorophenylisocyanate, 1-isocyanatonaphthalene, or pyrazine-2-carboxylic acid, respectively, to afford compounds **12a**−**12e** (Scheme 1).

Compounds **13** and **14** were designed to explore replacement of ring D with the benzofuran-3-one fragment, found in the TB drug rifampicin. In the presence of iodobenzene diacetate, tetrabutylammonium iodide, and AcONa, **1a** and **1c** yielded the target compounds **13** and **14**, respectively.[12] Using compound **14**, we also attained compounds **15**, **16**, and **17** by the method reported recently by our laboratory (Scheme 1).[13]

Although considered a major structural alert due to potential mutagenicity, 5-nitrofuran and 2-nitrobenzofuran moieties have found utility in some antibacterials.[14] A precedent for species-specific nitroreductases that confer specificity at the drug activation stage led us to explore nitrofurano analogues of calanolides as antimycobacterial agents. For example, reduction of a nitrofuran-containing molecule by an endogenous bacterial nitro-reductase caused DNA lesions in *E. coli*.[14a] The mechanism of action of nitrofurans remains to be elucidated. One possible mechanism is through cleavage of the nitrofuran ring to form the specific tail group with the potential to covalently bind biological molecules.[14a] In human cells, recent research demonstrated that aldehyde dehydrogenase (ALDH) 2 was involved in off-target toxic side effects of 5-nitrofurans. Combining 5-nitrofurans with ALDH2 inhibitors to block off-target 5-nitrofuran biological activity may alleviate clinical side effects caused by 5-nitrofurans.[15] Reduction of a nitro group to a hydroxylamino group may generate reactive intermediates that covalently interact with DNA.[16] This reduction is expected to occur upon exposure of the nitro compound to the huge diversity of organisms comprising the human gut microbiome and can be evaluated during in vitro toxicity and genotoxicity assays of nitrofuran-containing compounds.

To examine the potential anti-*Mtb* activity of nitrofurano analogues of calanolides, we replaced ring D with a nitrofurano moiety by reacting intermediate **1d** with bromonitromethane in the presence of $K_2CO_3$, followed by dehydration in acetic anhydride to afford the anticipated 2-nitrobenzofuran **18** (Scheme 1).[17]

Table 1 illustrates the activity of compounds against *Mtb* in round 1 of design and synthesis. Compound **14** inhibited NR-*Mtb* with $MIC_{90}$ of 6 μg/mL, and compounds **4f**, **4k**, and **8c** inhibited NR-*Mtb* when incubated for extended periods (7 days), with $MIC_{90}$ values of 7, 2, and 2 μg/mL, respectively. These compounds showed no effect on R-*Mtb*. Compounds **4e**, **4g**, **7a**, and **9** showed R-*Mtb* inhibitory activity with $MIC_{90}$ values of 20, 19, 19, and 23 μg/mL, respectively, but exhibited no effects on NR-*Mtb*.

When ring D was replaced with a benzofuran-3-one ring, compound **14** showed only activity against NR-*Mtb*. However, replacement of ring D with the 2-nitrobenzofuran pharmacophore (**18**) led to potent inhibition of both R-*Mtb* and NR-*Mtb*, with $MIC_{90}$ values of 0.3 and 0.6 μg/mL, respectively. Figure 1 summarizes the SAR at this round. As the most potent analogue in the first round of design and synthesis with submicrogram per milliliter $MIC_{90}$ value and improved SI compared to (+)-calanolide A, compound **18** was selected for further SAR studies.

*Round 2 of Design and Synthesis: Synthesis of 2-Nitrobenzofuran **18** Analogues.* Criteria for the design and synthesis of **18** analogues (Table 2) were the following: (1) to explore various double bonds and to improve the clogP value of the target compound (**22a**−**22g**, **26a**−**26c**, Scheme 2); (2) to increase the molecular flexibility, including saturating the C−C double bond (**30**, **32**, and **33**, Scheme 2), as well as removing ring C (**34**−**40**, Scheme 3); (3) to define the role of the nitro group (**42**, Scheme 4); and (4) to explore various nitrofurans (**46**, **49**, **52**, **56**, Schemes 5−8).

Using the same procedure as for the synthesis of **18**, compounds **22a**−**22g** bearing various substituents at C3 and C4 positions were synthesized through intermediates **19**−**21**. The reaction of intermediate **20j** and (*E*)-(3,3-diethoxyprop-1-enyl)benzene[10a] provided intermediate **23**, which led to target compound **24** containing a phenyl substitution at the C6 position in place of the dimethyl substitution found on **18** (Scheme 2).

We next tried to improve the water solubility of the compounds. Hydrophilic groups were introduced by 4-methyl substitution in **22a** using intermediate **21i** with chloromethyl substitution at the C4 position, which reacted with either morpholine, thiomorpholine, or *N*-methylpiperazine, to produce intermediates **25a**−**25c**, respectively. When these *O*-hydroxybenzaldehydes (**25a**−**25c**) were treated with bromonitromethane as was done for **18** (Scheme 1, reaction condition n, (ii)), mixed products were observed in the dehydration step in acetic anhydride. The optimal condition in the presence of $PPh_3$ and DEAD was finally employed, resulting in **26a, 26b**, and **26c** (Scheme 2).[18]

To investigate the effect of ring C modifications, ring C-saturated analogues **30a** and **30b** were synthesized. Hydrogenation of **1d** with Pd/C catalyst gave a complicated product mixture due to an aldehyde moiety. Protecting **1d**'s aldehyde as the diacetyl acetal also acetylated the hydroxyl group to give **27a**, which was hydrogenated with Pd/C catalyst to afford **28a**. When treated with $K_2CO_3$ in methanol, **28a** was deprotected to **29a**. Then **29a** was reacted with $BrCH_2NO_2$ in the presence of $K_2CO_3$ followed by dehydration with acetic anhydride to give the target compound **30a**. Using the same method, the


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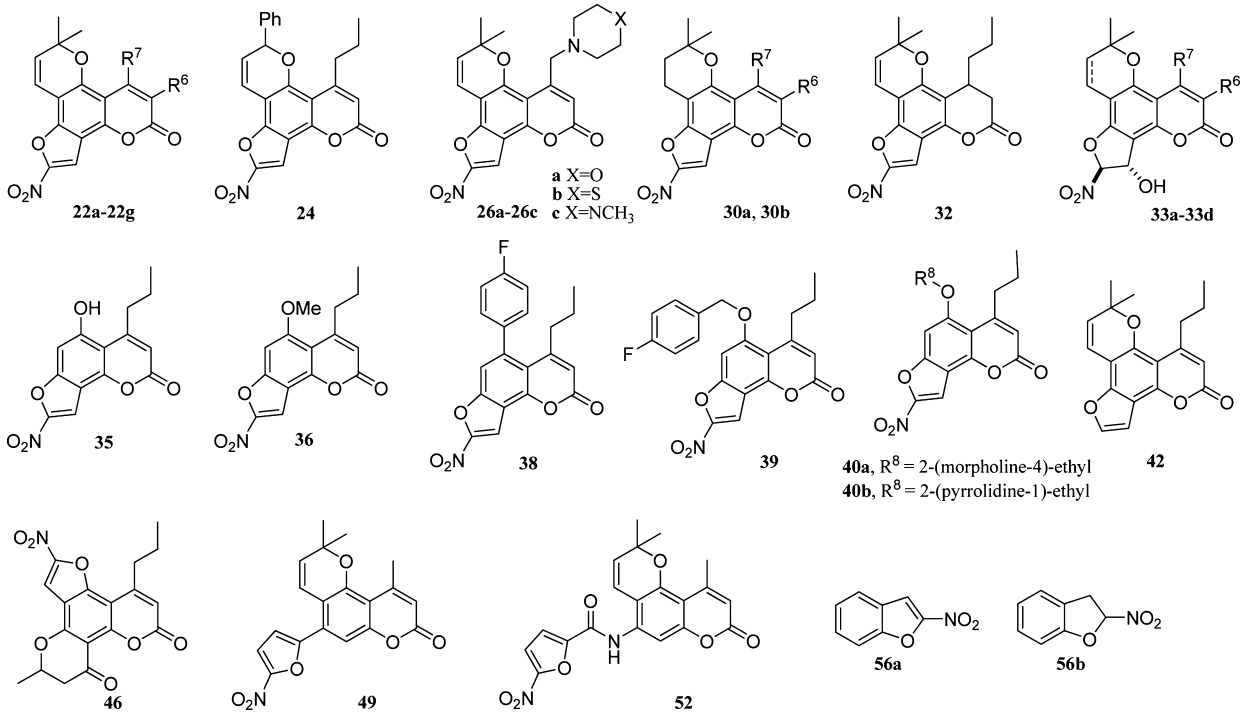
**Table 2. In Vitro Activity of the Analogues in Round 2 of Design and Synthesis**




| compd | $MIC_{90}$ ($\mu$g/mL) R, NR | $LD_{50}$, SI | compd | $MIC_{90}$ ($\mu$g/mL) R, NR | $LD_{50}$, SI |
|---|---|---|---|---|---|
| **22a**** | 0.08, 0.2 | 1, 16 | **22b** | 2, 19 | ND |
| **22c** | 2, >21 | ND | **22d** | <0.1, 0.2 | >50, >510 |
| **22e**** | 0.6, 1 | >25, >40 | **22f** | 19, 19 | ND |
| **22g** | <0.2, 2 | ND | **24** | 20, 6 | 3, 0.1 |
| **26a** | <0.2, <0.2 | 1, >5 | **26b** | <0.3, <0.3 | 1, >5 |
| **26c**** | 0.08, 0.3 | 3, 31 | **30a**** | 0.6, 1 | >50, >80 |
| **30b** | 0.8, 2 | >50, >64 | **32** | >18, >18 | ND |
| **33a** | <0.2, <0.2 | 3, >11 | **33b** | <0.1, <0.1 | 13, > 128 |
| **33c** | 0.4, 0.8 | 25, 64 | **33d** | 0.2, 0.4 | 25, 128 |
| **35** | >14, >14 | ND | **36** | <0.2, <0.2 | 0.3, >2 |
| **38** | 0.7, 18 | ND | **39**** | 0.6, 3 | >100, >160 |
| **40a**** | 0.08, 0.2 | 10, 125 | **40b** | <0.2, <0.2 | 1, >5 |
| **42** | >100, >100 | ND | **46** | >50, 6 | ND |
| **49** | >18, >18 | >18, CN | **52** | >20, >20 | ND |
| **56a** | 0.2, > 100 | 3, 16 | **56b** | 0.4, 50 | 3, 6 |

*See Scheme 2 for $R^6$ and $R^7$ substitutions. **The activity was confirmed in WT *Mtb* H37Rv; MICs in the table are indicative of WT *Mtb* H37Rv bacteria. ND, not determined. CN, cannot be calculated SI, selectivity index determined by $LD_{50}$ for HepG2 cells/$MIC_{90}$ for R-*Mtb*.

3-fluoro-4-methyl analogue with saturated ring C was synthesized, resulting in **30b** (Scheme 2).

Synthesis of the target compound **32** permitted study of the antitubercular activity of the dihydrocoumarin analogue. One aspect of interest leading to the preparation of this compound was to explore possible removal of the potential Michael acceptor at this site of the coumarin. 3,4-Dihydro-5,7-dihydroxycoumarin **31** was synthesized by hydrogenation of 5,7-dihydroxycoumarin **19j**, employing Pd/C as catalyst aided by ultrasound. Bis-phenol **31** was then converted to target compound **32** in three steps, as was done for **18** (Scheme 2).

*trans*-11-Hydroxy-10,11-dihydro-10-nitrobenzofuran **33a**, the synthetic precursor of compound **18**, was also prepared for SAR studies. The relative *trans* configuration was confirmed by H10−H11 coupling in $^1$H NMR.[19] For further SAR evaluation of the dihydronitrobenzofuran, **33b−33d** were synthesized using the same method as was done for **33a** (Scheme 2). Several attempts to synthesize 11-dehydroxylated dihydronitrofurano analogue of **33a** as well as the ring D saturated analogue of **18** failed.

Analogues **34−40** which lack ring C were designed to reduce planarity of the target compounds. Synthesis of compound **34** started from **20j**. The 5-hydroxy of **20j** was selectively reacted with chloromethyl methyl ether followed by the same method that was used to make **18** from **1d** to yield **34**. Removal of the protective group of **34** in acid conditions produced **35**. Reaction of **35** with either dimethyl sulfate or 1-bromomethyl-4-fluorobenzene produced the anticipated compounds **36** and **39**, respectively. Compound **35** was also converted to triflate **37**, which was used to prepare compound **38** employing the Suzuki cross-coupling reaction. Mitsunobu reaction of **35** with commercially available alcohols 2-morpholinoethanol and 2-(pyrrolidin-1-yl)ethanol afforded target compounds **40a** and **40b**, respectively (Scheme 3).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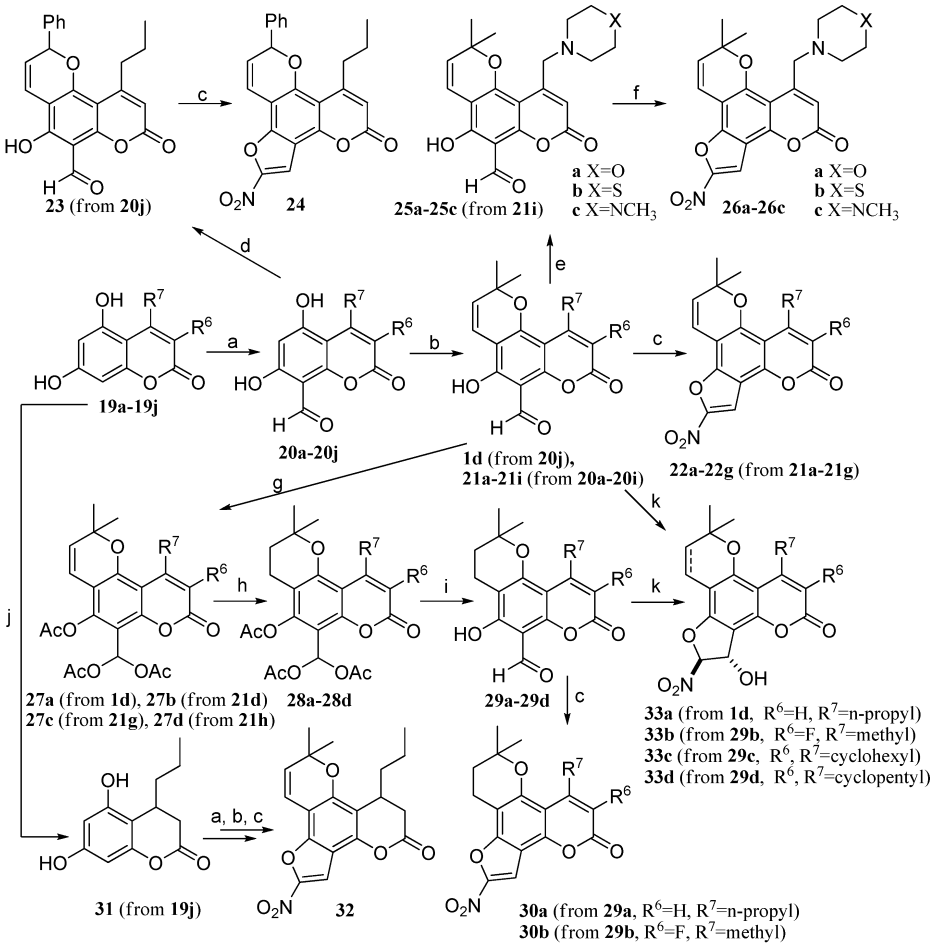
**Scheme 2. Synthesis of Compounds 22a−22g, 24, 26a−26c, 30a, 30b, 32, and 33a−33d**[a]




$R^6$ and $R^7$ substitutions of compounds **19a-19j**, **20a-20j**, **21a-21i**, and **22a-22g**.

| Compd | 19a-22a | 19b-22b | 19c-22c | 19d-22d | 19e-22e | 19f-22f | 19g-22g | 19h-21h | 19i-21i | 19j-20j |
|---|---|---|---|---|---|---|---|---|---|---|
| $R^6$ | H | H | benzyl | F | F | F | cyclohexyl | cyclopentyl | H | H |
| $R^7$ | methyl | phenyl | methyl | methyl | ethyl | *n*-propyl | | | chloromethyl | n-propyl |

[a]Reagents and conditions: (a) $POCl_3$, DMF, $CH_2Cl_2$, rt, 24 h; (b) $(CH_3)_2CCHCH(OEt)_2$, pyridine, toluene, reflux, 3 h, 24−72% in two steps; (c) i, $NO_2CH_2Br$, $K_2CO_3$, acetone, rt, 3 h; ii, acetic anhydride, 100 °C, 1 h, 40−51%; (d) (*E*)-(3,3-diethoxyprop-1-enyl)benzene, pyridine, toluene, reflux, 3 h, 50%; (e) amine, DIPEA, DMF, rt, 12 h, 58−69%; (f) i, $NO_2CH_2Br$, $K_2CO_3$, acetone, rt, 3 h; ii, $PPh_3$, DEAD, THF, rt, 1 h, 25−34%; (g) acetic anhydride, Amberlite IR-120, rt, 1 h, 72−81%; (h) $H_2$, Pd/C, $CH_3CH_2OH$, rt, 1 h, 90−96%; (i) $K_2CO_3$, $CH_3OH$, rt, 1 h, 91−92%; (j) $H_2$, Pd/C, $CH_3CH_2OH$, ultrasound, rt,10 h, 60%; (k) $BrCH_2NO_2$, $K_2CO_3$, acetone, rt, 3 h, 51−99%.

Compound **42**, a 10-denitro analogue of **18**, was designed to determine if the nitro group of compound **18** was essential for antitubercular activity. To obtain **42**, compound **41** was prepared from **1b** in a reaction with *N*-iodosuccinimide, followed by a Sonogashira cross-coupling reaction with trimethylsilylacetylene, intramolecular cyclization, and removal of the trimethylsilyl group to provide target compound **42** (Scheme 4).[20]

As shown by compound **46**, the 5-nitrofurano moiety was incorporated at the ring C position, whereas the ring D was 2-methyl-2,3-dihydro-4-pyranone. This compound was useful to study the impact of having a nitrofurano moiety in ring C. Similar to intermediate **41**, **44** was prepared from **43**[10a] by reaction with *N*-iodosuccinimide, followed by a Sonogashira cross-coupling reaction with trimethylsilylacetylene, intramolecular cyclization, and nitration to yield target compound **46** (Scheme 5).[21]

To further study the impact of the nitrofuran group, a 5-nitrofuran-2yl moiety was directly linked at the C9 position as shown by compound **49** (Scheme 6). Its synthesis benefited from triflate **47**, which was synthesized from coumarin **19a** using the same procedure as that for triflate **5**. Triflate **47** subjected to a Suzuki coupling reaction gave compound **48**, which was then nitrated with $HNO_3$ to produce compound **49**.[22]

For SAR studies, 5-nitrofuran was also linked at the C9 position as shown by amide **52** (Scheme 7). Amine **50** was synthesized in a similar method as **11**. The reaction of **50** with 5-nitrofuran-2-carbonyl chloride **51** resulted in compound **52**.

To clarify the role of the 2-nitrobenzofuran unit in antitubercular activity, 2-nitrobenzofuran **56a** and 2-nitro-2,3-dihydrobenzofuran **56b** were also synthesized by the method recently reported by our laboratory (Scheme 8).[23] All of the analogues obtained in the second round of design and synthesis were screened for their activity against R-*Mtb* and NR-*Mtb* (Table 2), and the SAR analyses are summarized in the subsequent sections.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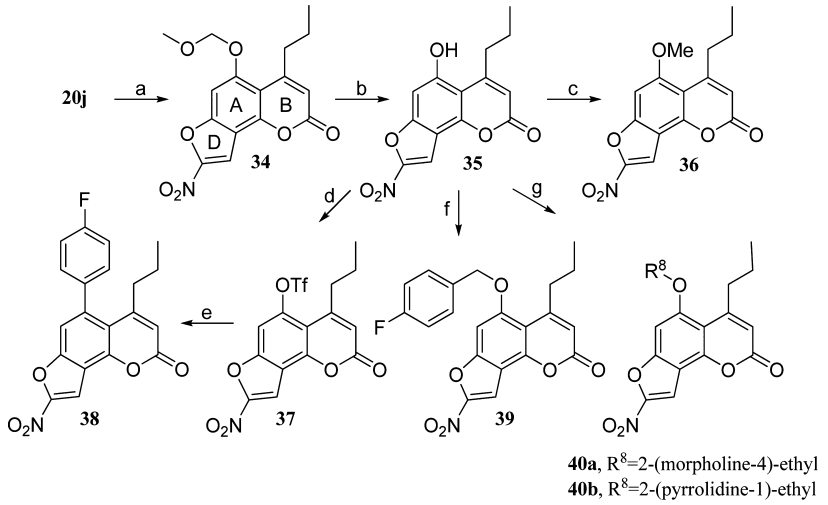
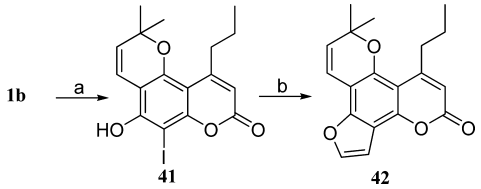
**Scheme 3. Synthesis of Compounds 34−40 Belonging to the ABD Scaffold**[a]




**40a**, $R^8$=2-(morpholine-4)-ethyl
**40b**, $R^8$=2-(pyrrolidine-1)-ethyl

[a] Reagents and conditions: (a) i, $CH_3OCH_2Cl$, $K_2CO_3$, acetone, rt, 5 h; ii, $NO_2CH_2Br$, $K_2CO_3$, acetone, rt, 3 h; iii, acetic anhydride, 100 °C, 1 h, 41%; (b) 2 M HCl, THF, rt, 1 h, 91%; (c) $Me_2SO_4$, $K_2CO_3$, acetone, rt, 12 h, 95%; (d) $Tf_2O$, 2,6-di-*tert*-butyl-4-methylpyridine, $CH_2Cl_2$, 0 °C, 30 min, 51%; (e) 4-fluorophenylboronic acid, $PdCl_2(PPh_3)_2$, $Na_2CO_3$, $H_2O$, DME, Ar, 55 °C, 24 h, 49%; (f) 1-(bromomethyl)-4-fluorobenzene, $K_2CO_3$, acetone, reflux, 3 h, 51%; (g) For **40a**: 2-morpholinoethanol, $PPh_3$, DEAD, THF, rt, 12 h, 56%; For **40b**: 2-(pyrrolidin-1-yl)ethanol, $PPh_3$, DEAD, THF, rt, 12 h, 17%.

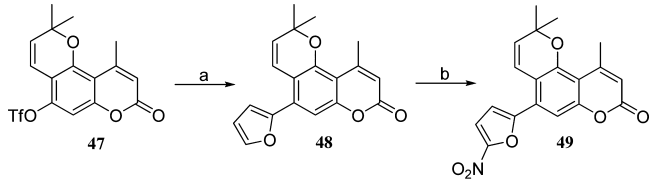
**Scheme 4. Synthesis of Compound 42**[a]




[a]Reagents and conditions: (a) NIS, $CH_2Cl_2$, rt, 3 h, 55%; (b) i, $Me_3SiCCH$, $PdCl_2(PPh_3)_2$, CuI, THF, reflux, 12 h; ii, TFA, $CH_2Cl_2$, rt, 2 h, 37% for two steps.

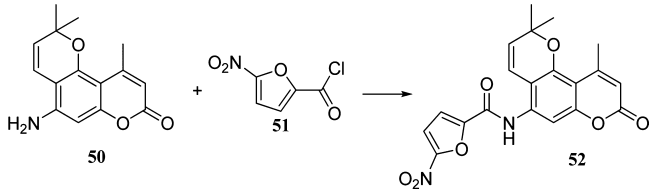
*Modification of Ring B (compounds **22a−22g, 26a−26c, 32**, Table 2).* Compound **22a**, in which C4 was methyl substituted, markedly improved anti-*Mtb* activity, but its SI did not improve compared to compound **18**. C4-phenyl analogue **22b** showed reduced potency against R-*Mtb* and NR-*Mtb*. Introduction of benzyl at the C3 position of compound **22a**, making compound **22c**, decreased R-*Mtb* potency and abrogated activity against NR-*Mtb*. Introduction of hydrophilic groups at the C4-methyl, such as 4-morpholinomethyl- (**26a**), 4-thiomorpholinomethyl- (**26b**), and 4-(4-methylpiperazino-methyl)- (**26c**) gave rise to comparable potencies and SIs to **22a**. Introduction of fluoro substitution at the C3 position on SI. Compounds **22e** and **22f**, as C4 ethyl and C4 propyl analogues of **22d**, respectively, had decreased potency, which suggested that the substitution effect was *n*-propyl < ethyl < methyl. The analogue **22g**, in which C3−C4 had formed a six-membered ring, was less active against NR-*Mtb* compared to the

**Scheme 6. Synthesis of Target Compound 49**[a]



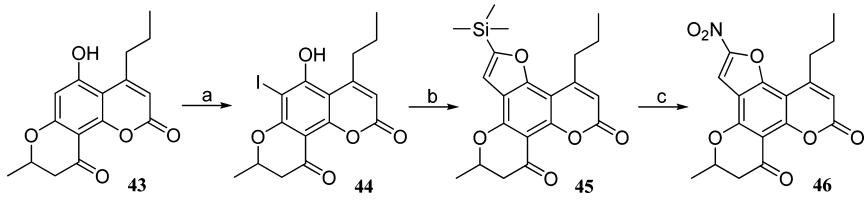

[a]Reagents and conditions: (a) furan-2-boronic acid, $PdCl_2(PPh_3)_2$, $Na_2CO_3$, $H_2O$, DME, Ar, 55 °C, 24h, 73%; (b) $HNO_3$, acetic anhydride, 20 °C, 2h, 31%.

**Scheme 7. Synthesis of Target Compound 52**[a]




[a]Reagents and conditions: $CH_2Cl_2$, rt, 3 h, 78%.

**Scheme 5. Synthesis of Target Compound 46**[a]




[a]Reagents and conditions: (a) NIS, $CH_2Cl_2$, rt, 3 h, 84%; (b) $Me_3SiCCH$, $PdCl_2(PPh_3)_2$, CuI, THF, reflux, 12 h; (c) $HNO_3$, $Ac_2O$, rt, 2 h, 36% in 2 steps.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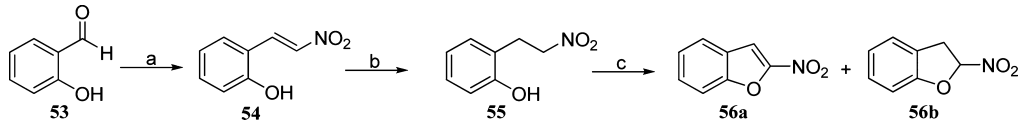
**Scheme 8. Synthesis of Target Compounds 56a, 56b**[a]




[a]Reagents and conditions: (a) $AcONH_4$, $CH_3NO_2$, 120 °C, 70 min, 70%; (b) $NaBH_4$, $CH_3OH$, 0 °C, 30 min, 82%; (c) DIB, TBAI, AcONa, $CH_3CN$, rt, 5 h, **56a** (60%), **56b** (10%).

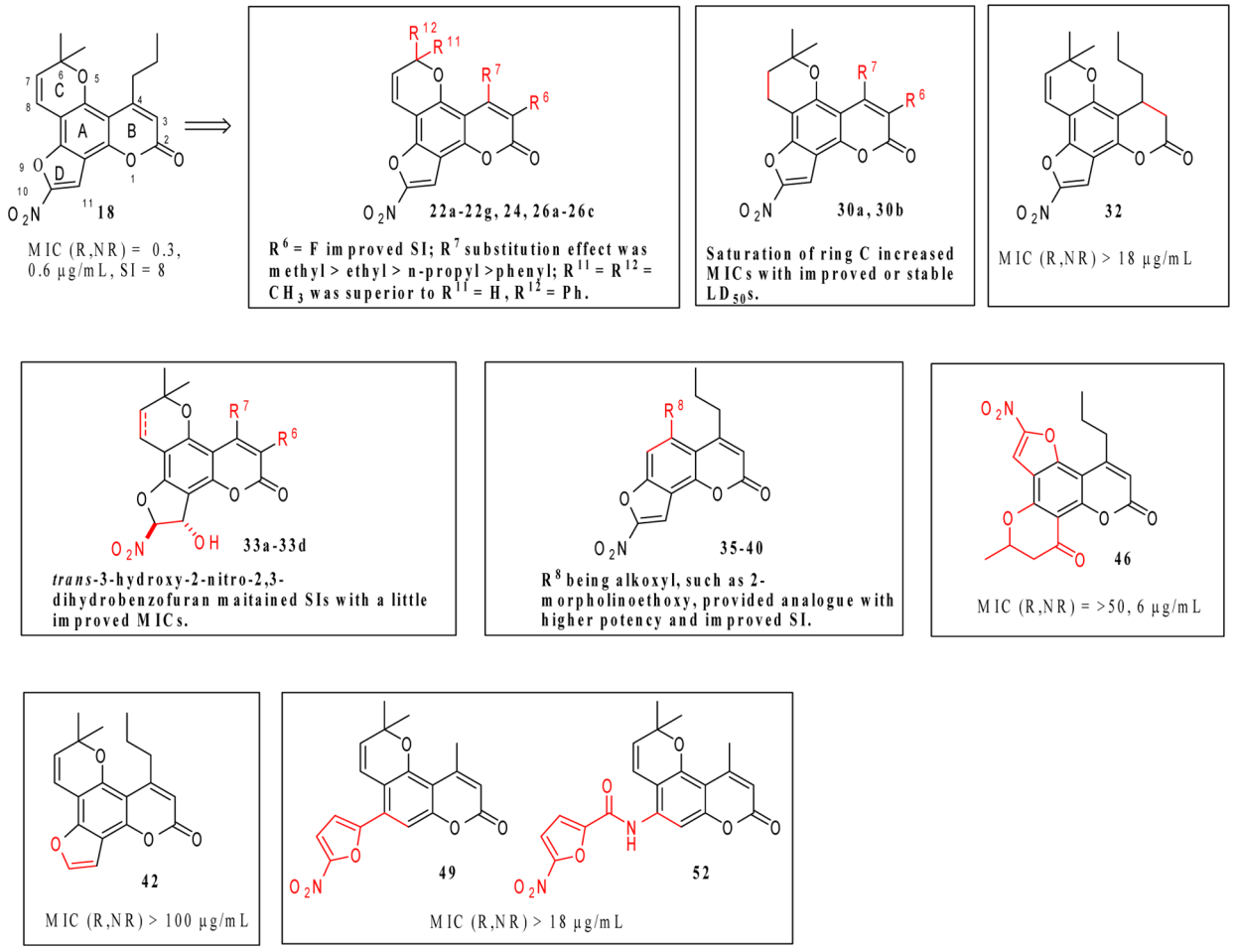



**Figure 2.** Summary of SARs from round 2 of design and synthesis.

parent compound **18**. Compound **32**, a 3,4-dihydro derivative of **18**, had an R and NR $MIC_{90}$ > 18 μg/mL, demonstrating the importance of the coumarin unit for anti-*Mtb* activity. This loss of activity may either be due to the lower stability of the six-membered lactone than that of the coumarin structure or simply reflect the necessity for the α,β-unsaturated lactone moiety as a component involved in its mode of action or multiple modes of action.

In summary, the SAR studies from these analogues revealed that (a) the unsaturated coumarin structure (ring B) must be maintained; (b) introduction of fluoro substitution at C-3 position improved $LD_{50}$; (c) introduction of a C3 benzyl or C3−C4 fused six-membered ring was detrimental to potency; (d) the sequence of positive effects of C4 substitution on R-*Mtb* potency was phenyl < *n*-propyl < ethyl < methyl. When methyl was substituted with 4-morpholinomethyl-, 4-thiomorpholinomethyl-, and 4-(4-methylpiperazinomethyl)-, the resulting compounds generally maintained potency.

*Modification of Ring C (Compounds **24**, **30**, **35**, **36**, **38**, **39**, **40a**, **40b**, Table 2).* Replacement of the dimethyl substitution at the C6 position of **18** with phenyl monosubstitution gave **24** with decreased potency and SI. Compound **30a**, formed by 7,8-dihydrogenation of **18**, reduced potency by 2-fold but improved SI >10-fold due to improved $LD_{50}$. Compound **30b**, formed by 7,8-dihydrogenation of **22d**, displayed reduced potency, which resulted in a reduced SI despite similar $LD_{50}$ values. These data suggested that saturation of ring C reduced potency with improved or stable $LD_{50}$ values. Compounds **35**, **36**, and **38**−**40** were generated as analogues of the ABD scaffold with various C5 substitutions. The 5-hydroxyl analogue (compound **35**) was inactive and 5-(4-fluorophenyl) analogue (**38**) lost most NR-*Mtb* activity. However, the 5-OMe analogue (**36**), 5-(4-fluorobenzyloxy) analogue (**39**), 5-(2-morpholinoethoxy) analogue (**40a**), and 5-(2-(pyrrolidin-1-yl)ethoxy) analogue (**40b**) all exhibited potent activity against *Mtb*. In particular, **40a** had a 16-fold improved SI value compared to **18**. SAR around ring C modifications can be summarized as follows: (a) saturation of ring C reduced potency with improved or stable $LD_{50}$ values; (b) monophenyl at the C6 position was inferior to dimethyl for both potency and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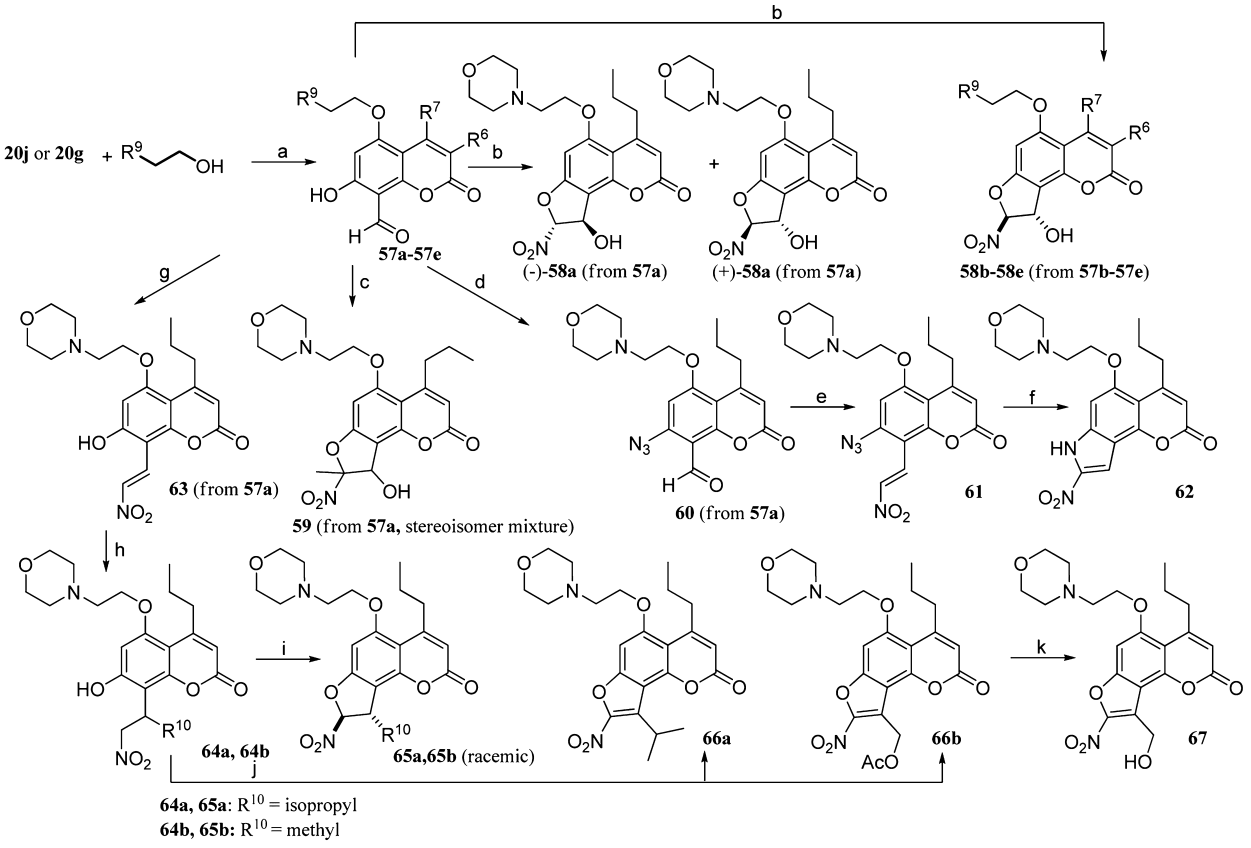
**Scheme 9. Synthesis of Compounds 58, 59, 62, 65−67**[a]




$R^6$, $R^7$, and $R^9$ substitutions of compounds **57a-57e, 58a-58e**.

| Compds | 57a, 58a | 57b, 58b | 57c, 58c | 57d, 58d | 57e, 58e |
|---|---|---|---|---|---|
| $R^6$ | H | H | H | cyclohexyl | cyclohexyl |
| $R^7$ | *n*-propyl | *n*-propyl | *n*-propyl | | |
| $R^9$ |  |  | hydroxyl |  |  |

[a]Reagents and conditions: (a) $PPh_3$, DEAD, THF, rt, 12 h, for **57a**: 77%, for **57b−57e**: crude product were obtained; (b) $BrCH_2NO_2$, $K_2CO_3$, acetone, rt, 3 h, for (±)**58a**: 59%, for **58b−58e**: 42−63% (from **20j** or **20g**); (c) $CH_3CHBrNO_2$, $K_2CO_3$, acetone, rt, 3 h, 55%; (d) DPPA, DIPEA, DMF, rt, 2 h, 74%; (e) i. $CH_3NO_2$, $K_2CO_3$, rt, 1 h, ii. $Ac_2O$, pyridine, rt, 1 h, 67%; (f) xylene, reflux, 3 h, 52%; (g) $AcONH_4$, $CH_3NO_2$, 120 °C, 70 min, 92%; (h) for **64a**: isopropylmagnesium chloride, THF, −78 °C, 1 h; for **64b**: methylmagnesium bromide, THF, −78 °C, 1 h; (i) TBAI, $H_2O_2$, $CH_3CN$, rt, 6 h, for **65a**, 49% (from **63**); **67**, 50% (from **63**); (j) iodobenzene diacetate, TBAI, $Et_3N$, $CH_3CN$, 35 °C, 1 h, for **66a**: 63% (from **63**), for **66b**: 65% (from **63**); (k) HCl/$CH_3OH$, 75 °C, 2 h, 83%.

SI; and (c) introduction of an alkoxyl substitution for $R^8$ such as 2-morpholinoethoxy produced a more potent analogue with improved SI.

*Modification of Ring D (Compounds **33a−33d, 42, 46, 49, 52, 56a, and 56b**, Table 2).* During the synthesis of compound **18**, intermediate compound **33a** was prepared as an inseparable mixture of *trans* isomers. This mixture exhibited a comparable MIC and SI to compound **18**. Compound **33b**, as the synthetic intermediate of compound **30b**, had improved potency and SI compared to **30b**. Other dihydronitrobenzofurans, such as **33c** and **33d** had comparable potency and SI to **33b**. In conclusion, this class of *trans*-3-hydroxy-2-nitro-2,3-dihydronitrobenzofurans had stable SIs and slightly improved potency when compared to nitrobenzofurans. Compound **42**, a 10-denitro analogue of **18** lacked activity against R-*Mtb* and NR-*Mtb*, demonstrating the high significance of **18**'s nitro group for its antitubercular activity. Analogue **46**, with a 5-nitrofurano on ring C, had significantly reduced activity, emphasizing the importance of the location of the nitro group. Compounds **49**, a ring D acyclic analogue with a C9 5-nitrofuran-2-yl substituent, and **52**, another ring D acyclic analogue with a 5-nitrofuran-2-carboxamide side chain at the C9 position, were inactive. These analogues were consistent with 2-nitrobenzofuran being the pharmacophore of the potent compounds. Therefore,


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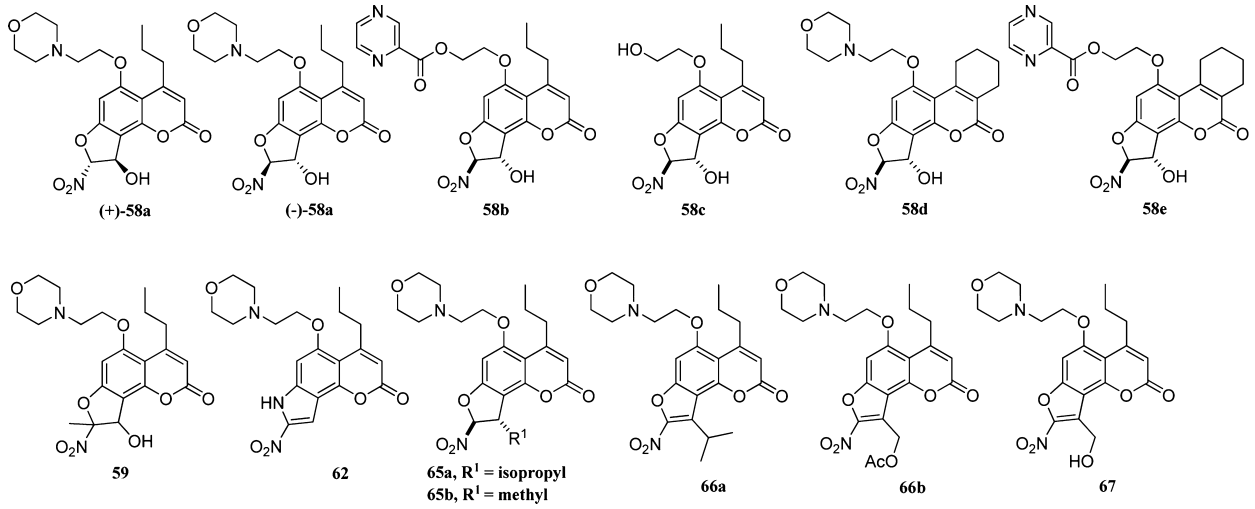
**Table 3. In Vitro Activity of the Analogues in Round 3 of Design and Synthesis**




| compd | $MIC_{90}$ (μg/mL) R, NR | $LD_{50}$, SI | compd | $MIC_{90}$ (μg/mL) R, NR | $LD_{50}$, SI |
|---|---|---|---|---|---|
| (+)-58a** | 0.08, 0.08 | 0.8, 10 | (−)-58a** | 0.08, 0.08 | 2, 20 |
| **58b** | <1, <1 | <1, CN | **58c** | <1, <1 | <1, CN |
| **58d** | <1, <1 | 0.4, >0.3 | **58e** | <1, <1 | 0.4, >0.3 |
| **59** | 100, 100 | >100, >1 | **62** | >50, >50 | 25, <0.5 |
| **65a** | 22, >22 | >22, >1 | **65b** | >21, >21 | >21, CN |
| **66a** | >22, >22 | 22, <1 | **66b** | 1, 3 | <0.3, <0.3 |
| **67** | 100, 100 | ND | | | |

**The activity was confirmed in WT *Mtb* H37Rv; MICs in the table were indicative of WT *Mtb* H37Rv bacteria. ND, not determined. CN, cannot be calculated. SI, selectivity index was determined by $LD_{50}$ for HepG2 cells/$MIC_{90}$ for R-*Mtb*.

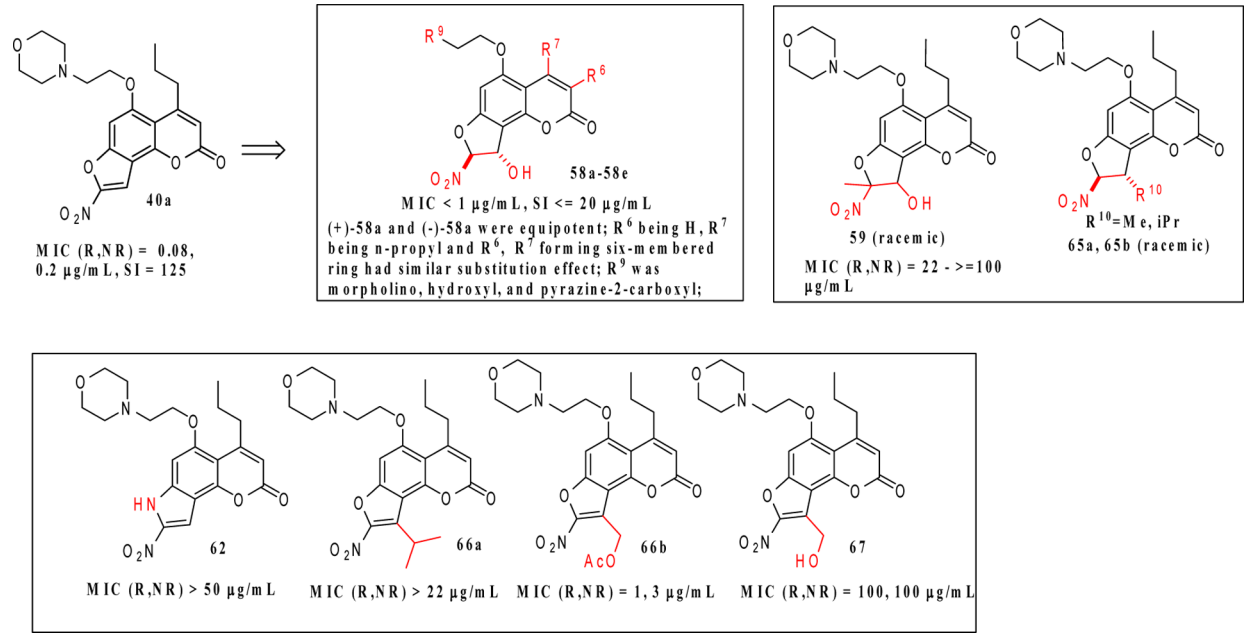



**Figure 3.** Summary of SARs from round 3 of design and synthesis.

2-nitrobenzofuran **56a** and 2-nitro-2,3-dihydrobenzofuran **56b** were evaluated for anti-*Mtb* activity. These two compounds exhibited potent R-*Mtb* inhibitory activity but lacked NR-*Mtb* inhibitory activity. This result demonstrated the significance of the coumarin unit in the synthetic calanolide nitrobenzofurans with potent dual activity toward both R-*Mtb* and NR-*Mtb*, such as **18**, **30a**, and **40a**.

In summary (Figure 2), the SAR studies of analogues exploring modifications of rings B, C, and D informed us that both the 2-nitrobenzofuran and coumarin moieties were essential for potent dual inhibitory activity against R-*Mtb* and NR-*Mtb*.

*Round 3 of Design and Synthesis: Synthesis of 2-Nitrobenzofuran **40a** Analogues.* Analysis of the SAR studies based on the 2-nitrobenzofuran **18** showed that the 2-nitrobenzofuran **40a** had improved potency and SI values (Table 2). Additionally, it had satisfactory skeletal flexibility, because the rigid ring C was replaced with a morpholinoethoxyl substitution. Therefore, we next carried out modifications based on **40a**.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Based on our conclusion from the second round of design and synthesis that *trans*-3-hydroxy-2-nitro-2,3-dihydronitrobenzofurans maintained SI with slightly improved potency compared to nitrobenzofurans, the synthetic precursor of compound **40a** was used to obtain *trans*-enantiomeric (±)-**58a**. To determine the MIC and SI of the enantiomeric isomer respectively, (+)- and (−)-**58a** were separated by chiral HPLC and their absolute configurations were proposed on the basis of the CD spectra (see Supporting Information). Using a similar procedure, racemic **58b** with pyrazine-2-carboxylethoxyl at C5 position, racemic **58c** with 2-hydroxylethoxyl at C5 position, and racemic **58d** and **58e** with $R^1$ and $R^2$ fused as a six-membered ring were also prepared for SARs studies (Scheme 9).

We desired to explore whether, under certain conditions (for example, in the antitubercular assays), compounds such as **58a**, having a 3-hydroxy-2-nitro-2,3-dihydrobenzofuran moiety in place of the 2-nitrobenzofuran, could eliminate water in situ, to give back a 2-nitrobenzofuran. Thus, additional compounds blocking this elimination were prepared, such as compound **59**, where a methyl was introduced to the nitro-substituted carbon atom of **58a** to block elimination of the hydroxyl. 1-Bromo-1-nitroethane was employed to obtain compound **59** as a stereoisomeric mixture (Scheme 9).

On the basis of the structure of **40a**, 2-nitroindole was explored to replace the 2-nitrobenzofuran moiety yielding compound **62**. To do this, compound **57a** was treated with diphenyl azidophosphate to produce azide **60**.[24] To the best of our knowledge, this method for azide preparation was first applied to *O*-formyl phenols, such as compound **57a**. Subsequently, condensation between **60** and nitromethane produced compound **61**, which cyclized to nitroindole **62** in refluxing xylene (Scheme 9).[25]

To evaluate the impact of the hydroxyl group in compounds **58a**, compounds **65a**/**65b** were synthesized to substitute the hydroxyl with a methyl (**65b**) or isopropyl group (**65a**). Catalyzed by ammonium acetate, compound **57a** and nitromethane were condensed to **63**, which underwent Michael additions with methylmagnesium bromide or isopropylmagnesium chloride to produce **64a**/**64b**, respectively. Upon treatment with hydrogen peroxide and tetrabutylammonium iodide, **64a**/**64b** produced **65a**/**65b** (Scheme 9).[23]

To further substitute the furan ring in **40a**, **64a**/**64b** were treated with iodobenzene diacetate and tetrabutylammonium iodide to produce **66a**/**66b** with isopropyl and acetoxylmethyl substitutions,[23] respectively, which may sterically block the reduction of the nitro group. Compound **66b** underwent deacetylation under hydrogen chloride treatment to afford **67**, having a hydroxymethyl on the nitrobenzofuran ring (Scheme 9).

Analogues obtained in the third round of design and synthesis were assayed for their ability to kill R- and NR-*Mtb* (Table 3). These data suggested that (1) the nitroindole analogue, and analogues with substitution of isopropyl, acetoxymethyl, or hydroxymethyl at the C3 position of 2-nitrobenzofuran such as compounds **62**, **66a**, **66b**, and **67**, showed markedly reduced potency or no potency; (2) *trans*-3-hydroxy-2-nitro-2,3-dihydrobenzofuran analogues **58a**−**58e** had MICs < 1 μg/mL with $LD_{50}$'s < 2. Among these compounds, activities of the enantiomers (+)-**58a** and (−)-**58a** were surprisingly equipotent. $R^6$ as H, $R^7$ as *n*-propyl (**58a, 58b, 58c**) and $R^6$, $R^7$ forming a six-membered ring (**58d, 58e**) had similar effects on MIC and SI. When $R^9$ was morpholino, hydroxyl, or pyrazine-2-carboxyl, the resulting analogues had comparable MICs and SIs; (3) 3-hydroxy-2-methyl-2-nitro-2,3-dihydrobenzofuran **59** and 3-isopropyl (or methyl)-2-nitro-2,3-dihydrobenzofuran **65a, 65b** showed no significant potency. These results may provide circumstantial evidence that the unblocked 3-hydroxy-2-nitro-2,3-dihydrobenzofuran analogues may eliminate water to give back a nitrobenzofuran, although no experimental evidence has been obtained to confirm such elimination (Figure 3).

**Additional Aspects of Biological Activity of Synthetic Calanolides.** *Select Calanolides Are Cidal against Replicating and Nonreplicating Mtb.* The synthetic calanolides with potent R and NR activity and acceptable selectivity indices were chosen for further bactericidal evaluation. A colony-forming unit (CFU) assay was employed to determine the impact of several growth-inhibitory nitrofurano analogues of calanolides on wild-type *Mtb*. Compounds **18** and **30a** were bactericidal against both R- and NR-*Mtb*. Compound **18** reduced survival of R-*Mtb* by 3.1 $\log_{10}$ at 0.6 μg/mL (2× MIC) and NR-*Mtb* by 2.6−4.5 $\log_{10}$ at 0.6 μg/mL (1× MIC) (Figure 4a,b). Similarly, compound **30a**

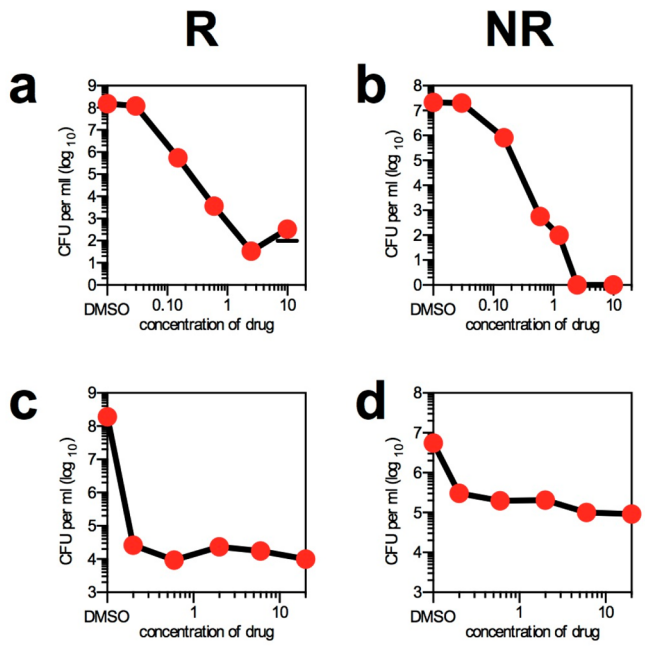


**Figure 4.** Nitrofurano analogues of calanolides kill R and NR-*Mtb*. Compounds **18** (a, b), **30a** (c, d) or DMSO were coincubated with *Mtb* in R conditions (200 μL of 3.4 × $10^6$/mL bacteria) (a, c) or NR conditions (200 μL of 3 × $10^7$/mL bacteria) (b, d). After 7−8 days, bacteria were enumerated on 7H11 agar plates supplemented with OADC and glycerol and incubated an additional ~3 weeks, after which colonies were counted. Results are means ± SD of triplicates of one experiment representative of two.

reduced R-*Mtb* by 2.6 $\log_{10}$, at 0.6 μg/mL (1× MIC), and NR bacilli by 2.1 $\log_{10}$, at 0.6 μg/mL (0.5−1× MIC) (Figure 4c,d), when compared to the inoculum plated.

*Calanolides Spare Gram-Positive/-Negative Bacteria and Yeast.* The activity of nitrofurano analogues of calanolide **30a** was determined against a panel of Gram-positive bacteria, Gram-negative bacteria, and yeast. Despite its potent activity against *Mtb*, **30a** failed to kill *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* or *Candida albicans* at concentrations ≤25 μg/mL. Compound **30a** was weakly active against a nonpathogenic mycobacterial strain, *Mycobacterium smegmatis* (~20−70% inhibition of growth at 10 μg/mL) (Figure 5).

*Calanolides Kill Mtb within Primary Human Macrophages.* The majority of the intracellular *Mtb* in the infected


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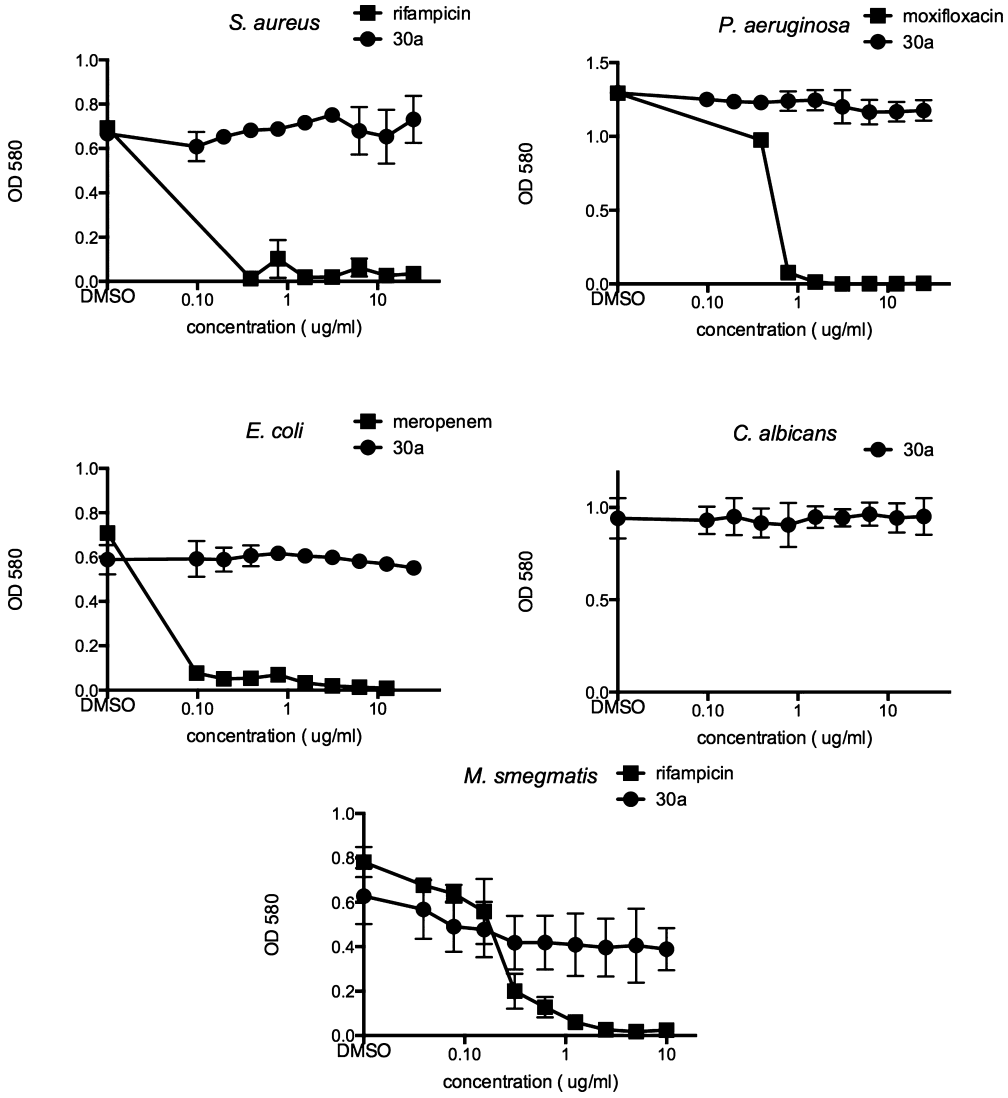


**Figure 5.** Nitrofurano analogues of calanolides are selectively active against *Mtb*. Compound **30a**, DMSO vehicle, or control antibiotics (moxifloxacin, Meropenem, and rifampicin) were coincubated with log-phase Gram positive (a) or negative bacteria (b, c) or yeast (d) for 6−18 h. At the end of the incubation, $OD_{600}$ was measured. Results are means ± SD for triplicates, representative of two similar experiments. *Mycobacterium smegmatis* (e) was coincubated with compounds indicated (**30a** and rifampicin) for 24−48 h. At the end of the incubation, $OD_{580}$ was measured. Results are means ± SD of triplicates, representative of two similar experiments.

host resides within macrophages.[26] We evaluated the activity of select synthetic calanolides for their ability to kill wild-type *Mtb* infecting primary monocyte-derived human macrophages.[27] Compound **18** reduced the number of *Mtb* in human macrophages by ~1.8 $\log_{10}$ at 1 μg/mL [3× MIC]. Compound **30a**, which had minimal toxicity to HepG2 cells, reduced the number of viable intracellular *Mtb* by 2−4 $\log_{10}$ (Figure 6). The extent of bactericidal activity of these calanolides was greater than that of the first-line TB drug, rifampicin (1−1.5 $\log_{10}$ killing at 30× MIC, data not shown). Although compounds **39** and **40a** decreased *Mtb* viability in human macrophages by 1−2 $\log_{10}$ of killing (data not shown), **30a** was the most potent calanolide tested against *Mtb* in the human macrophage model.

*Certain Calanolides Are Genotoxic.* The nitrofuran moiety has been associated with DNA damage and is regarded as a major structural alert in medicinal chemistry.[14a] Although compound **30a** and other select nitrofurano analogues of calanolides were either weakly toxic or nontoxic to HepG2 cells, our concern regarding the nitrofurano moiety prompted us to evaluate the DNA damaging capacity of these compounds. Thus, compounds **30a** and **40a** were subjected to genotoxicity testing in a mouse lymphoma assay (kindly performed by GlaxoSmithKline) and, not surprisingly, were found to be genotoxic (data not shown), precluding their progression as lead compounds. However, the potent activity of select synthetic calanolides against both R- and NR-*Mtb*, narrow microbicidal spectrum, and potency against *Mtb* residing in human macrophages make them attractive candidates as tool compounds for target identification.

## ■ SUMMARY

Newly synthesized ring-D-modified analogues led to identification of several series of novel synthetic calanolides with selective activity against R-*Mtb* and/or NR-*Mtb*. In particular, analogues bearing 2-nitrofurano at the ring D position had markedly improved in vitro efficacy and reduced mammalian cell toxicity compared with the parent compound, (+)-calanolide A. For example, compound **39** had MIC values of 0.6 μg/mL and 3 μg/mL against R-*Mtb* and NR-*Mtb*, respectively,


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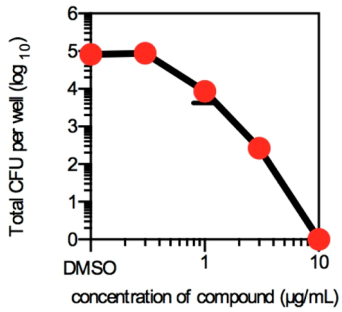


**Figure 6.** Compound **30a** kills wild-type *Mtb* infecting human macrophages. Primary human macrophages were differentiated, activated by IFN-gamma, and infected with wild-type *Mtb* at a multiplicity of infection (MOI) of 0.1. After 24 h of infection, compound or vehicle (DMSO) was added for an additional 7 days. Macrophages were evaluated by microscopy for evidence of toxicity, after which they were washed twice with PBS to remove residual compound. Intracellular bacteria were released by lysing the macrophages and enumerated by plating serial dilutions on 7H11 agar plates supplemented with OADC and glycerol. Colonies were counted after ∼3 weeks incubation. Results are means ± SD of triplicates of one of three similar experiments.

with $LD_{50}$ values> 100 μg/mL to HepG2 cells. Similarly, compound **40a** was improved over (+)-calanolide A both in potency (39-fold against R-*Mtb;* $MIC_{90}$ = 0.08 μg/mL) and SI (60-fold). Compound **22d** had an MIC of 0.08 μg/mL (R-*Mtb*) (a 40-fold improvement over calanolide A) and was nontoxic to HepG2 cells, with an $LD_{50}$ value of >50 μg/mL. Several of the active analogues (**40a**, **30a**) had potent bactericidal activity against *Mtb* H37Rv residing in primary human macrophages. Their eukaryotic genotoxicities preclude them from clinical progression. However, the remarkable bactericidal properties of these synthetic calanolides should enable their use as tool compounds in identifying pathways essential for *Mtb* during replication, nonreplication, and infection of human macrophages. In addition, these potent calanolides will aid further structure-based design of more effective and drug-like antimycobacterial agents.

## EXPERIMENTAL SECTION

**General Chemical Methods.** Details on the synthesis of all chemical entities described in this article are in the Supporting Information. All chemicals were purchased as reagent grade and used without further purification unless otherwise noted. Reactions were monitored by analytical thin-layer chromatography on silica gel GF254 precoated on glass plates (10−40 μm, Yantai, China). Spots were detected under UV (254 nm). Solvents were evaporated under reduced pressure and below 45 °C (water bath). Column chromatography was performed on silica gel (200−300 mesh, Qingdao, China). The automatic LC-MS analysis was also performed on a Thermo Finnigan LCQ Advantage mass spectrometer equipped with an Agilent HPLC system and an eluent splitter (5% eluent was split into the MS system). A Kromasil C18 column (4.6 μm, 4.6 mm × 50 mm) from DIKMA was employed. The eluent was a mixture of acetonitrile and water containing 0.05% formic acid with a linear gradient from 5:95 v/v acetonitrile−$H_2O$ to 95:5% acetonitrile−$H_2O$ within 10 min at 1 mL/min. The UV detection wavelength was 254 nm. Mass spectra were recorded in either positive or negative-ion mode using electrospray ionization. High-resolution mass spectrometry (HRMS) was obtained using the time-of-flight method. The ion source is electrospray ionization (ESI). $^1$H NMR spectra were recorded at 25 °C on 300, 400, or 500 MHz spectrometers. $^{13}$C NMR spectra were at 25 °C on 500 MHz, or 600 MHz spectrometers. Unless otherwise noted below, all compounds were >95% pure by analytical HPLC.

*6,6-Dimethyl-10-nitro-4-propyl-2H-5H-furo[2,3-h]-pyrano-[2,3-f]chromen-2-one (**18**).* 5-Hydroxy-2,2-dimethyl-10-propyl-8-oxo-2*H*,8*H*-pyrano[2,3-f]chromen-8-one-6-carbaldehyde (**1d**) was synthesized by the reported procedure:[11b] $^1$H NMR (300 MHz, DMSO) δ: 12.66 (s, 1H), 10.16 (s, 1H), 6.52 (d, *J* = 10.1, 1H), 6.11 (s, 1H), 5.78 (d, *J* = 10.1, 1H), 2.92−2.70 (m, 2H), 1.63−1.42 (m, 8H), 0.97 (t, *J* = 7.3, 3H). $^{13}$C NMR (75 MHz, DMSO) δ: 192.56, 160.58, 158.56, 158.41, 158.21, 157.97, 128.37, 114.60, 111.91, 105.25, 104.16, 102.80, 81.00, 38.29, 28.41, 23.54, 14.41. ESI-MS (*m*/*z*): 315 $[M + H]^+$ (MW = 314.1).

To a solution of the **1d** (53 mg, 0.17 mmol) in 1.5 mL anhydrous acetone was added $K_2CO_3$ (24 mg, 0.17 mmol) and $BrCH_2NO_2$ (15 μL, 0.21 mmol) successively at room temperature, and the reaction mixture was stirred for 3 h. Upon completion as shown by TLC, the reaction mixture was filtered and concentrated in vacuum. The residue was treated with 1 mL of acetic anhydride and stirred for 1 h at 100 °C. Upon completion as shown by TLC, the reaction mixture was cooled, concentrated, and purified by column chromatography on silica gel (petroleum ether/ethyl acetate) to provide the compound **18**: yellow solid (55 mg, 92% yield); mp =183−185 °C. $^1$H NMR (500 MHz, DMSO) δ: 8.35 (s, 1H), 6.75 (d, *J* = 9.9, 1H), 6.32 (s, 1H), 5.99 (d, *J* = 9.9, 1H), 3.05−2.77 (m, 2H), 1.70−1.58 (m, 2H), 1.56 (s, 6H), 1.03 (t, *J* = 7.2, 3H). $^{13}$C NMR (126 MHz, DMSO) δ: 158.69, 157.97, 152.91, 150.46, 150.08, 130.73, 114.04, 113.99, 109.72, 107.67, 107.26, 103.00, 80.33, 38.10, 27.77, 23.38, 14.21. ESI-MS (*m*/*z*): 356 $[M + H]^+$ (MW = 355.1). HRMS (*m*/*z*): ($[M + H]^+$) calcd 356.1134; found, 356.1142.

*6,6-Dimethyl-10-nitro-4-propyl-7,8-dihydro-2H-5H-furo-[2,3-h]-pyrano[2,3-f]chromen-2-one (**30a**).* 5-Acetoxy-2,2-dimethyl-10-propyl-8-oxo-2*H*,8*H*-pyrano[2,3-f]chromen-8-one-6-methylene diacetate (**27a**). To a solution of the **1d** (300 mg, 0.95 mmol) in 3 mL of acetic anhydride was added 150 mg of Amberlite IR-120 at room temperature, and the reaction mixture was stirred for 1 h. Upon completion as shown by TLC, the reaction mixture was filtered and concentrated in vacuum. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate) to provide the compound as a white solid (355 mg, 81% yield). $^1$H NMR (300 MHz, $CDCl_3$) δ: 8.09 (s, 1H), 6.21 (d, *J* = 9.9, 1H), 6.12 (s, 1H), 5.66 (d, *J* = 10.0, 1H), 2.93−2.87 (m, 2H), 2.42 (s, 3H), 2.08 (s, 6H), 1.70−1.62 (m, 2H), 1.51 (s, 6H), 1.04 (t, *J* = 7.2, 3H). ESI-MS (*m*/*z*): 459 $[M + H]^+$ (MW = 458.2).

5-Acetoxy-3,4-dihydro-2,2-dimethyl-10-propyl-8-oxo-2*H*,8*H*-pyrano[2,3-f]chromen-8-one-6-methylene diacetate (**28a**). To the solution of 0.30 g **27a** in 5 mL of ethanol was added 0.15 g of Pd/C, and the resulting mixture was stirred under atmospheric pressure of $H_2$ at room temperature for 1 h. Upon completion as shown by TLC, the reaction mixture was filtered. The filtrate was concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate) to provide the compound to give a white solid (289 mg, 96% yield). $^1$H NMR (300 MHz, DMSO) δ: 8.02 (s, 1H), 6.20 (s, 1H), 2.97−2.74 (m, 2H), 2.37 (s, 3H), 2.02 (s, 6H), 1.86−1.73 (m, 2H), 1.66−1.46 (m, 2H), 1.42−1.16 (m, 8H), 0.97 (t, *J* = 7.3, 3H). ESI-MS (*m*/*z*): 461 $[M + H]^+$ (MW = 460.2).

5-Hydroxy-3,4-dihydro-2,2-dimethyl-10-propyl-8-oxo-2*H*,8*H*-pyrano[2,3-f]chromen-8-one-6-carbaldehyde (**29a**). To a solution of 50 mg (0.16 mmol) of **28a** in 1.5 mL of methanol was added $K_2CO_3$ (66 mg, 0.478 mmol) at room temperature, and the reaction mixture was stirred for 1 h. Upon completion as shown by TLC, the reaction mixture was filtered and concentrated in vacuum. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate) to provide the compound as a white solid (32 mg, 91% yield). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 12.93 (s, 1H), 10.40 (s, 1H), 6.01 (s, 1H), 2.98−2.79 (m, 2H), 2.70 (t, $J$ = 6.8, 2H), 1.85 (t, $J$ = 6.8, 2H), 1.73−1.48 (m, 2H), 1.44 (s, 6H), 1.03 (t, $J$ = 7.3, 3H). ESI-MS ($m/z$): 317 $[M + H]^+$ (MW = 316.1).

To a solution of **29a** (25 mg, 0.08 mmol) in 1.5 mL of anhydrous acetone was added $K_2CO_3$ (11 mg, 0.08 mmol) and $BrCH_2NO_2$ (7 $\mu$L, 0.10 mmol) successively at room temperature, and the reaction mixture was stirred for 3 h. Upon completion as shown by TLC, the reaction mixture was filtered and concentrated in vacuum. The residue was treated with 1 mL of acetic anhydride and stirred for 1 h at 100 °C. Upon completion as shown by TLC, the reaction mixture was cooled, concentrated, and purified by column chromatography on silica gel (petroleum ether/ethyl acetate) to provide compound **30a**, a yellow solid (12 mg, 45% yield); mp = 212−213 °C. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 7.90 (s, 1H), 6.17 (s, 1H), 3.00 (m, 4H), 1.94 (t, $J$ = 6.7, 2H), 1.67 (m, 2H), 1.48 (s, 6H), 1.05 (t, $J$ = 7.3, 3H). $^{13}$C NMR (126 MHz, DMSO) $\delta$: 158.95, 158.55, 154.31, 153.66, 152.64, 149.47, 113.81, 108.67, 107.80, 107.47, 102.33, 78.54, 38.56, 30.25, 26.63, 23.48, 16.43, 14.14. ESI-MS ($m/z$): 358 $[M + H]^+$ (MW = 357.1). HRMS ($m/z$): ($[M + H]^+$) calcd 358.1291; found, 358.1292.

*10,11-trans-6,6-Dimethyl-11-hydroxyl-10-nitro-4-propyl-10,11-dihydro-2H-5H-furo[2,3-h]-pyrano[2,3-f]chromen-2-one (**33a**).* To a solution of **1d** (63 mg, 0.20 mmol) in 1.5 mL of anhydrous acetone was added $K_2CO_3$ (28 mg, 0.20 mmol) and $BrCH_2NO_2$ (22 $\mu$L, 0.30 mmol) successively at room temperature, and the reaction mixture was stirred for 3 h. Upon completion as shown by TLC, the reaction mixture was filtered and concentrated in vacuum. The residue was triturated with ethyl acetate to break up lumps, and the suspension was filtered. The solid thus obtained was washed with ethyl acetate, air-dried, and then dried under reduced pressure overnight to give compound **33a**: white solid (74 mg, 99% yield); mp = 145−150 °C. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 6.38 (d, $J$ = 9.9, 1H), 6.02 (s, 1H), 5.77 (s, 1H), 5.48 (m, 2H), 2.70 (t, $J$ = 7.2, 2H), 1.38 (s, 3H), 1.33 (s, 3H), 0.85 (t, $J$ = 7.1, 3H). ESI-MS ($m/z$): 374 $[M + H]^+$ (MW = 373.1). HRMS ($m/z$): ($[M + H]^+$) calcd 374.1240; found, 374.1239.

*5-(2-Morpholinoethoxy)-8-nitro-4-propyl-2H-furo[2,3-h]-chromen-2-one (**40a**) and 8-Nitro-4-propyl-5-(2-(pyrrolidin-1-yl)ethoxy)-2H-furo[2,3-h]chromen-2-one (**40b**).* To a solution of **35** (30 mg, 0.10 mmol) and 2-morpholinoethanol (14 mg, 0.11 mmol) or 2-(pyrrolidin-1-yl)ethanol (13 mg, 0.11 mmol) in 1.5 mL of THF was added $PPh_3$ (35 mg, 0.13 mmol) and diethyl diazenedicarboxylate (23 $\mu$L, 0.15 mmol) successively at room temperature, and the reaction mixture was stirred for 12 h. Upon completion as shown by TLC, the reaction mixture was concentrated in vacuum and purified by column chromatography on silica gel (petroleum ether/ethyl acetate) to provide the compound **40a** as a brown solid (23 mg, 56% yield); mp = 204−206 °C. $^1$H NMR (300 MHz, DMSO) $\delta$: 8.34 (s, 1H), 7.50 (s, 1H), 6.30 (s, 1H), 4.32 (m, 2H), 3.60 (m, 4H), 3.02 (t, $J$ = 7.3, 2H), 2.80 (m, 2H), 2.50−2.35 (m, 4H), 1.63 (m, 2H), 0.98 (t, $J$ = 7.2, 3H). ESI-MS ($m/z$): 403 $[M + H]^+$ (MW = 402.1). HRMS ($m/z$): ($[M + H]^+$) calcd 403.1505; found, 403.1511. **40b**: red solid (7 mg, 17% yield). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 7.90 (s, 1H), 7.01 (s, 1H), 6.22 (s, 1H), 4.39 (s, 2H), 3.16 (s, 2H), 3.06−2.95 (m, 2H), 2.79 (s, 4H), 1.93 (s, 4H), 1.67 (m, 2H), 1.04 (t, $J$ = 7.3, 3H). ESI-MS ($m/z$): 387 $[M + H]^+$ (MW = 386.2). HRMS ($m/z$): ($[M + H]^+$) calcd 387.1556; found, 387.1558.

*(8R,9R)-9-Hydroxy-5-(2-morpholinoethoxy)-8-nitro-4-propyl-8,9-dihydrofuro[2,3-h]chromen-2-one ((+)-**58a**) and (8S,9S)-9-Hydroxy-5-(2-morpholinoethoxy)-8-nitro-4-propyl-8,9-dihydrofuro[2,3-h]chromen-2-one ((−)-**58a**).* The compound (±)-**58a** was synthesized as a white solid (69 mg, 59% yield) from compound **57a** (100 mg, 0.28 mmol) as done for compound **33a** from **1d**. $^1$H NMR (500 MHz, acetone) $\delta$: 6.89 (s, 1H), 6.54 (br, 1H), 6.04 (s, 1H), 5.77 (s, 1H), 4.56−4.19 (m, 2H), 3.68−3.66 (m, 4H), 3.13−3.01 (m, 2H), 2.92−2.90 (m, 2H), 2.57 (s, 4H), 1.74−1.70 (m, 2H), 1.05 (t, $J$ = 7.4 Hz, 3H). ESI-MS ($m/z$): 421 $[M + H]^+$ (MW = 420.2). HRMS $m/z$: ($[M + H]^+$) calcd 421.1611; found, 421.1619.

Sino-Chiral AD 0P10003-A was used to separate the enantiomeric mixture of the enantiomers to provide (+)-**58a** and (−)-**58a**. Chromatography condition: mobile phase = isopropanol/*n*-hexane; flow velocity = 3 mL/min. Two fraction peaks were observed eluting at 28.3 min (compound (−)-**58a** $[\alpha]D^{20} = -16.7°$) and 39.2 min (compound (+)-**58a** $[\alpha]D^{20} = +14.3°$). On the basis of the calculated and experimental CD spectrum, the absolute configuration of compound (−)-**58a** was proposed to be (8*R*, 9*R*) and compound (+)-**58a** was proposed to be (8*S*, 9*S*) (see the Supporting Information for the calculated and experimental CD spectrum).

**Bacterial Strains and Growth Conditions.** *Mtb* H37Rv was grown in Middlebrook 7H9 supplemented with 0.2% glycerol, 0.02% tyloxapol, and 10% ADN (albumin, dextrose, NaCl) supplement. An attenuated *Mtb* strain mc$^2$6220 Δ*panCD*Δ*lysA* was used for HTS assays and grown in Middlebrook 7H9 supplemented with 0.5% glycerol, 0.02% tyloxapol, 10% OADC (oleic acid, albumin, dextrose, catalase), 0.05% casein hydrolysate (CAS) amino acids, 240 $\mu$g/mL L-lysine, and 24 $\mu$g/mL pantothenate.

For replicating (R) conditions, *Mtb* was propagated at 37 °C with 20% $O_2$ and 5% $CO_2$. For nonreplicating (NR) conditions, bacteria were incubated at 37 °C with 1% $O_2$ and 5% $CO_2$ in a Sauton's based medium ("NR medium") consisting of 0.5 g of $MgSO_4$, 0.05 g of ferric ammonium citrate, 0.5 g of $KH_2PO_4$, 0.5% BSA, 0.085% NaCl, 0.02% tyloxapol, 50 $\mu$M butyrate, and 0.5 mM $NaNO_2$ at pH 5.0. Strain *Mtb* mc$^2$6220 Δ*panCD*Δ*lysA* (a kind gift of W. Jacobs, Jr., Albert Einstein College of Medicine, New York) was grown with an additional 240 $\mu$g/mL L-lysine and 24 $\mu$g/mL pantothenate.

Other bacterial strains were growth in *Mycobacterium smegmatis* (Middlebrook 7H9 supplemented with 0.2% glycerol, 0.02% tyloxapol), *Candida albicans* (YM), uropathogenic *Escherichia coli* (Luria Broth), *Pseudomonas aeruginosa* PAO1 (Luria Broth), and *Staphyloccus aureus* ATCC 29213 (Mueller-Hinton broth). All of these strains were grown in a shaking incubator at 37 °C at 200 rpm except for *Candida albicans*, which was grown at 30 °C.

**High-Throughput Screening.** High throughput screening (HTS) was performed as described previously with BSL2+-rated attenuated strain of *Mtb*, mc$^2$6220 Δ*panCD*Δ*lysA*.[5b] Briefly, to assay R activity, bacteria in mid log growth were diluted to an $OD_{580}$ of 0.01 and dispensed into 384-well plates


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at 50 μL/well. Test compound (500 nL) in DMSO was added to each well. After 7 days of co-incubation, the optical density was determined at 580 nm. For NR activity, log phase bacteria were washed 2× with PBS/0.02% tylaxopol and resuspended in NR medium. Bacteria were diluted to an $OD_{580}$ of 0.1, and 15 μL/well was dispensed into 384-well plates, and 150 nL of test compound was added per well. After 3 days of co-incubation at 37 °C with 1% $O_2$ and 5% $CO_2$, an outgrowth stage was initiated by addition of 60 μL of R medium (effectively diluting the test compound 5-fold) and incubation at 37 °C with 20% $O_2$ and 5% $CO_2$. The $OD_{580}$ was read after 7 days of outgrowth. $MIC_{90}$ (minimal inhibitory concentration) is the measure of activity of compound against *Mtb*, defined as 90% inhibition of growth when compared to vehicle control (DMSO).

**Antibacterial Activity.** For select compounds, as indicated in the tables, activity was confirmed against wild-type *Mtb* H37Rv in 96 well plates. The setup for this was similar to HTS as described above, except the final volume was 200 μL per well and during the outgrowth stage, instead of 1:5 dilution, a 1:21 dilution was performed into a new 96-well plate using 7H9-ADN medium. To test selectivity against the panel of Gram-negative and Gram-positive microorganisms, log phase bacteria were diluted to OD 0.01 in their respective media, and 200 μL of cells were dispensed per well of 96-well tissue-culture plates. Two microliters of compound or DMSO vehicle control was added. After drug addition, bacteria were incubated for 8−48 h at 37 °C with shaking, after which the $OD_{600}$ was determined. Colony-forming units (CFU) assays were performed by plating wild-type *Mtb* (200 μL at OD 0.01 or 0.1 for R and NR conditions, respectively of a single-cell suspension in 96-well plates and co-incubating with compound). After 7 days, bacteria were enumerated by serially diluting in 7H9-AND and plating onto Middlebrook 7H11 agar plates containing 0.5% glycerol and 10% OADC.

**Toxicity Assays.** Human hepatoma HepG2 cells were grown in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS) supplemented with 1 mM pyruvate, 2 mm glutamine, 10 mM HEPES, and 1% nonessential amino acids. When the cell monolayer became confluent, HepG2 cells were removed from the flask and seeded at 3000 cells per well in a tissue-culture treated 384-well Greiner plate. The mixture was incubated for 2 days after the compound was added while keeping the final DMSO concentration at 1%. The CellTiter-Glo kit from Promega was used to measure ATP content of the cell represented by a luminescent signal as an indicator of viability. $LD_{50}$ was defined as the concentration of compound that caused a 50% decrease in the ATP signal compared to the DMSO control. The selectivity index (SI) was calculated as $LD_{50}$ divided by the $MIC_{90}$.

**Macrophage Assays.** Human macrophages were generated as described previously[27] under an IRB-approved protocol. Briefly, monocytes were isolated from healthy donors by CD14 positive selection using magnetic beads. For the assay, 100 000 cells/well were differentiated in 200 μL/well in 40% human plasma containing 0.5 ng/mL human GM-CSF and 0.5 ng/mL human TNF-alpha at 10% $O_2$, 5% $CO_2$. After 2 weeks, cells were stimulated with 5 ng/mL IFN-gamma overnight [ACTIMMUNE] and infected with wild-type *Mtb* at multiplicity of infection (MOI) of 0.1. After 24 h of infection, either DMSO vehicle control or test compound was added, maintaining the final DMSO concentration at 1%. Infected human macrophages containing DMSO or drug were then coincubated for 7 days. Macrophages were visually inspected for viability. If drug-treated macrophages looked healthy, they were lysed with 0.55% Triton-X for enumeration of intracellular bacteria by plating on Middlebrook 7H11 agar plates containing OADC supplement.

## ■ ASSOCIATED CONTENT

### Supporting Information

Details for preparation of all new compounds are available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

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## ■ ABBREVIATIONS

AIDS, acquired immunodeficiency syndrome; BINAP, 2,2′-bis(diphenylphosphino)-1,1′- binaphthalene; Boc, *tert*-butoxycarbonyl; BOP, (benzotriazol-1-yloxy)tris(dimethylamino)-phosphonium hexafluorophosphate; DBU, 1,8-diazabicyclo-[5.4.0]undec-7-ene; DCC, dicyclohexylcarbodiimide; DEAD, diethyl azodicarboxylate; DIB, iodobenzene diacetate; DMAP, 4-dimethylaminopyridine; DME, 1,2-dimethoxyethane; DMF, dimethylformamide; HIV, human immunodeficiency virus; HOBt, hydroxybenzotriazole; INH, isoniazid; $LD_{50}$, median lethal dose; MDR, multidrug-resistant; MIC, minimum inhibitory concentration; NIS, N-iodosuccinimide; NMR, nuclear magnetic resonance; NR-*Mtb*, nonreplicating *Mycobacterium tuberculosis*; RIF, rifampicin; R-*Mtb*, replicating *Mycobacterium tuberculosis*; SAR, structure−activity relationship; SI, selective index; TB, tuberculosis; TBAI, tetrabutylammonium iodide; TFA, trifluoroacetic acid; THF, tetrahydrofuran

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