Journal of Medicinal Chemistry

Article

Identification of Novel Coumestan Derivatives as Polyketide Synthase 13 Inhibitors against Mycobacterium tuberculosis. Part II

Wei Zhang, Shichun Lun, Ling-Ling Liu, Shiqi Xiao, Guanfu Duan, Hendra Gunosewoyo, Fan Yang, Jie Tang, William R. Bishai, and Li-Fang Yu

J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.9b00010 • Publication Date (Web): 15 Mar 2019

Just Accepted

ACS Publications

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Identification of Novel Coumestan Derivatives as Polyketide Synthase 13 Inhibitors against *Mycobacterium tuberculosis*. Part II

Wei Zhang,[†, #] Shichun Lun,[§, #] Ling-ling Liu,[†] Shiqi Xiao,[§] Guanfu Duan,[†] Hendra Gunosewoyo,[⊥] Fan Yang,[*, †] Jie Tang,[□] William R. Bishai,[*, §] and Li-Fang Yu[*, †]

[†]Shanghai Engineering Research Center of Molecular Therapeutics and New Drug Development, School of Chemistry and Molecular Engineering, East China Normal University, 3663 North Zhongshan Road, Shanghai 200062, China

[§]Center for Tuberculosis Research, Department of Medicine, Division of Infectious Disease, Johns Hopkins School of Medicine, Baltimore, Maryland 21231-1044, United States

[⊥]School of Pharmacy and Biomedical Sciences, Faculty of Health Sciences, Curtin University, Bentley, Perth, WA 6102, Australia

[□]Shanghai Key Laboratory of Green Chemistry and Chemical Process, School of Chemistry and Molecular Engineering, East China Normal University, 3663 North Zhongshan Road, Shanghai 200062, China

ABSTRACT

Our group recently reported the identification of novel coumestan derivatives as *Mycobaterium tuberculosis* Pks13 thioesterase (TE) domain inhibitors, with mutations observed (D1644G and N1640K) in the generated coumestan-resistant *Mtb* colonies. Herein, we report a further SAR exploration exploiting the available Pks13 TE X-ray co-crystal structure that resulted in the discovery of extremely potent coumestan analogues **48** and **50**. These molecules possess excellent anti-TB activity against both the drug susceptible (MIC = 0.0039 μg/mL) and drug resistant *Mtb* strains (MIC = 0.0078 μg/mL). Moreover, the excellent *in vitro* activity is translated to the *in vivo* mouse serum inhibitory titration (SIT) assay, with administration of coumestan **48** given at 100 mg/kg showing an 8-fold higher activity than isoniazid or

TAM 16 given at 10 or 100 mg/kg, respectively. Preliminary ADME-Tox data for the coumestans were promising, and coupled with the practicality of synthesis, warrant further *in vivo* efficacy assessments of the coumestan derivatives.

INTRODUCTION

Tuberculosis (TB) is an age-old infectious disease caused by *Mycobacterium tuberculosis (Mtb)*, that typically affects the lungs (pulmonary TB).[1] TB is responsible for ill-health with approximately 10 million incidence each year and is one of the top ten causes of death worldwide.[2] According to the 2018 World Health Organization (WHO) report, there were 10.0 million people suffering from TB worldwide in 2017, accompanied with 1.6 million deaths, including 0.3 million co-infected with HIV. It is estimated that 40% of HIV deaths were associated with TB, making it the leading infectious disease killer in HIV-positive people. Of alarming global concern, the emergence of drug-resistant *Mtb* remains a public health crisis. Approximately 558,000 new cases with resistance to rifampicin were reported, 82% of which had multidrug-resistant (MDR) TB and 8.5% of MDR-TB classified as extensively drug-resistant (XDR) TB.[3]

The currently recommended treatment for drug-susceptible TB is a 6-month regimen containing four first-line drugs: isoniazid, rifampicin, ethambutol, and pyrazinamide. Although treatment success rates of >85% is seen for cases of drug-susceptible TB, the front-line anti-TB drugs are still limited in terms of available options and they need to be delivered in long treatment course.[4] These observations, coupled with patient non-compliance and over 50 years of overuse, render the development of drug-resistant *Mtb* more problematic. Treatment for rifampicin-resistant TB (RR-TB), MDR-TB, and XDR-TB is longer (>20 months), and requires combination therapy with second-line drugs which are generally associated with poor curative effect, higher toxicity, more expensive, and difficulty in administration.[5] Only in the past

five years, two new anti-TB drugs, bedaquiline[6] (**1**) and delamanid[7] (**2**, Figure 1), are approved as combination therapy against MDR-TB and XDR-TB infections when other effective treatment regimen is deemed not responsive.[8] However, their clinical use has been linked with various adverse side effects especially irregular heart rhythms.[9, 10] Therefore, it is imperative that the discovery and development of safe, highly effective new anti-TB chemotherapeutics with novel mechanisms of action continue to push forward.

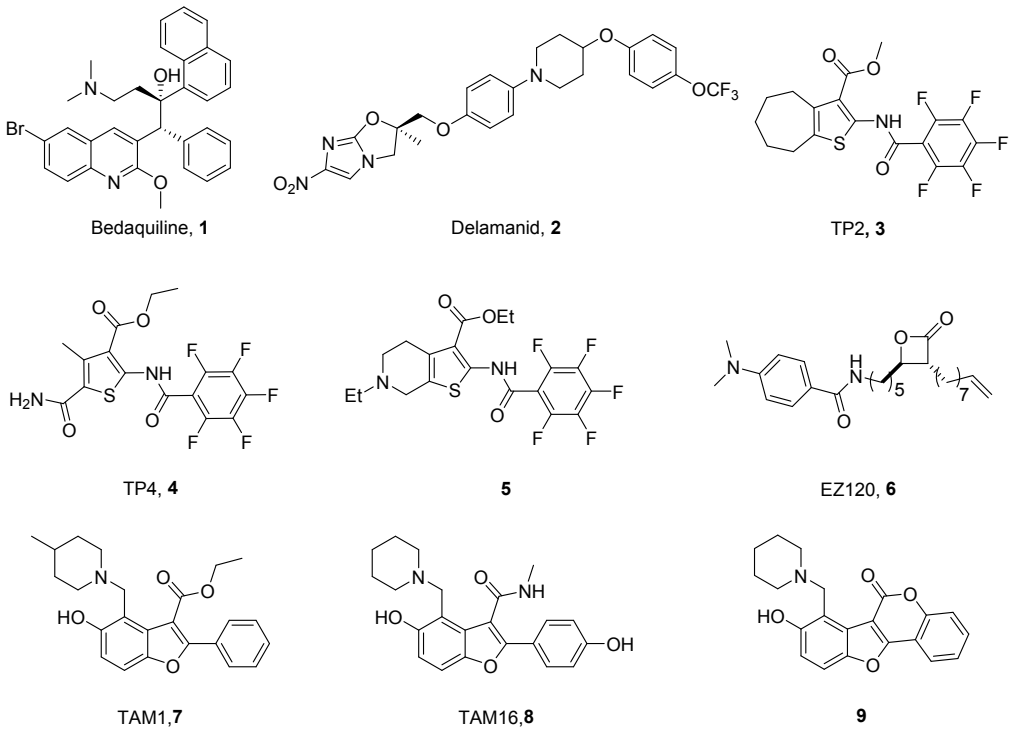


Figure 1. Chemical structures of bedaquiline (**1**), delamanid (**2**), thiophene-based Pks13 inhibitors (**3–5**), β-lactones-based Pks13 inhibitor EZ120P (**6**), benzofuran-based Pks13 inhibitor TAM1 (**7**) and TAM16 (**8)**, and coumestan-based Pks13 inhibitor (**9**).

Mycolic acids are major and specific lipid components of the mycobacterial cell envelope and are essential for the survival of *Mtb*. Its biosynthesis occurs through the coordination of more than 20 enzymes.[11, 12] The decades of anti-TB drug development have proven that the mycolic acid biosynthesis is a rich source

of druggable targets.[13-16] Briefly, the biosynthesis of mycolic acids can be categorized into five distinct steps, involving the initial syntheses of C_{24}-C_{26} saturated α-alkyl branch by FAS-I and C_{50}-C_{60} meromycolate chain by FAS-II,[17] followed by the subsequent incorporation of double bonds, cyclopropyl and keto functionalities, before the final Pks13-catalyzed condensation[18] of the two chains and translocation[19, 20] of the mature mycolic acid building blocks into the periplasm.[13] Isoniazid (INH), ethionamide (ETH), and delamanid all target various enzymatic machineries responsible for the mycolic acid biosynthesis pathway. Of increasing interest in the past five years, the polyketide synthase 13 (Pks13), a member of the type-I PKS family, was identified as the key enzyme for the final assembly step of mycolic acid synthesis via Claisen-type condensation.[21] Several studies have shown the importance of Pks13 for mycobacterial viability,[22, 23] and recently Pks13 inhibitors have been reported in succession.

The Alland group reported a class of thiophene compounds with whole-cell activity against *Mtb*, with the most active compound, TP2 (**3**, MIC = 1.0 μM) and TP4 (**4**, MIC = 0.5 μM), when combined with isoniazid exhibited sterilization of *Mtb* activity.[24] These thiophene compounds were subsequently identified to target Pks13 by interfering with the function of the *N*-ACP domain of Pks13, thus blocking the loading of the meromycolyl-AMPs onto the P-pant attachment site (Ser-55) of Pks13 (Figure 1).[24] The 2-aminothiophenes-based compound **5** (Figure 1) with potency against *Mtb* H37Rv (MIC = 0.23 μM) was also reported by the Sucheck group.[25] However, the thiophene ring containing an ester group is prone to hydrolysis, likely suggesting a metabolic liability. More recently, β-lactone EZ120 (Figure 1, compound **6**, MIC = 0.68 μg/mL) was reported to effectively mimic mycolic acid and block serine hydrolases via acylation of the active site located in the Pks13-thioesterase (TE) domain, thus exhibiting anti-mycobacterial and bactericidal activity.[26] Utilizing an activity-based protein profiling (ABPP) approach, ser/Pks13 and ser/Ag85 enzymes were verified as major targets.[26]

From high-throughput screening, benzofuran derivative TAM1 (Figure 1, compound **7**, MIC = 1.0 μg/mL) was active against *Mtb* H37Rv and identified to also target Pks13 through whole-genome sequencing and recombineering of the resistance mutations.[27] The mutations observed (D1644G and D1607N) in the resistant colonies were located in the *C*-terminus of the TE domain of Pks13 which is in accordance with the obtained X-ray co-crystal structure. However, TAM1 bearing an ester group is prone to hydrolysis to the inactive carboxylic acid in mouse serum. To circumvent this issue, bioisosteric replacement of the ester with amide strategy had significantly improved the metabolic stability. Benzofuran derivative TAM16 (Figure 1, compound **8**, MIC = 0.0313 μg/mL) was efficacious in both acute and chronic mouse TB infection models and has favorable drug-like properties, pharmacokinetic and safety profiles.[28]

In our own previous work, coumestan was identified as a natural scaffold that showed antitubercular activity *in vitro* and oral bioavailability in a mouse model of serum inhibition titration (SIT) assay.[29] Whole genome deep sequencing of the resistant mutants confirmed that these coumestans target the Pks13 as shown by the N1640K, N1640S, D1644G, and A1667V mutations observed in the resistant mutants. Herein, we report further SAR studies on the development of coumestan analogues, taking into consideration the structural insights of the Pks13-TE domain. Together with microsomal stability analysis and preliminarily evaluation for their *in vivo* bioavailability in the SIT assay head-to-head with compound **8**, herein we report the development of second-generation highly potent anti-TB coumestan analogues.

RESULTS AND DISCUSSION

Chemistry

The coumestan derivatives **12-15** and **17-24** were synthesized in 2-3 steps utilizing the synthetic routes shown in Scheme 1. Compounds **12-15** were formed starting from the previously reported hydroxyl ester

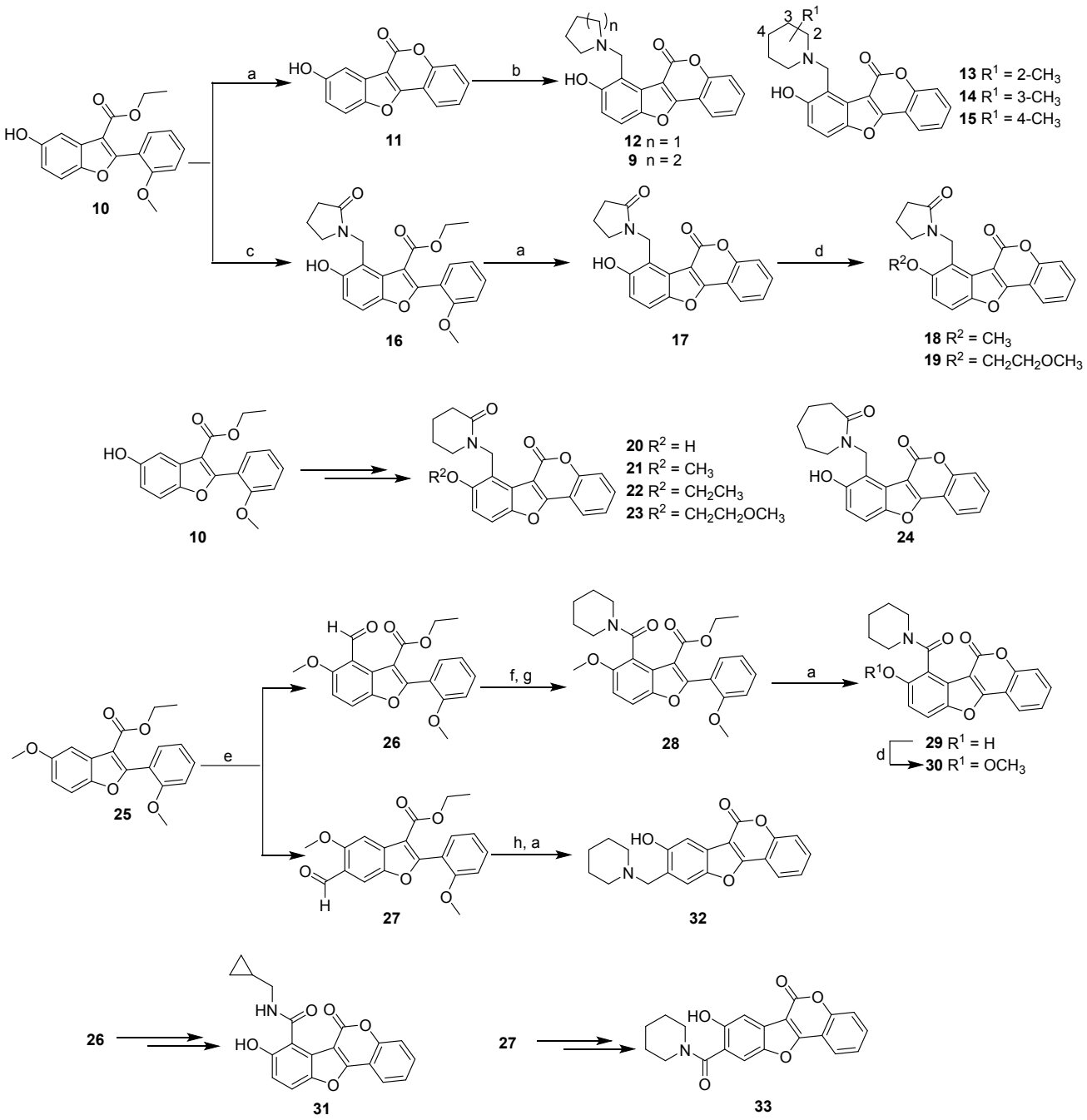
10 [29] through demethylation by boron tribromide and the subsequent Mannich reaction with 37% aqueous formaldehyde and piperidine analogues. The hydroxyl ester **10** was also reacted with 37% aqueous formaldehyde and a delta lactam using solid acid catalyst-silica-H_2SO_4 (50% w/w) via Mannich-type condensation to obtain compound **16.** Compound **16** was demethylated using boron tribromide and lactonization in refluxing ethanol. Next, alkylation of the phenolic group in the presence of K_2CO_3 afforded compounds **18** and **19.** Following a method similar to the synthesis of compounds **17** and **18** utilising various lactams and alkyl iodides, compounds **20-24** were successfully formed starting from hydroxyl ester **10.**

Regioisomers **26** and **27** were obtained via formylation of compound **25** in the presence of dichloromethyl methyl ether and $TiCl_4$.[30] Pinnick oxidation of aldehyde **26** afforded the carboxylic acid intermediate which was subsequently reacted with piperidine in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl) and 1-hydroxybenzotriazole (HOBt) as coupling agents and 3-methylpyridine as a base to obtain compound **28.** Subsequent demethylation by boron tribromide and cyclization of the ester afforded compound **29**, which underwent alkylation to give compound **30.** The 6-carboxaldehyde **27** was subjected to reductive amination using piperidine and sodium cyanoborohydride, followed by demethylation/lactonization to afford compound **32**. Synthesis of compounds **31** and **33** is similar to compound **29** starting from intermediates **26** and **27**.

The coumestan derivatives **34-37, 41-44, 48-51, 55,** and **56** were synthesized utilizing the synthetic routes shown in Scheme 2. Compound **9** underwent nitration or bromination to give **34** or the bromide **35**. Compound **35** next served as an Ullmann-type C-O coupling partner with sodium methoxide. The resultant compound **36** was then demethylated using boron tribromide to give the hydroxyl compound **37.**

Compound **39** was formed via a copper-catalyzed cyclization of 2-*tert*-butyl-1,4-benzoquinone and ethyl 3-(2-methoxyphenyl)-3-oxopropanoate which was prepared as described in the literature from commercially available 2-acetoanisole.[31] Standard demethylation/lactonization afforded compound **40**, which then underwent Mannich reaction with 37% aqueous formaldehyde and piperidine to yield coumestan derivative **41.** To access the bis-phenolic analogues, compound **46** was formed via a copper-catalyzed cyclization of 1,4-benzoquinone and ethyl 3-(2,4-dimethoxyphenyl)-3-oxopropanoate. Compound **48** was obtained via intermediate **47** through Mannich reaction/demethylation/lactonization sequence. Coumestan derivatives **42-44, 49-51** were synthesized in a similar manner from commercially available substituted ethyl 3-(2-methoxyphenyl)-3-oxopropanoate and the substituted 1,4-benzoquinone. Compounds **55** and **56** were formed via sequential bromination, nucleophilic substitution using sodium methoxide and boron tribromide demethylation respectively from compounds **52** and **47**.

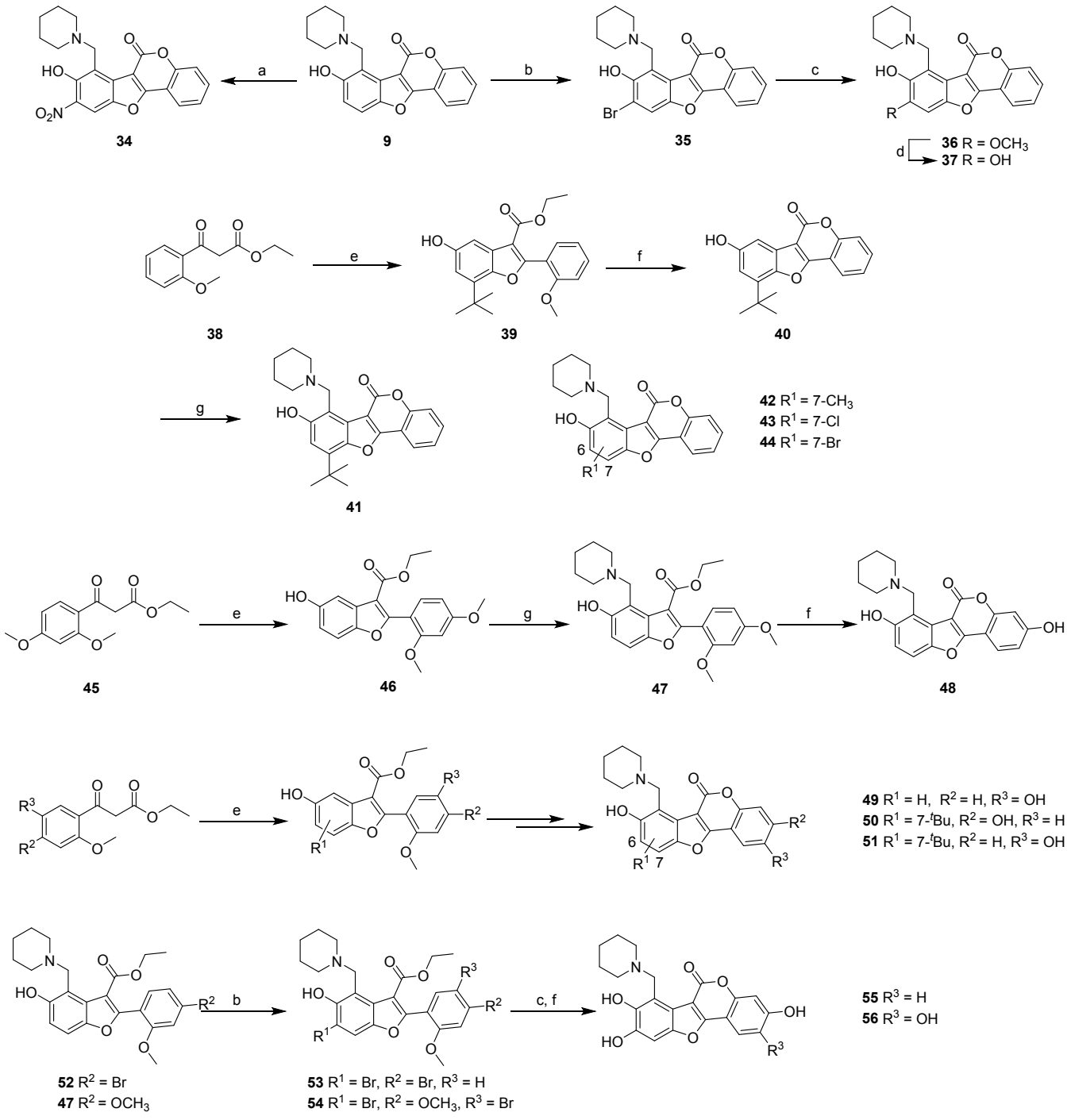
Scheme 1. Synthesis of compounds **10−33** [a]



[a] Reagents and conditions: (a) BBr_3 in CH_2Cl_2 (1 M), CH_2Cl_2, rt, overnight, then EtOH, reflux, 1 h; (b) formaldehyde (37% aq), corresponding primary amines, EtOH, reflux, 8–12 h; (c) formaldehyde (37% aq), corresponding lactam, silica−H_2SO_4 (50% w/w), acetonitrile, 50 °C for 2−5 h; (d) CH_3I/$CH_3OCH_2CH_2Br$, K_2CO_3, THF, rt - 80 ºC, 4–6 h; (e) Cl_2CHOCH_3, $TiCl_4$, $CHCl_3$, rt, 40 min; (f)

$NaClO_2$, NH_2SO_3H, dioxane/H_2O (7/3), rt, 1 h; (g) EDC·HCl, HOBt, 3-methylpyridine, piperidine, DMF, rt, 8 h; (h) piperidine, CH_3COOH, CH_3OH, rt, 10 min then $NaBH_3CN$, rt, 16 h.

Scheme 2. Synthesis of compounds **34–56** [a]



[a] Reagents and conditions: (a) HNO_3, AcOH, rt, 1 h; (b) Br_2, CH_2Cl_2, rt, overnight; (c) CuCl, $NaOCH_3$, DMF, 140 ºC, 1 h; (d) BBr_3 in CH_2Cl_2 (1 M), CH_2Cl_2, rt, overnight; (e) 1,4-benzoquinone or 2-^{t}Bu-1,4-benzoquinone, $Cu(OTf)_2$ (5 mol %), toluene, reflux, 7–12 h; (f) BBr_3 in CH_2Cl_2 (1 M), CH_2Cl_2, rt, overnight, then EtOH, reflux, 1 h; (g) formaldehyde (37% aq), piperidine, EtOH, reflux, 8–12 h.

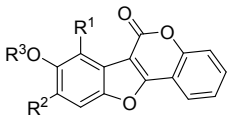
Structure−Activity Relationships.

All final compounds were initially evaluated for their MIC values against *Mtb* strain H37Rv in a standard microplate alamar blue assay (MABA).[32, 33] As shown in Table 1, benzofuran TAM16 potently inhibited *Mtb* H37Rv with an MIC value of 0.0313 μg/mL (0.09 μM), consistent with data reported by the Sacchettini group.[28] We first examined the substitutions at position 4 of the coumestans (the R^1 group). Deletion of the (methylpiperidin-1-yl) methyl to give compound **11** resulted in a total loss of activity, indicating the importance of R^1 substitution, which is consistent with the SAR trend observed previously in the benzofuran series of Pks13 inhibitors. A smaller pyrrolidin-1-yl-methyl (**12**) and various methyl substituted piperidin-1-yl-methyl substituents at R^1 decreased activity by 2-8 fold compared to compound **9**. Shifting the R^1 substituent from position 4 to position 6 (**32**) resulted in loss of activity (MIC value > 64 μg/mL), further supporting the importance of R^1 substitution at position 4 of coumestan.

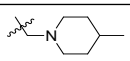
The coumestan derivatives contain phenolic Mannich base substructure that were reported to be possible Pan Assay Interference Compounds (PAINS).[34] However, Sacchettini group reported that no adducts of benzofuran derivatives were formed in mouse plasma and HLMs upon incubation with glutathione and methoxylamine, suggesting that the Mannich substructure in benzofuran derivatives does not represent a significant false-positive.[28] Initially, deletion or alteration of the Mannich structure was attempted by replacing the piperidin-1-yl-methyl with lactams (**17-24**) or amides (**28-31** and **33**), which are expected to

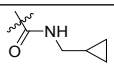
slightly lower the clogP while potentially maintaining the hydrogen bonding. However, these compounds were found to be completely inactive (MIC value > 64 μg/mL), regardless of the ring size (5-7 membered ring) or if the 5-hydroxyl group was free or masked with various alkyl groups such as methyl, ethyl, or methoxyethyl. This is presumably due to the loss of intra-molecular hydrogen bond with the carbonyl oxygen and the inter-molecular hydrogen bond with the side chain oxygen of Asn1640.

Table 1. Antitubercular activity of coumestan derivatives against the *M. tuberculosis* strain H37Rv [a]



9, 11-15, 17-24, 29-33

Compound	R^1	R^2	R^3	MIC[a], μg/mL	cLogP[b]
7	-	-	-	1.0	6.53
8	-	-	-	0.0313	2.98
9		H	H	0.125-0.25	4.17
11	H	H	H	> 64	3.19
12		H	H	0.5	3.60
13		H	H	1.0	4.69
14		H	H	2.0	4.69
15		H	H	0.5	4.69
17		H	H	> 64	2.04

18		H	CH_3	> 64	2.52
19		H	$CH_2CH_2OCH_3$	> 64	2.41
20		H	H	> 64	2.60
21		H	CH_3	> 64	3.08
22		H	CH_2CH_3	>64	3.08
23		H	$CH_2CH_2OCH_3$	> 64	2.97
24		H	H	> 64	3.16
29		H	H	> 64	3.21
30		H	CH_3	> 64	3.46
31		H	H	> 64	4.00
32	H		H	> 64	4.17
33	H		H	> 64	3.21
Isoniazid[c]	-	-	-	0.04	-0.67
Rifampin	-	-	-	0.125	4.25
Ethambutol	-	-	-	1	0.12
Levofloxacin	-	-	-	0.25	-0.5

Moxifloxacin	-	-	-	0.0625-0.125	-0.08
Kanamycin	-	-	-	2	-3.88
Capreomycin	-	-	-	1	-8.40
Amikacin	-	-	-	1	-4.12

[a]See Experimental Section. The lowest concentration of compounds leading to at least 90% inhibition of bacterial growth signal by the MABA. MIC values are reported as an average of three individual measurements. [b]cLogP was calculated using ChemBioDraw Ultra 14.0. [c]MIC values for reference compounds isoniazid, rifampin, ethambutol, levofloxacin, moxifloxacin, kanamycin, capreomycin, and amikacin were obtained from our previous publication.[14]

Next, keeping the piperidin-1-yl-methyl (position 4) and hydroxyl group (position 5) intact, we then investigated the SAR at positions 6 and 7 of the coumestan derivative (Table 2). It is suspected that the hydroxyl groups play significant roles for the observed pharmacological activities of coumestans such as those found in the naturally occurring coumestans, examplified by coumestrol, wedelolactone, and plicadin.[35-37] We speculated that an introduction of additional hydroxyl group to the phenyl rings would possibly increase the antitubercular activity. Initially, hydroxyl group along with other electron-donating groups (EDGs; CH_3, ^{t}Bu, OCH_3) and various electron-withdrawing groups (EWGs; F, Br, Cl, NO_2) were examined. Consistent with the SAR in Table 1, substituents at position 6 were generally found to be detrimental to the activity, as seen in the nitro (**34**) and bromo compounds (**35**) that were completely inactive (MIC value > 64 μg/mL). EDG substitutions (**36** and **37**) had at least 16-32 fold increase in activity with an MIC value of 2-4 μg/mL, whereas in contrast, EDGs substituents (**41, 42**) at position 7 favored the antitubercular activity with MIC values of 0.125 μg/mL and 0.0625-0.125 μg/mL, which are very similar to the parent coumestan **9**. Halogen substituents (**43** and **44**), however, resulted in 16-fold reduction

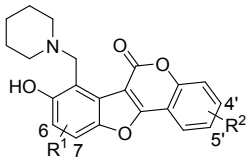
of antitubercular activity (MIC = 2 μg/mL), suggesting the importance of EDG at this position. Molecular docking studies suggested that the substituents at 6-position may interfere with Arg1641 resulting in unfavorable ligand binding (data not shown), while CH_3 and ^{t}Bu substituents at 7-position (**41** and **42**) seem to accommodate well with the adjacent amino acid Tyr1637.

Investigating the right-hand side benzene ring of the coumestans (R^2 substitutions), we further probed positions 4' or 5' of with various EDGs and EWGs. In our previous findings, 4'-Br, 4'-OCH_3, 4'-F, 5'-CH_3, 5'-OCH_3 were reported to possess MIC values ranging from 0.25 to 4 μg/mL.[29] We found that monosubstitutions on the right-hand side benzene ring of the coumestans with EDG and EWG were generally tolerated. Utilizing the structural information obtained from the co-crystal structure of the Pks13-TE-TAM16 complex and the vastly improved activity of TAM16, we speculated that an introduction of hydroxyl group to the phenyl ring would increase the anti-TB activity, likely through an optimal hydrogen bond to the carboxylate group of D1644. To our delight, introduction of hydroxyl group at the 4'-position to give compound **48** resulted in an extremely potent analogue with an MIC value of 0.0039 μg/mL, whereas a hydroxyl walk to the 5'-position (**49**) resulted in significantly decreased MIC value of 0.5 μg/mL. Similarly, the *tert*-butyl analogue **50** showed similar activities with **48**, whereas **51** showed a 4-fold increase in activity compared to **49**. Introduction of poly-hydroxyl group on the benzene ring of the coumestans to give compounds **55** and **56** resulted in reduction of activity with MIC values of 2 and 32 μg/mL.

Bactericidal activity of **48** on Mtb H37Rv was evaluated by minimum bactericidal concentration assay (MBC) using broth dilution and colony forming unit determination methods. The minimum concentration

that killed 99% of the inoculum was 0.0039-0.0078 μg/mL, demonstrating **48** to be a potent bactericidal compound for Mtb.

Table 2. Antitubercular activity of coumestan derivatives against the *M. tuberculosis* strain H37Rv. [a]



34-37, 41-44, 48-51, 55, 56

Compound	R^1	R^2	MIC[a], μg/mL	cLogP[b]
34	6-NO_2	H	> 64	4.36
35	6-Br	H	> 64	4.86
36	6-OCH_3	H	2.0	3.87
37	6-OH	H	4.0	3.63
41	7-^{t}Bu	H	0.0625-0.125	5.94
42	7-CH_3	H	0.125	4.67
43	7-Cl	H	2.0	4.99
44	7-Br	H	2.0	5.14
48	H	4'-OH	0.0039	4.11
49	H	5'-OH	0.5	4.11
50	7-^{t}Bu	4'-OH	0.0039	5.89
51	7-^{t}Bu	5'-OH	0.125	5.89
55	6-OH	4'-OH	2.0	3.52
56	6-OH	4',5'-di-OH	32	3.08

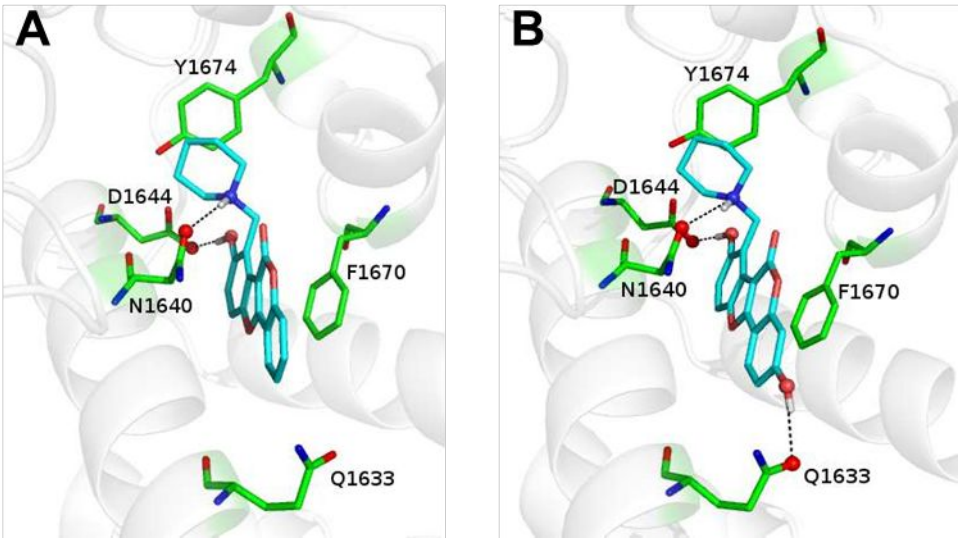
[a]See Experimental Section. The lowest concentration of compounds leading to at least 90% inhibition of bacterial growth signal by the MABA. MIC values are reported as an average of three individual measurements. [b]cLogP was calculated using ChemBioDraw Ultra 14.0.

Molecular Docking

For the molecular docking of compounds **9** and **48**, we used the solved crystal structures of Pks13-TE (PDB ID 5V3Y). The protein structure was prepared by Protein Preparation Wizard. In this step, missing atoms and all hydrogen atoms were added to the protein according to their local environment. All compounds were flexibly docked into the binding pocket defined by residues Q1633, F1670, Y1674, and N1640 using Glide with XP scoring function. Top scored poses were used for visualization analysis. Pks13 interactions with each compound were pictured using Pymol. All the molecular docking work was performed using the Maestro software.

The proposed binding modes are shown in Figure 2, demonstrating the aminophenol part of coumestan binding favorably to the TE domain and the coumarin portion relatively exposed to the solvent. Interaction of the 4'-hydroxyl group with the side chain amide of Q1633 is proposed to be responsible for the significant improvement in the MIC value. This is also consistent with the proposed binding mode of the compound **8** (TAM16).[28]

Figure 2. Proposed binding modes of compounds **9** (colored cyan) (A) and **48** (colored cyan) (B). The key amino acid residues are colored green in the active site of Pks13 (PDB ID 5V3Y). Relative Binding Affinity Estimated by Glide-XP scoring Function. **9** (-8.7), **48** (-10.7).



Encouraged by the vast improvement of the MIC values of our most potent coumestans (**48** and **50**) as well as their bactericidal activity thus far, their activities against clinical drug sensitive (DS, V4207), MDR-TB (KZN494 and V2475), and XDR-TB (TF274 and R506) strains (Table 3) were evaluated.[38] The MDR and XDR strains are resistant to the first-line anti-TB drugs isoniazid and rifampin, and second-line anti-TB drugs levofloxacin, ofloxacin and kanamycin were used as positive controls. Again, to our delight, coumestans **48** and **50** demonstrated potent activities against susceptible clinical isolates of *Mtb* (MICs 0.0039-0.0078 μg/mL) as well as all the MDR and XDR-TB strains examined.

Table 3. Antitubercular activity of compounds **48** and **50** against susceptible, MDR, and XDR strains of *M. tuberculosis*.

Mtb MIC[a] (μg/mL)					
strain/phenotype	V4207/DS[b]	V2475/MDR[c]	KZN494/MDR	TF274/XDR[d]	R506/XDR
Isoniazid	0.04	16	16	8	8
Rifampin	0.0625	4	128	>128	>128

Levofloxacin	0.125	NT[e]	NT	1	2
Ofloxacin	0.5	0.5	0.5	8	4
Kanamycin	2	2	2	>128	>128
48	0.0078	0.0156	0.0039	0.0078	0.0078
50	0.0039-0.0078	0.0156	0.0010-0.0020	0.0039	0.0039

[a]The lowest concentration of drug leading to at least a 90% reduction of bacterial growth signal by microplate alamar blue assay (MABA); MIC values are reported as an average of three individual measurements. MIC values for reference compounds isoniazid, rifampin, levofloxacin, ofloxacin, and kanamycin were obtained from our previous publication.[39] [b]Drug susceptible strain of *M. tuberculosis*. [c]Multidrug resistant strain of *M. tuberculosis*, resistance to isoniazid and rifampin. [d]Extensively drug resistant strain of *M. tuberculosis*, resistance to isoniazid, rifampin, levofloxacin, ofloxacin, and kanamycin. [e]NT = not tested.

The follow-up investigation was then to examine if these compounds possess improved selectivity index (SI) against normal cells by examining their cytotoxicity profiles. In our previous work, we have evaluated the potential cytotoxicity of the coumestans on the human-derived cell lines and found that the coumestans were not cytotoxic to these at concentrations of more than 500-fold their MIC values for *Mtb*.[29] As seen in Tables 4 and 5, the cytotoxicity of both coumestans **48** and **50** were tested against Vero cells as well as 4 human derived cell lines. Gratifyingly, coumestans **48** and **50** were non-cytotoxic to these mammalian cells at concentrations of more than 1000-fold their MIC values for *Mtb*, unlike the cytotoxic profile towards breast cancer cells displayed by wedelolactone for example.[40, 41]

Table 4. Vero toxicity and selectivity index (SI) of selected compounds.

Compd	Toxicity to Vero cells IC_{50}, μg/mL	MIC H37Rv, μg/mL	SI [a]
12	8	0.5	16
13	16	1	16
15	16	0.5	32
41	16	0.0625-0.125	128-256
42	8	0.125	64
48	4	0.0039	1024
49	4	0.5	8
50	8	0.0039	2048
51	8	0.125	64

[a] $SI = IC_{50\ (Vero\ cells)} / MIC_{(H37Rv)}$

Table 5. Toxicity of selected coumestans **48** and **50** in 4 human-derived cell lines.

	IC_{50} [a], μM			
Compound	MRC-5 cells	HFL-1 cells	QSG-7701 cells	HEK-293 cells
48	> 50	> 50	40.1±3.5	> 50
50	30.6 ±4.5	22.3 ± 3.9	34.1 ±1.7	33.6 ±3.4

[a] IC_{50} values were obtained as an average from three independent experiments and are expressed in μM.

To further probe their microbial selectivity, coumestans **48** and **50** were tested in a panel of gram positive and gram negative bacteria. As shown in Table 6, no appreciable inhibition of growth was observed in the

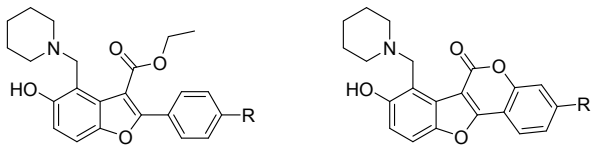
4 tested strains, suggesting that these coumestans were selective against actinobacteria and development of potential drug resistance to nonpathogenic bacteria is unlikely.

Table 6. Assessment of coumestans **48** and **50** against a panel of gram positive and gram negative bacteria.

Compounds	MIC, μg/mL			
	S. aureus Newman	*B. subtilis* 168	*E. coli* AB1157	*E. coli* DH5α
Vancomycin	0.39-0.78	> 25	> 25	> 25
Tetracycline	0.2-0.39	> 25	1.56-3.13	0.78-1.56
Kanamycin	2.34-4.68	9.39-18.75	9.39-18.75	2.34-4.68
48	> 25	> 25	> 25	> 25
50	6.25-12.5	> 25	> 25	> 25

Microsomal stability profile of the coumestan **48** was improved compared to the benzofuran counterpart **9** (Table 7), with a half-life of 120 min. Introduction of hydroxyl group at the 4-position therefore not only resulted in drastic improvement of anti-TB potency but also improved metabolic stability.

Table 7. Microsomal stability of compounds **57**, **58**, **9**, and **48** on human liver microsomes.



57 R = H
58 R = OH

9 R = H
48 R = OH

Compound	**$T_{1/2}$[a], min**	**$CL_{int\ (mic)}$[b], mL/min/kg**	**$CL_{int\ (liver)}$[c], mL/min/kg**	**Remaining (T = 60 min)**

57 [d]	18.8	73.7	66.3	10.8%
58 [d]	53.0	26.2	23.6	46.6%
9	21.4	64.9	58.4	14.5%
48	120.1	11.5	10.4	73.6%
Testosterone	19.9	69.8	62.8	12.5%
Diclofenac	11.9	116.4	104.8	3.2%
Propafenone	7.5	184.5	166.1	0.4%

[a] $T_{1/2}$ is half life, [b] $CL_{int\ (mic)}$ is the intrinsic clearance. [b] $CL_{int\ (mic)}$ = 0.693/half life/mg microsome protein per mL. [c] $CL_{int(liver)} = CL_{int(mic)}$ * mg microsomal protein/g liver weight * g liver weight/kg body weight; mg microsomal protein /g liver weight: 45 mg/g; liver weight: 20 g/kg. [d] See reference 28 and 29 for synthetic details.

Serum Inhibition Titration (SIT) Assay.

Selected coumestans **48** and **50**, as well as benzofuran **8** for head-to-head comparison, were evaluated for their *in vivo* bioavailability in the serum inhibition titration (SIT) assay.[33] Carboxymethylcellulose (0.5%) was used as vehicle and isoniazid included as a positive control. In this assay, BALB/c mice were orally gavaged with 100 mg/kg (carboxymethylcellulose as vehicle) and 50 mg/kg (Propylene glycol and Tween 80 at 4:1 as vehicle) of compounds **48**, **50**, or 10 mg/kg of INH, with blood collected at 30 min. The sera were then separated and prepared in 2-fold dilutions and incubated with a bacterial suspension for 7 days. Growth inhibition of serially diluted serum on H37Rv was determined using the microplate alamar Blue assay. As shown in Figure 3, compound **8** (TAM16, 100 mg/kg) displayed a very similar profile to isoniazid (10 mg/kg). On the other hand, coumestan **48** showed improved bioavailability up to 8-fold higher than that of TAM16 when tested at a dose of 100 mg/kg. The *tert*-butyl analogue **50** was

significantly less effective in this assay, most likely due to increased lipophilicity (clogP difference of 1.8) by introduction of *tert*-butyl group on the benzene ring. Administration of the coumestan **48** at 50 mg/kg using PG ether still maintained inhibitory activity up to 1:1024 dilution, but this was not the case for the *tert*-butyl analogue **50.**

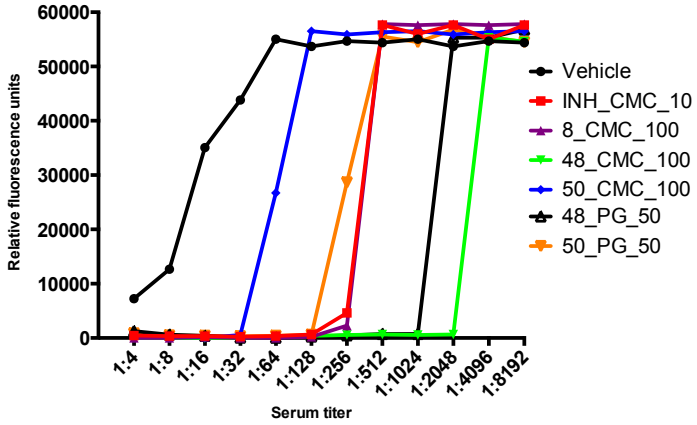


Figure 3. Serum inhibition titration for compounds **8, 48,** and **50.**

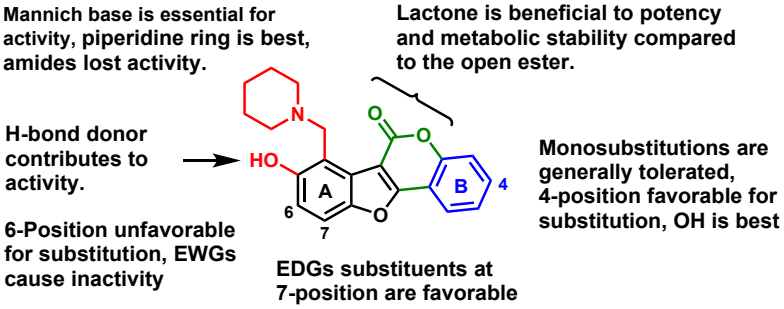


Figure 4. SAR summary on the coumestans.

Conclusions

In summary, rational SAR studies on the coumestan series (Figure 4) as mycobacterial Pks13 inhibitors resulted in the discovery of highly potent compound **48**, with MIC value of 0.0039 μg/mL. Preliminary ADME-Tox evaluation of this compound revealed favorable human microsomal stability and selectivity against normal cells as shown in the cytotoxicity data. More importantly, coumestan **48** maintained potent

activities against the clinical isolates of MDR and XDR-TB and showed excellent microbial selectivity to nonpathogenic bacteria strains. Furthermore, compound **48** is orally bioavailable as shown by the serum inhibition titration assay. In the SIT assay, coumestan **48** given at 100 mg/kg showed 8-fold higher activity than INH or **8** given at 10 or 100 mg/kg, respectively. Taken together, these data strongly suggest the translational utility of coumestan-based Pks13 inhibitors as novel anti-TB medications.

Experimental Section

General Methods: Starting materials, reagents, and solvents were purchased from commercial suppliers and used without further purification, unless otherwise stated. Anhydrous CH_2Cl_2 and tetrahydrofuran (THF) were obtained by distillation over calcium hydride or sodium wire, respectively. All non-aqueous reactions were run under a nitrogen atmosphere with exclusion of moisture from reagents. The progress of reactions was monitored by liquid chromatography mass spectrometry (LCMS) and thin layer chromatography (TLC) on SiO_2. Silica gel for column chromatography (CC) was of 200-300 mesh particle size, and an EtOAc/petroleum ether mixture or gradient was used unless stated otherwise. High resolution mass spectra (HRMS) were performed using a Bruker ESI-TOF high-resolution mass spectrometer. ^{1}H NMR spectra were recorded at a spectrometer frequency of 400 MHz, and ^{13}C NMR spectra were recorded at 101 MHz. Chemical shifts were reported in δ (ppm) using the δ 0 signal of tetramethylsilane (TMS), δ 7.26 signal of $CDCl_3$, δ 3.31 signal of CD_3OD or δ 2.50 signal of $(CD_3)_2SO$ as internal standards. Purities of all final compounds were established by analytical HPLC (> 95%), which was carried out on a Waters HPLC system using InertSustain-C18 column (5micron, 250×4.6 mm) with detection at 280, 254, and 220 nm on a variable wavelength detector 2998 PDA. InertSustain-C18 column, flow rate = 1.0 mL/min, gradient of 30-95% acetonitrile in water or 10-95% acetonitrile in water (containing 0.05 vol% of HCO_2H)

over 25 min. Melting points and analytical HPLC purities for all final compounds were included in the Supporting Information.

General procedure for the synthesis of substituted 5-hydroxy-2-phenyl benzofurans from substituted ethyl benzoylacetates (method A) were reported previsouly. [29]

General procedure for the Mannich reaction of hydroxy-2-phenyl benzofuran or coumestan with amine and formaldehyde (method B) were reported previsouly. [29]

General procedure for the preparation of coumestan derivatives from substituted ethyl 5-hydroxy-2-(2-methoxyphenyl)benzofuran-3-carboxylate or ethyl 2-(2,4-dimethoxyphenyl)-5-hydroxy-4-(piperidin-1-ylmethyl)benzofuran-3-carboxylate (method C) were reported previously.[29]

General procedure for the synthesis of Mannich-type products from ethyl 5-hydroxy-2-(2-methoxyphenyl)benzofuran-3-carboxylate (method D). To a solution of ethyl 5-hydroxy-2-(2-methoxyphenyl)benzofuran-3-carboxylate (1 mmol) in acetonitrile (5 mL) was added formaldehyde (3 mmol), lactam (1.5 mmol) and silica-H_2SO_4 (50% w/w). Reaction mixture was then stirred at 50 °C for 2-5 h. Reaction mixture was cooled to rt and filtered. Filtrate was evaporated. The residue was purified by flash chromatography to get Mannich-type products.

8-Hydroxy-7-(pyrrolidin-1-ylmethyl)-6H-benzofuro[3,2-c]chromen-6-one (12). This compound was obtained from **11** and pyrrolidine employing method B. Yield 71%; pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ = 2/1) δ 8.02 (d, J = 7.8 Hz, 1H), 7.65 (t, J = 7.8 Hz, 1H), 7.58 (d, J = 8.9 Hz, 1H), 7.50-

7.42 (m, 2H), 7.10 (d, J = 8.9 Hz, 1H), 5.03 (s, 2H), 3.38 (s, 4H), 2.11 (s, 4H). ^{13}C NMR (101 MHz, $CDCl_3/CD_3OD$ = 2/1) δ 162.1, 160.0, 155.8, 153.9, 150.0, 133.2, 125.7, 124.6, 122.6, 117.6, 116.2, 114.7, 112.6, 110.5, 106.2, 54.2, 52.2, 23.5. HRMS (ESI) m/z: Calcd for $C_{20}H_{18}NO_4$ $(M+H)^+$ 336.1230, found 336.1217.

8-Hydroxy-7-((2-methylpiperidin-1-yl)methyl)-6H-benzofuro[3,2-c]chromen-6-one (13). This compound was obtained from **11** and 2-methylpiperidine employing method B. Yield 61%; white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.05 (d, J = 8.0 Hz, 1H), 7.72 (t, J = 7.1 Hz, 1H), 7.63-7.56 (m, 2H), 7.50 (t, J = 7.5 Hz, 1H), 6.88 (d, J = 8.9 Hz, 1H), 4.77-4.64 (m, 2H), 2.83 (d, J = 12.4 Hz, 1H), 2.68 (br, 1H), 2.34 (t, J = 9.2 Hz, 1H), 1.82-1.28 (m, 6H), 1.16 (d, J = 6.1 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 160.2, 157.3, 156.8, 152.8, 148.6, 132.4, 124.9, 121.9, 121.8, 116.7, 116.0, 115.0, 111.7, 111.0, 105.4, 55.4, 53.7, 50.4, 33.0, 25.1, 21.9, 16.9. HRMS (ESI) m/z: Calcd for $C_{22}H_{22}NO_4$ $(M+H)^+$ 364.1543, found 364.1538.

8-Hydroxy-7-((3-methylpiperidin-1-yl)methyl)-6H-benzofuro[3,2-c]chromen-6-one (14). This compound was obtained from **11** and 3-methylpiperidine employing method B. Yield 61%; pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.05 (d, J = 8.0 Hz, 1H), 7.72 (t, J = 7.3 Hz, 1H), 7.63-7.57 (m, 2H), 7.49 (t, J = 7.5 Hz, 1H), 6.92 (d, J = 8.9 Hz, 1H), 4.63-4.52 (m, 2H), 2.89 (br, 2H), 2.14 (t, J = 11.0 Hz, 1H), 1.87 (t, J = 10.6 Hz, 1H), 1.68 (br, 3H), 1.54-1.44 (m, 1H), 1.02-0.93 (m, 1H), 0.85 (d, J = 6.1 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 160.3, 157.2, 156.5, 152.8, 148.6, 132.4, 124.9, 122.3, 121.8, 116.7, 115.9, 114.4, 111.7, 111.2, 105.4, 60.1, 57.1, 52.6, 31.9, 30.6, 24.7, 19.1. HRMS (ESI) m/z: Calcd for $C_{22}H_{22}NO_4$ $(M+H)^+$ 364.1543, found 364.1550.

8-Hydroxy-7-((4-methylpiperidin-1-yl)methyl)-6H-benzofuro[3,2-c]chromen-6-one (15). This compound was obtained from **11** and 4-methylpiperidine employing method B. Yield 89%; pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ = 3/1) δ 7.96 (d, *J* = 7.7 Hz, 1H), 7.56 (t, *J* = 7.5 Hz, 1H), 7.47-7.28 (m, 3H), 6.89 (d, *J* = 8.8 Hz, 1H), 4.64 (s, 2H), 2.98 (d, *J* = 10.5 Hz, 2H), 2.29 (t, *J* = 10.3 Hz, 2H), 1.66 (d, *J* = 13.0 Hz, 2H), 1.44 (s, 1H), 1.30-1.20 (m, 2H), 0.91 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3/CD_3OD$ = 3/1) δ 161.0, 158.8, 157.0, 153.4, 149.6, 132.0, 124.8, 122.6, 122.0, 117.0, 116.5, 114.8, 112.5, 111.2, 106.0, 58.0, 53.1, 34.1, 30.4, 21.6. HRMS (ESI) m/z: Calcd for $C_{22}H_{22}NO_4$ $(M+H)^+$ 364.1543, found 364.1532.

Ethyl 5-hydroxy-2-(2-methoxyphenyl)-4-((2-oxopyrrolidin-1-yl)methyl)benzofuran-3-carboxylate (16). This compound was obtained from ethyl 5-hydroxy-2-(2-methoxyphenyl)benzofuran-3-carboxylate and pyrrolidin-2-one employing method D. Yield 24%; light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.65 (s, 1H), 7.59 (d, *J* = 7.4 Hz, 1H), 7.44 (t, *J* = 7.6 Hz, 1H), 7.38 (d, *J* = 8.8 Hz, 1H), 7.08 (t, *J* = 7.4 Hz, 1H), 7.02 (d, *J* = 8.8 Hz, 1H), 6.97 (d, *J* = 8.3 Hz, 1H), 5.12 (s, 2H), 4.15 (q, *J* = 7.1 Hz, 2H), 3.81 (s, 3H), 3.49 (t, *J* = 7.1 Hz, 2H), 2.42 (t, *J* = 8.1 Hz, 2H), 2.03-1.93 (m, 2H), 1.04 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.6, 166.1, 157.1, 156.9, 153.9, 148.8, 131.4, 130.4, 125.9, 120.7, 120.2, 116.8, 114.5, 112.4 (2), 110.9, 61.0, 55.7, 48.2, 38.4, 30.4, 18.0, 13.8.

1-((8-Hydroxy-6-oxo-6H-benzofuro[3,2-c]chromen-7-yl)methyl)pyrrolidin-2-one (17). This compound was obtained from **16** employing method C. Yield 71%; pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.06 (s, 1H), 8.04 (d, *J* = 7.7 Hz, 1H), 7.63 (t, *J* = 7.8 Hz, 1H), 7.54-7.48 (m, 2H), 7.43 (t, *J* = 7.5 Hz, 1H), 7.14 (d, *J* = 8.9 Hz, 1H), 5.44 (s, 2H), 3.65 (t, *J* = 7.1 Hz, 2H), 2.45 (t, *J* = 8.2 Hz, 2H), 2.07-1.92 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.0, 161.4, 158.8, 155.4, 153.5, 149.7, 132.3, 124.9, 124.0,

122.2, 118.6, 117.2, 115.9, 112.9, 112.6, 106.0, 48.3, 39.6, 30.6, 18.0. HRMS (ESI) m/z: Calcd for $C_{20}H_{15}NNaO_5$ $(M+Na)^+$ 372.0842, found 372.0858.

1-((8-Methoxy-6-oxo-6H-benzofuro[3,2-c]chromen-7-yl)methyl)pyrrolidin-2-one (18). To a stirred solution of **17** (1.0 mmol) and K_2CO_3 (2.1 mmol) in anhydrous DMF (20 mL) was added CH_3I (1.2 mmol) under N_2. After stirring overnight at rt, the reaction mixture was quenched with H_2O. The mixture was extracted with EtOAc (2×20 mL), and the combined organic phases were washed with water (2×20 mL), dried over Na_2SO_4, and evaporated. The residue was purified by flash chromatography to give the product **18**. Yield 52%; white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, *J* = 7.2 Hz, 1H), 7.64-7.54 (m, 2H), 7.47 (d, *J* = 8.3 Hz, 1H), 7.46 (d, *J* = 8.4 Hz, 1H), 7.39(t, *J* = 7.5 Hz, 1H), 7.05(d, *J* = 9.0 Hz, 1H), 5.40 (s, 2H), 3.88 (s, 3H), 3.16 (t, *J* = 7.0 Hz, 2H), 2.37 (t, *J* = 8.1 Hz, 2H), 1.90-1.78 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.1, 161.8, 158.2, 156.7, 153.8, 150.0, 132.4, 125.3, 124.8, 122.1, 118.9, 117.2, 112.4, 111.5, 110.9, 106.1, 56.9, 46.94 39.0, 31.3, 17.9. HRMS (ESI) m/z: Calcd for $C_{21}H_{17}NNaO_5$ $(M+Na)^+$ 386.0999, found 386.0985.

1-((8-(2-Methoxyethoxy)-6-oxo-6H-benzofuro[3,2-c]chromen-7-yl)methyl)pyrrolidin-2-one (19). To a stirred solution of **17** (1.0 mmol) and K_2CO_3 (2.1 mmol) in anhydrous DMF (20 mL) was added 1-bromo-2-methoxyethane (1.2 mmol) under N_2. After stirring 5 h at 80 ℃, the reaction mixture was quenched with H_2O. The mixture was extracted with EtOAc (2×20 mL), and the combined organic phases were washed with brine (1×20 mL), dried over Na_2SO_4, and evaporated. The residue was purified by flash chromatography to give the product **19**. Yield 51%; white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, *J* = 7.8 Hz, 1H), 7.63 (t, *J* = 7.8 Hz, 1H), 7.57 (d, *J* = 9.0 Hz, 1H), 7.49 (d, *J* = 8.3 Hz, 1H), 7.42 (t, *J* = 7.6 Hz, 1H), 7.10 (d, *J* = 9.0 Hz, 1H), 5.44(s, 2H), 4.16 (t, *J* = 4.8 Hz, 2H), 3.82 (t, *J* = 4.8 Hz, 2H), 3.47 (s,

3H), 3.19 (t, *J* = 7.1 Hz, 2H), 2.38 (t, *J* = 8.1 Hz, 2H), 1.92-1.82 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.8, 161.6, 158.0, 155.9, 153.6, 150.1, 132.4, 125.2, 124.7, 122.0, 119.3, 117.1, 112.3, 112.2, 111.5, 105.9, 71.2, 69.3, 59.2, 46.2, 39.0, 31.2, 17.8. HRMS (ESI) m/z: Calcd for $C_{23}H_{21}NNaO_6$ $(M+Na)^+$ 430.1261, found 430.1254.

1-((8-Hydroxy-6-oxo-6H-benzofuro[3,2-c]chromen-7-yl)methyl)piperidin-2-one (20). This compound was obtained from **10** and piperidin-2-one according to the methodology described for **17**. Yield 45%, 92%; white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (dd, *J* = 7.8, 1.0 Hz, 1H), 7.65-7.59 (m, 1H), 7.54-7.47 (m, 2H), 7.42 (t, *J* = 7.6 Hz, 1H), 7.10 (d, *J* = 8.9 Hz, 1H), 5.62 (s, 2H), 3.47 (t, *J* = 5.2 Hz, 2H), 2.46 (t, *J* = 6.4 Hz, 2H), 1.81-1.63 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.9, 161.5, 158.8, 155.6, 153.4, 149.4, 132.3, 124.9, 124.6, 122.1, 118.0, 117.2, 115.2, 112.9, 112.5, 106.0, 47.5, 43.3, 31.9, 22.8, 20.6. HRMS (ESI) m/z: Calcd for $C_{21}H_{17}NNaO_5$ $(M+Na)^+$ 386.0999, found 386.0980.

1-((8-Methoxy-6-oxo-6H-benzofuro[3,2-c]chromen-7-yl)methyl)piperidin-2-one (21). This compound was obtained from **20** according to the methodology described for **18**. Yield 52%; white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, *J* = 7.6 Hz, 1H), 7.65-7.54 (m, 2H), 7.47 (d, *J* = 8.3 Hz, 1H), 7.40 (t, *J* = 7.5 Hz, 1H), 7.06 (d, *J* = 8.9 Hz, 1H), 5.60 (s, 2H), 3.89 (s, 3H), 3.16 (t, *J* = 5.3 Hz, 2H), 2.40 (t, *J* = 5.9 Hz, 2H), 1.77-1.62 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.2, 161.8, 158.2, 156.8, 153.8, 150.1, 132.4, 126.0, 124.7, 122.1, 120.2, 117.2, 112.5, 111.3, 111.0, 106.4, 56.9, 46.9, 42.9, 32.7, 23.5, 21.5. HRMS (ESI) m/z: Calcd for $C_{22}H_{19}NNaO_5$ $(M+Na)^+$ 400.1155, found 400.1161.

1-((8-Ethoxy-6-oxo-6H-benzofuro[3,2-c]chromen-7-yl)methyl)piperidin-2-one (22). This compound was obtained from **20** and CH_3CH_2I according to the methodology described for **18**. Yield 63%; pale

yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, *J* = 7.7 Hz, 1H), 7.60 (t, *J* = 7.8 Hz, 1H), 7.53 (d, *J* = 8.9 Hz, 1H), 7.46 (d, *J* = 8.3 Hz, 1H), 7.39 (t, *J* = 7.5 Hz, 1H), 7.03 (d, *J* = 9.0 Hz, 1H), 5.60 (s, 2H), 4.07 (q, *J* = 6.9 Hz, 2H), 3.15 (t, *J* = 5.7 Hz, 2H), 2.40 (t, *J* = 6.4 Hz, 2H), 1.76-1.62 (m, 4H), 1.43 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.9, 161.7, 158.2, 156.1, 153.8, 150.0, 132.3, 125.9, 124.7, 122.1, 120.2, 117.2, 112.5, 111.7, 111.2, 106.4, 65.1, 46.8, 42.9, 32.8, 23.4, 21.6, 15.2. HRMS (ESI) m/z: Calcd for $C_{23}H_{21}NNaO_5$ $(M+Na)^+$ 414.1312, found 414.1319.

1-((8-(2-Methoxyethoxy)-6-oxo-6H-benzofuro[3,2-c]chromen-7-yl)methyl)piperidin-2-one (23). This compound was obtained from **20** according to the methodology described for **19**. Yield 45%; white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (d, *J* = 7.7 Hz, 1H), 7.58 (t, *J* = 7.7 Hz, 1H), 7.52 (d, *J* = 8.9 Hz, 1H), 7.44 (d, *J* = 8.3 Hz, 1H), 7.37 (t, *J* = 7.5 Hz, 1H), 7.05 (d, *J* = 8.9 Hz, 1H), 5.59 (s, 2H), 4.13 (t, *J* = 4.7 Hz, 2H), 3.78 (t, *J* = 4.8 Hz, 2H), 3.44 (s, 3H), 3.14 (t, *J* = 5.7 Hz, 2H), 2.38 (t, *J* = 6.4 Hz, 2H), 1.77-1.60 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.9, 161.7, 158.1, 156.1, 153.7, 150.2, 132.3, 125.9, 124.7, 122.1, 120.5, 117.1, 112.4, 112.3, 111.3, 106.3, 71.3, 69.2, 59.2, 46.7, 42.7, 32.7, 23.4, 21.5. HRMS (ESI) m/z: Calcd for $C_{24}H_{23}NNaO_6$ $(M+Na)^+$ 444.1418, found 444.1433.

1-((8-Hydroxy-6-oxo-6H-benzofuro[3,2-c]chromen-7-yl)methyl)azepan-2-one (24). This compound was obtained from **10** according to the methodology described for **17**. Yields 17 and 81%; white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.16 (s, 1H), 8.03 (d, *J* = 7.7 Hz, 1H), 7.62 (t, *J* = 7.5 Hz, 1H), 7.52-7.46 (m, 2H), 7.42 (t, *J* = 7.4 Hz, 1H), 7.09 (d, *J* = 8.9 Hz, 1H), 5.54 (s, 2H), 3.46 (s, 2H), 2.60 (s, 2H), 1.70 (s, 4H), 1.60 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.1, 160.3, 157.8, 154.5, 152.3, 148.3, 131.1, 123.8, 123.0, 121.0, 116.5, 116.0, 114.4, 111.6, 111.4, 104.7, 47.5, 42.6, 35.4, 28.7, 26.3, 22.2. HRMS (ESI) m/z: Calcd for $C_{22}H_{19}NNaO_5$ $(M+Na)^+$ 400.1155, found 400.1148.

Ethyl 5-methoxy-2-(2-methoxyphenyl)benzofuran-3-carboxylate (25). This compound was obtained from **10** according to the methodology described for **18**. Yield 91%; yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56-7.51 (m, 2H), 7.49-7.43 (m, 1H), 7.41 (d, *J* = 8.9 Hz, 1H), 7.06 (t, *J* = 7.5 Hz, 1H), 7.00 (d, *J* = 8.4 Hz, 1H), 6.94 (dd, *J* = 8.9, 2.6 Hz, 1H), 4.27 (q, *J* = 7.1 Hz, 2H), 3.90 (s, 3H), 3.81 (s, 3H), 1.23 (t, *J* = 7.1 Hz, 3H).

Ethyl 4-formyl-5-methoxy-2-(2-methoxyphenyl)benzofuran-3-carboxylate (26) and ethyl 6-formyl-5-methoxy-2-(2-methoxyphenyl)benzofuran-3-carboxylate (27). To a solution of **25** (1 mmol) and Cl_2CHOCH_3 (5.0 mmol) in $CHCl_3$ (5 mL) were added slowly $TiCl_4$ (2.5 mmol) at rt. The reaction mixture was stirred at r t for 40 min and quenched with H_2O. The mixture was extracted with EtOAc (2×20 mL), washed with saturated aqueous $NaHCO_3$ solution (1×20 mL), dried over Na_2SO_4, and evaporated. The residue was purified by silica gel column chromatography to give the product **26** and **27** in 22% and 34% yield respectively. **26**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.61 (s, 1H), 7.65 (t, *J* = 8.3 Hz, 2H), 7.44 (t, *J* = 7.9 Hz, 1H), 7.06 (t, *J* = 7.5 Hz, 1H), 7.02-6.95 (m, 2H), 4.38 (q, *J* = 7.2 Hz, 2H), 3.97 (s, 3H), 3.82 (s, 3H), 1.28 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 189.4, 165.2, 159.7, 157.3, 156.7, 149.4, 131.8, 130.9, 125.3, 120.7, 118.6, 117.7, 117.4, 114.1, 111.3, 109.4, 61.2, 57.0, 55.5, 14.2. **27:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.54 (s, 1H), 7.97 (s, 1H), 7.61 (s, 1H), 7.55 (d, *J* = 7.6 Hz, 1H), 7.49 (t, *J* = 7.9 Hz, 1H), 7.08 (t, *J* = 7.5 Hz, 1H), 7.01 (d, *J* = 8.4 Hz, 1H), 4.28 (q, *J* = 7.1 Hz, 2H), 4.02 (s, 3H), 3.82 (s, 3H), 1.22 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 189.8, 163.7, 162.2, 159.2, 157.8, 148.8, 133.7, 132.3, 131.2, 122.7, 120.4, 119.1, 111.6, 111.2, 110.7, 103.5, 60.6, 56.3, 55.8, 14.2.

Ethyl 5-methoxy-2-(2-methoxyphenyl)-4-(piperidine-1-carbonyl)benzofuran-3-carboxylate (28). To a solution of **26** (1 mmol) in 10 ml of dioxane/H_2O (7/3) was added $NaClO_2$ (6 mmol) and NH_2SO_3H (5.7 mmol) at rt. The reaction mixture was stirred at rt for 1 h and quenched with saturated aqueous $Na_2S_2O_3$ solution. The mixture was extracted with EtOAc (2×20 mL), washed with saturated aqueous NaCl solution, dried over Na_2SO_4, and evaporated. The residue was dissolved in anhydrous DMF (5 mL), EDC·HCl (1.3 mmol), HOBt (1.3 mmol), 3-methylpyridine (2.1 mmol) and piperidine (1.2 mmol) under N_2 were added at rt. After stirring overnight at rt, the reaction mixture was quenched with saturated NH_4Cl solution and extracted with EtOAc (2×30 mL). The combined organic phases were washed with HCl (5% aqueous solution), saturated aqueous $NaHCO_3$ solution (1×30 mL), dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by flash chromatography to give the amide product **28**. Overall yield 90%; white semisolid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (dd, J = 7.6, 1.5 Hz, 1H), 7.47-7.38 (m, 2H), 7.05 (t, J = 7.3 Hz, 1H), 6.95 (d, J = 8.9 Hz, 2H), 4.27-4.17 (m, 1H), 4.15-4.03 (m, 2H), 3.87 (s, 3H), 3.78 (s, 3H), 3.56-3.45 (m, 1H), 3.41-3.32 (m, 1H), 3.24-3.13 (m, 1H), 1.73-1.50 (m, 6H), 1.10 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.6, 163.7, 157.3, 156.9, 152.2, 149.5, 131.4, 130.8, 124.7, 120.5, 119.7, 118.7, 112.4, 111.6, 111.0, 109.8, 60.6, 57.2, 55.5, 48.1, 42.4, 26.1, 25.4, 24.9, 14.0.

8-Hydroxy-7-(piperidine-1-carbonyl)-6H-benzofuro[3,2-c]chromen-6-one (29). This compound was obtained from **28** employing method C. Yield 98%; white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.83 (s, 1H), 8.06 (d, J = 7.7 Hz, 1H), 7.75-7.70 (m, 2H), 7.57 (d, J = 8.3 Hz, 1H), 7.50 (t, J = 7.5 Hz, 1H), 7.07 (d, J = 9.0 Hz, 1H), 3.77-3.72 (m, 1H), 3.65-3.53 (m, 1H), 3.21-3.02 (m, 2H), 1.84-1.70 (m, 1H), 1.62-1.48 (m, 3H), 1.41-1.31 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.9, 160.3, 155.7, 153.1, 150.8, 148.7, 132.6, 124.9, 121.9, 120.4, 117.1, 117.0, 115.4, 112.2, 111.8, 104.9, 47.0, 41.5, 25.4, 24.5, 24.2. HRMS (ESI) m/z: Calcd for $C_{21}H_{17}NNaO_5$ $(M+Na)^+$ 386.0999, found 386.0976.

8-Methoxy-7-(piperidine-1-carbonyl)-6H-benzofuro[3,2-c]chromen-6-one (30). This compound was synthesized from **29** and CH_3I according to the methodology described for **18**. Yield 63%; white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, *J* = 7.8 Hz, 1H), 7.64-7.57 (m, 2H), 7.47 (d, *J* = 8.4 Hz, 1H), 7.39 (t, *J* = 7.6 Hz, 1H), 7.08 (d, *J* = 9.1 Hz, 1H), 3.94-3.89 (m, 5H), 3.28-3.12 (m, 2H), 2.00-1.89 (m, 1H), 1.73-1.59 (m, 3H), 1.53-1.39 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.0, 161.5, 156.8, 154.0, 153.2, 150.5, 132.4, 124.7, 122.1, 121.5, 119.9, 117.6, 112.4, 112.0, 110.8, 105.6, 57.0, 48.0, 42.5, 26.0, 25.1, 24.9. HRMS (ESI) m/z: Calcd for $C_{22}H_{19}NNaO_5$ $(M+Na)^+$ 400.1155, found 400.1135.

N-(cyclopropylmethyl)-8-hydroxy-6-oxo-6H-benzofuro[3,2-c]chromene-7-carboxamide (31). This compound was synthesized from **26** and cyclopropylmethanamine according to the methodology described for **29**. Yields 87 and 78%; white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.42 (s, 1H), 8.98 (br, 1H), 8.09 (d, *J* = 7.9 Hz, 1H), 7.74-7.64 (m, 2H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.47 (t, *J* = 7.6 Hz, 1H), 7.18 (d, *J* = 9.1 Hz, 1H), 3.45 (dd, *J* = 7.0, 5.3 Hz, 2H), 1.21-1.13 (m, 1H), 0.58-0.53 (m, 2H), 0.35-0.31 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.9, 161.8, 161.3, 159.7, 153.0, 149.4, 133.0, 125.3, 122.4, 119.5, 119.3, 117.1, 117.0, 112.2, 109.9, 105.7, 45.4, 10.2, 3.9. HRMS (ESI) m/z: Calcd for $C_{20}H_{15}NNaO_5$ $(M+Na)^+$ 372.0842, found 372.0832.

8-Hydroxy-9-(piperidin-1-ylmethyl)-6H-benzofuro[3,2-c]chromen-6-one (32). To a solution of **27** (1 mmol) in CH_3OH (5 mL) were added piperidine (1.2 mmol) and CH_3COOH (cat., 1 drop) at rt. The reaction mixture was stirred at rt for 10 min and then added with $NaBH_3CN$ (2.0 mmol). The mixture was stirred at rt for 16 h then evaporated under vacuum. The mixture was extracted with EtOAc (2×20 mL), washed with saturated aqueous $NaHCO_3$ solution (20 mL), dried over Na_2SO_4, and evaporated. The

residue was dissolved in CH_2Cl_2 (5 mL) and BBr_3 (1M in CH_2Cl_2, 2.0 mmol) was added at rt under N_2. After stirring overnight, the reaction mixture was quenched with EtOH. The resulting mixture was allowed to reflux for 1 h and evaporated. The residue was purified by flash chromatography. Overall yield 34%; white solid. ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ =3/1) δ 8.07 (s, 1H), 8.01 (d, *J* = 7.6 Hz, 1H), 7.64 (t, *J* = 7.8 Hz, 1H), 7.51-7.40 (m, 3H), 4.39 (s, 2H), 3.49 (br, 2H), 2.94 (br, 2H), 1.93 (br, 6H). ^{13}C NMR (101 MHz, $CDCl_3/CD_3OD$ = 3/1) δ 162.2, 159.1, 154.9, 154.0, 149.6, 133.0, 126.5, 125.5, 122.5, 117.8, 116.5, 115.2, 112.7, 106.7, 105.5, 55.7, 53.0, 23.1, 22.0. HRMS (ESI) m/z: Calcd for $C_{21}H_{20}NO_4$ $(M+H)^+$ 350.1387, found 350.1392.

8-Hydroxy-9-(piperidine-1-carbonyl)-6H-benzofuro[3,2-c]chromen-6-one (33). This compound was synthesized from **27** according to the methodology described for **29**. Yields 90 and 74%; white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.12 (s, 1H), 8.04 (d, *J* = 7.7 Hz, 1H), 7.73 (t, *J* = 7.8 Hz, 1H), 7.66 (s, 1H), 7.60 (d, *J* = 8.3 Hz, 1H), 7.50 (t, *J* = 7.5 Hz, 1H), 7.43 (s, 1H), 3.61 (br, 2H), 3.21 (br, 2H), 1.60 (br, 2H), 1.51 (br, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.7, 160.4, 157.2, 153.1, 151.5, 148.5, 132.5, 125.1, 124.6, 123.9, 121.8, 117.2, 112.1, 111.0, 105.7, 104.9, 47.2, 41.9, 25.6, 24.1 (2C). HRMS (ESI) m/z: Calcd for $C_{21}H_{17}NNaO_5$ $(M+Na)^+$ 386.0999, found 386.1019.

8-Hydroxy-9-nitro-7-(piperidin-1-ylmethyl)-6H-benzofuro[3,2-c]chromen-6-one (34). To the solution of **9** (1 mmol) in CH_3COOH (5 mL), HNO_3 (0.5 mL) under N_2 was added at rt. The reaction mixture was stirred at rt for 1 h and evaporated. The residue was purified by flash chromatography to give the product. Yield 80%; red solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (s, 1H), 8.06 (d, *J* = 7.8 Hz, 1H), 7.69 (t, *J* = 7.7 Hz, 1H), 7.51 (d, *J* = 8.4 Hz, 1H), 7.46 (t, *J* = 7.6 Hz, 1H), 4.97 (s, 2H), 2.93 (br, 4H), 1.89-1.75 (m, 4H), 1.62 (br, 2H). ^{13}C NMR (101 MHz, $CDCl_3/CD_3OD$ = 3/1) δ 165.1, 162.1, 159.0, 154.1,

144.0, 136.4, 133.9, 129.8, 125.6, 122.9, 117.5, 114.1, 112.1, 110.3, 104.7, 57.0, 52.8, 23.6, 22.5. HRMS (ESI) m/z: Calcd for $C_{21}H_{19}N_2O_6$ $(M+H)^+$ 395.1238, found 395.1239.

9-Bromo-8-hydroxy-7-(piperidin-1-ylmethyl)-6H-benzofuro[3,2-c]chromen-6-one (35). To the solution of **9** (1 mmol) in CH_2Cl_2 (5 mL), Br_2 (1.2 mmol) under N_2 was added at rt. After stirring overnight at rt, the reaction mixture was quenched with saturated aqueous $Na_2S_2O_3$ solution and extracted with CH_2Cl_2 (2×30 mL). The combined organic phases were washed saturated aqueous $NaHCO_3$ solution (20 mL), brine (20 mL), dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by flash chromatography to give the product. Yield 77%; pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ = 3/1) δ 8.01 (dd, *J* = 7.8, 1.2 Hz, 1H), 7.79 (s, 1H), 7.67-7.59 (m, 1H), 7.49-7.37 (m, 2H), 4.82 (s, 2H), 2.81 (br, 4H), 1.73 (br, 4H), 1.56 (br, 2H). ^{13}C NMR (101 MHz, $CDCl_3/CD_3OD$ = 3/1) δ 161.4, 159.0, 156.6, 153.6, 148.3, 132.6, 125.2, 122.6, 122.3, 117.3, 115.4, 113.4, 112.5, 111.9, 105.8, 58.6, 53.4, 25.2, 23.6. HRMS (ESI) m/z: Calcd for $C_{21}H_{19}BrNO_4$ $(M+H)^+$ 428.0492, found 428.0496.

8-Hydroxy-9-methoxy-7-(piperidin-1-ylmethyl)-6H-benzofuro[3,2-c]chromen-6-one (36). To the solution of **35** (1 mmol) in anhydrous DMF (5 mL), CuCl (1.0 mmol) and $NaOCH_3$ (10 mmol) under N_2 were added at rt. After stirring 1 h at 140 °C, the reaction mixture cooled to rt and extracted with EtOAc (2×30 mL). The combined organic phases were washed with H_2O (3×15 mL), dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by flash chromatography to give the product. Yield 78%; pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ = 3/1) δ 7.97 (d, *J* = 7.6 Hz, 1H), 7.59 (t, *J* = 7.6 Hz, 1H), 7.49-7.37 (m, 2H), 7.24 (s, 1H), 4.86 (s, 2H), 3.96 (s, 3H), 3.07 (br, 4H), 1.80 (br, 4H), 1.62 (br, 2H). ^{13}C NMR (101 MHz, $CDCl_3/CD_3OD$ = 3/1) δ 160.2, 160.0, 153.1, 149.9, 149.0, 147.0,

132.2, 125.4, 121.9, 117.4, 115.8, 112.7, 110.0, 106.5, 96.4, 56.6, 55.4, 53.6, 24.1, 22.9. HRMS (ESI) m/z: Calcd for $C_{22}H_{22}NO_5$ $(M+H)^+$ 380.1492, found 380.1484.

8,9-Dihydroxy-7-(piperidin-1-ylmethyl)-6H-benzofuro[3,2-c]chromen-6-one (37). To a solution of substituted **36** (1.0 mmol) in anhydrous CH_2Cl_2 (4 mL), BBr_3 (1M in CH_2Cl_2, 2.0 mmol) was added at rt under N_2. After stirring overnight, the reaction mixture was quenched with EtOH. The residue was purified by recrystallization in EtOH to obtain the product. Yield 60%; white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.02 (d, *J* = 7.8 Hz, 1H), 7.68 (t, *J* = 7.6 Hz, 1H), 7.58-7.42 (m, 2H), 7.25 (s, 1H), 4.92 (s, 2H), 3.67-3.63 (m, 2H), 3.21 (t, *J* = 11.9 Hz, 2H), 2.00-1.96 (m, 2H), 1.86-1.69 (m, 3H), 1.65-1.58 (m, 1H). ^{13}C NMR (101 MHz, CD_3OD) δ 161.2, 160.7, 154.2, 151.1, 147.6, 146.8, 133.1, 126.4, 122.7, 118.2, 116.5, 113.6, 109.0, 108.0, 100.9, 54.3, 53.9, 23.9, 22.8. HRMS (ESI) m/z: Calcd for $C_{21}H_{20}NO_5$ $(M+H)^+$ 366.1336, found 366.1330.

Ethyl 7-(*tert*-butyl)-5-hydroxy-2-(2-methoxyphenyl)benzofuran-3-carboxylate (39). This compound was obtained from **38** and 2-(*tert*-butyl)cyclohexa-2,5-diene-1,4-dione employing method A. Yield 27%; pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, *J* = 7.6 Hz, 1H), 7.46 (t, *J* = 7.9 Hz, 1H), 7.37 (d, *J* = 2.4 Hz, 1H), 7.07 (t, *J* = 7.4 Hz, 1H), 7.01 (d, *J* = 8.3 Hz, 1H), 6.80 (d, *J* = 2.4 Hz, 1H), 4.27 (q, *J* = 7.1 Hz, 2H), 3.82 (s, 3H), 1.48 (s, 9H), 1.23 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.5, 158.0, 157.8, 152.4, 147.7, 136.1, 131.5 (2C), 128.1, 120.3, 120.0, 111.2, 111.0, 110.7, 104.4, 60.4, 55.7, 34.5, 29.9, 14.3.

10-(*tert*-Butyl)-8-hydroxy-6H-benzofuro[3,2-c]chromen-6-one (40). This compound was obtained from **39** employing method C. Yield 57%; white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.66 (s, 1H),

8.09 (dd, *J* = 7.8, 1.3 Hz, 1H), 7.72 (t, *J* = 7.9 Hz, 1H), 7.60 (d, *J* = 8.2 Hz, 1H), 7.51 (t, *J* = 7.6 Hz, 1H), 7.20 (d, *J* = 2.4 Hz, 1H), 6.88 (d, *J* = 2.4 Hz, 1H), 1.53 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 159.0, 157.3, 155.2, 153.0, 147.2, 136.0, 132.2, 125.0, 124.2, 121.6, 117.2, 112.6, 112.1, 104.9, 103.0, 34.1, 29.7.

10-(*tert*-Butyl)-8-hydroxy-7-(piperidin-1-ylmethyl)-6H-benzofuro[3,2-c]chromen-6-one (41). This compound was obtained from **40** employing method B. Yield 86%; white solid. ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ = 4/1) δ 8.06 (dd, *J* = 7.8, 1.3 Hz, 1H), 7.67-7.60 (m, 1H), 7.51-7.42 (m, 2H), 6.90 (s, 1H), 4.68 (s, 2H), 2.70 (br, 4H), 1.69 (br, 4H), 1.56 (s, 11H). ^{13}C NMR (101 MHz, $CDCl_3/CD_3OD$ = 4/1) δ 160.5, 159.3, 156.9, 153.6, 148.2, 135.3, 132.2, 125.1, 123.3, 122.1, 117.2, 114.4, 112.9, 112.2, 106.1, 58.4, 53.9, 34.6, 30.1, 26.1, 24.1. HRMS (ESI) m/z: Calcd for $C_{25}H_{28}NO_4$ $(M+H)^+$ 406.2013, found 406.2016.

8-Hydroxy-10-methyl-7-(piperidin-1-ylmethyl)-6H-benzofuro[3,2-c]chromen-6-one (42). This compound was obtained from **38** and 2-methylcyclohexa-2,5-diene-1,4-dione employing methods A, C, and B. Yields 50, 57, and 71%; white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, *J* = 7.8 Hz, 1H), 7.57 (t, *J* = 7.7 Hz, 1H), 7.46 (d, *J* = 8.3 Hz, 1H), 7.38 (t, *J* = 7.5 Hz, 1H), 7.26 (s, 1H), 4.65 (s, 2H), 2.74 (br, 4H), 2.54 (s, 3H), 1.66 (br, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.5, 158.6, 157.2, 153.5, 148.7, 131.8, 124.6, 122.2, 122.0, 121.5, 117.7, 117.1, 112.8, 112.2, 106.5, 58.4, 53.7, 26.0, 24.1, 15.0. HRMS (ESI) m/z: Calcd for $C_{22}H_{22}NO_4$ $(M+H)^+$ 364.1543, found 364.1544.

10-Chloro-8-hydroxy-7-(piperidin-1-ylmethyl)-6H-benzofuro[3,2-c]chromen-6-one (43). This compound was obtained from **38** and 2-chlorocyclohexa-2,5-diene-1,4-dione employing methods A, C, and B. Yields 52, 33, and 69%; yellow solid. ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ =3/1) δ 8.07 (d, *J* = 7.6

Hz, 1H), 7.62 (t, *J* = 7.9 Hz, 1H), 7.48-7.38 (m, 2H), 6.91 (s, 1H), 4.69 (s, 2H), 2.71 (br, 4H), 1.67 (br, 4H), 1.55 (br, 2H). ^{13}C NMR (101 MHz, $CDCl_3/CD_3OD$ = 3/1) δ 161.7, 159.0 (2C), 153.8, 145.4, 132.9, 125.4, 124.4, 122.6, 117.3 (2C), 117.2, 112.6, 112.4, 106.5, 57.9, 53.7, 25.5, 23.7. HRMS (ESI) m/z: Calcd for $C_{21}H_{19}ClNO_4$ $(M+H)^+$ 384.0997, found 384.0993.

10-Bromo-8-hydroxy-7-(piperidin-1-ylmethyl)-6H-benzofuro[3,2-c]chromen-6-one (44). This compound was obtained from **38** and 2-bromocyclohexa-2,5-diene-1,4-dione employing methods A, C and B. Yields 61, 65, and 46%; pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ = 3/1) δ 8.08 (d, *J* = 7.7 Hz, 1H), 7.64 (t, *J* = 7.7 Hz, 1H), 7.51-7.39 (m, 2H), 7.18 (s, 1H), 4.79 (s, 2H), 2.90 (br, 4H), 1.76 (br, 4H), 1.58 (br, 2H). ^{13}C NMR (101 MHz, $CDCl_3/CD_3OD$ = 3/1) δ 161.7, 159.3, 158.0, 153.8, 146.9, 133.2, 125.5, 124.9, 122.6, 119.6, 117.4, 112.2, 111.1, 106.7, 105.5, 56.0, 53.5, 24.4, 23.0. HRMS (ESI) m/z: Calcd for $C_{21}H_{19}BrNO_4$ $(M+H)^+$ 428.0492, found 428.0501.

Ethyl 2-(2,4-dimethoxyphenyl)-5-hydroxybenzofuran-3-carboxylate (46). This compound was obtained from **45** and benzoquinone employing method A. Yield 89%; pale solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, *J* = 1.7 Hz, 1H), 7.47 (d, *J* = 8.4 Hz, 1H), 7.35 (d, *J* = 8.8 Hz, 1H), 6.86 (dd, *J* = 8.8, 2.0 Hz, 1H), 6.59 (d, *J* = 8.5 Hz, 1H), 6.54 (s, 1H), 4.28 (q, *J* = 7.1 Hz, 2H), 3.87 (s, 3H), 3.79 (s, 3H), 1.23 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.0, 162.8, 159.5, 159.1, 153.0, 149.1, 132.3, 127.9, 113.5, 112.6, 111.8, 110.4, 106.8, 104.5, 98.7, 60.7, 55.7, 55.6, 14.2.

3,8-Dihydroxy-7-(piperidin-1-ylmethyl)-6H-benzofuro[3,2-c]chromen-6-one (48). This compound was obtained from **47** employing method C. Yield 94%; white solid. ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ = 3/1) δ 7.77 (d, *J* = 8.6 Hz, 1H), 7.45 (d, *J* = 8.9 Hz, 1H), 7.02 (d, *J* = 8.8 Hz, 1H), 6.89 (d, *J* = 8.6 Hz,

1H), 6.82 (s, 1H), 4.85 (s, 2H), 3.15 (br, 4H), 1.82 (br, 4H), 1.62 (br, 2H). ^{13}C NMR (101 MHz, $CDCl_3/CD_3OD$ = 3/1) δ 163.1, 162.8, 160.5, 155.9, 155.8, 149.5, 124.7, 123.6, 115.3, 114.8, 113.9, 109.8, 104.4, 103.4, 102.8, 54.7, 53.5, 23.7, 22.6. HRMS (ESI) m/z: Calcd for $C_{21}H_{20}NO_5$ $(M+H)^+$ 366.1336, found 366.1329.

2,8-Dihydroxy-7-(piperidin-1-ylmethyl)-6H-benzofuro[3,2-c]chromen-6-one (49). This compound was obtained from ethyl 3-(2,5-dimethoxyphenyl)-3-oxopropanoate and benzoquinone employing methods A, B, and C. Yields 53, 77, and 76%; pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ = 2/1) δ 7.58 (d, *J* = 8.9 Hz, 1H), 7.36 (d, *J* = 2.5 Hz, 1H), 7.32 (d, *J* = 9.0 Hz, 1H), 7.13 (dd, *J* = 9.0, 2.6 Hz, 1H), 7.06 (d, *J* = 9.0 Hz, 1H), 4.89 (s, 2H), 3.19 (br, 4H), 1.83 (br, 4H), 1.66 (br, 2H). ^{13}C NMR (101 MHz, $CDCl_3/CD_3OD$ = 2/1) δ 162.3, 160.6, 156.4, 155.2, 150.2, 147.8, 125.0, 121.8, 118.7, 116.2, 114.6, 113.1, 110.0, 106.6, 106.5, 54.7, 53.7, 23.9, 22.7. HRMS (ESI) m/z: Calcd for $C_{21}H_{20}NO_5(M+H)^+$ 366.1336, found 366.1336.

10-(*tert*-Butyl)-3,8-dihydroxy-7-(piperidin-1-ylmethyl)-6H-benzofuro[3,2-c]chromen-6-one (50). This compound was obtained from **45** and 2-(*tert*-butyl)cyclohexa-2,5-diene-1,4-dione employing methods A, B, and C. Yields 73, 96, and 81%; white solid. ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ = 3/1) δ 7.89 (d, *J* = 8.6 Hz, 1H), 7.01 (s, 1H), 6.97 (dd, *J* = 8.7, 2.0 Hz, 1H), 6.92 (d, *J* = 2.0 Hz, 1H), 4.93 (s, 2H), 3.61-3.57 (m, 2H), 3.06 (t, *J* = 11.0 Hz, 2H), 1.98-1.94 (m, 2H), 1.85-1.78 (m, 3H), 1.52 (s, 10H). ^{13}C NMR (101 MHz, $CDCl_3/CD_3OD$ = 3/1) δ 162.9, 162.5, 161.1, 156.0, 155.3, 148.1, 139.3, 125.7, 123.7, 115.0, 112.5, 105.4, 104.6, 103.6, 102.9, 53.3, 53.2, 35.1, 29.9, 23.0, 22.1. HRMS (ESI) m/z: Calcd for $C_{25}H_{28}NO_5$ $(M+H)^+$ 422.1962, found 422.1972.

10-(*tert*-Butyl)-2,8-dihydroxy-7-(piperidin-1-ylmethyl)-6H-benzofuro[3,2-c]chromen-6-one (51). This compound was obtained from ethyl 3-(2,5-dimethoxyphenyl)-3-oxopropanoate and 2-(*tert*-butyl)cyclohexa-2,5-diene-1,4-dione employing methods A, B, and C. Yields 23, 99, and 86%; white solid. ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ = 3/1) δ 7.41 (d, *J* = 2.7 Hz, 1H), 7.34 (d, *J* = 9.0 Hz, 1H), 7.15 (dd, *J* = 9.0, 2.8 Hz, 1H), 7.08 (s, 1H), 4.96 (s, 2H), 3.59-3.55 (m, 2H), 3.06 (t, *J* = 11.9 Hz, 2H), 1.97-1.93 (m, 2H), 1.88-1.76 (m, 3H), 1.52 (s, 10H). ^{13}C NMR (101 MHz, $CDCl_3/CD_3OD$ = 3/1) δ 161.3, 160.7, 155.3, 154.9, 148.4, 147.5, 139.4, 125.4, 121.5, 118.5, 113.4, 112.8, 106.4, 105.9, 105.5, 53.2, 53.0, 35.0, 29.8, 22.9, 22.0. HRMS (ESI) m/z: Calcd for $C_{25}H_{28}NO_5$ $(M+H)^+$ 422.1962, found 422.1948.

Ethyl 6-bromo-2-(4-bromo-2-methoxyphenyl)-5-hydroxy-4-(piperidin-1-ylmethyl)benzofuran-3-carboxylate (53). This compound was synthesized from **52** according to the methodology described for **35**. Yield 47%; Yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (s, 1H), 7.43 (d, *J* = 8.2 Hz, 1H), 7.22 (d, *J* = 8.2 Hz, 1H), 7.10 (s, 1H), 4.20 (q, *J* = 6.8 Hz, 2H), 4.14 (s, 2H), 3.80 (s, 3H), 2.59 (br, 4H), 1.68 (br, 6H), 1.15 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.5, 157.4, 154.9, 153.0, 148.0, 131.5, 125.1, 124.6, 124.0, 118.9, 114.7, 114.2, 113.3, 112.6, 109.2, 61.1, 58.1, 55.9, 53.8, 25.8, 23.9, 14.0.

Ethyl 6-bromo-2-(5-bromo-2,4-dimethoxyphenyl)-5-hydroxy-4-(piperidin-1-ylmethyl) benzofuran-3-carboxylate (54). This compound was synthesized from **47** according to the methodology described for **35**. Yield 29%; white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (s, 1H), 7.60 (s, 1H), 6.50 (s, 1H), 4.22 (q, *J* = 7.1 Hz, 2H), 4.12 (s, 2H), 3.97 (s, 3H), 3.82 (s, 3H), 2.72 (br, 4H), 1.68 (br, 6H), 1.17 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.6, 158.2, 157.7, 154.6, 152.9, 147.8, 134.3, 124.6, 114.2, 113.3, 113.2, 111.9, 108.9, 102.3, 96.1, 61.1, 58.1, 56.6, 56.0, 53.8, 25.7, 23.9, 14.1.

3,8,9-Trihydroxy-7-(piperidin-1-ylmethyl)-6H-benzofuro[3,2-c]chromen-6-one (55). This compound was obtained from **53** according to the methodology described for **36** and method C. Yields 43 and 52%; pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ = 1/1) δ 7.84 (d, *J* = 8.6 Hz, 1H), 7.24 (s, 1H), 6.93 (d, *J* = 8.7 Hz, 1H), 6.89 (s, 1H), 4.94 (s, 2H), 3.65-3.61 (m, 2H), 3.13 (t, *J* = 11.8 Hz, 2H), 2.00-1.95 (m, 2H), 1.86-1.70 (m, 3H), 1.64-1.49 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3/CD_3OD$ = 1/1) δ 162.7, 161.9, 161.5, 155.8, 150.1, 146.1, 145.9, 123.6, 116.3, 115.1, 108.1, 105.4, 104.1, 103.8, 100.9, 53.9, 53.7, 23.5, 22.5. HRMS (ESI) m/z: Calcd for $C_{21}H_{20}NO_6$ $(M+H)^+$ 382.1285, found 382.1265.

2,3,8,9-Tetrahydroxy-7-(piperidin-1-ylmethyl)-6H-benzofuro[3,2-c]chromen-6-one (56). This compound was obtained from **54** according to the methodology described for **36** and method C. Yields 57 and 65%; white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.62 (s, 1H), 10.45 (s, 1H), 9.86 (s, 1H), 9.42 (s, 1H), 8.55 (s, 1H), 7.35 (s, 1H), 7.27 (s, 1H), 6.96 (s, 1H), 4.85 (s, 2H), 3.45-3.41 (m, 2H), 3.19-3.00 (m, 2H), 1.84-1.80 (m, 2H), 1.69-1.59 (m, 3H), 1.52-1.38 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 159.6, 159.1, 150.8, 148.2, 147.4, 145.4, 144.7, 144.0, 115.4, 108.4, 105.0, 103.6, 103.4, 103.1, 100.0, 52.2 (2C), 22.2, 21.2. HRMS (ESI) m/z: Calcd for $C_{21}H_{20}NO_7$ $(M+H)^+$ 398.1234, found 398.1229.

General Procedures for Biological Studies. [32, 14, 29] Oral bioavailability was analyzed by using serum inhibition titration assay. Compounds were ground to homogenate suspension in 0.5% carboxymethyl cellulose (CMC), or in propylene glycol (PG) and tween 80 at 4:1. Six-week female BALB/c mice (20 g, 3 mice in each group) were administered orally with 100 mg/kg dose for each experimental compound in CMC, or 50 mg/kg for 48 and 50 in PG. Isoniazid at 10 mg/kg was used as positive control, and 0.5% CMC treatment was used as vehicle control.

Minimum bactericidal concentration (MBC) determination. Mtb H37Rv was cultured to mid-log phase (OD600 = 0.5) in 7H9 broth at 37ºC. The culture was diluted to OD600 of 0.01 using 7H9 without Tween 80 targeting 106 colony forming units (CFU) per mL. Two-fold serial dilution was made for compound 48 from 0.5 to 0.00098 μg/mL using 7H9 without Tween 80 in a total volume of 2.5 mL using 15 mL conical tubes. Three tubes of growth control (with bacteria, without compound) and three tubes of media control (without bacteria, without compound) were included in the assay as controls. One hundred μL of the above mentioned diluted culture was added to respective test tubes. Inoculum size was determined by plating the growth control culture on 7H11 agar on time zero and CFU were enumerated after incubation. Test tubes were incubated at 37 ºC stand still. After 14 days of incubation, tubes were observed for pellet formation at the bottom. The minimum concentration that resulted no visible pellet formation was defined as MIC. All cultures from tubes without visible pellets were plated on 7H11 agar plates and CFU were enumerated. The minimum concentration that resulted 99% CFU reduction compared with inoculum at time zero was defined as MBC.

For the cytotoxicity testing of compounds **48** and **50** at four human-derived cell lines were conducted according to the previously published procedure.[29] The IC_{50} values were calculated by GraphPad Prism software (GraphPad Software, CA, USA). All experiments were performed independently three times. Data was shown as mean ± SD.

Acknowledgements. This work has been supported by the National Natural Science Foundation of China (21778019). This project has also been funded in part with Federal funds from the National Institutes of Health and National Institute of Allergy and Infectious Diseases, Department of Health and Human Services under grants AI37856 and HL133190. H.G. is the recipient of an Australian Research Council

Discovery Early Career Researcher Award (DE160100482) funded by the Australian Government. The authors thank Prof. Cai-Guang Yang at Shanghai Institute of Material Medica for performing the nonpathogenic bacteria selectivity assays.

AUTHOR INFORMATION

Author Contributions

Wei Zhang [†,#] and Shichun Lun [§,#] contributed equally to this work.

ORCID ID:

Li-Fang Yu: 0000-0002-3250-6773

Hendra Gunosewoyo: 0000-0003-3897-1948

Corresponding Authors

*Li-Fang Yu, E-mail: lfyu@sat.ecnu.edu.cn

*William R. Bishai, E-mail: wbishai1@jhmi.edu

*Fan Yang, E-mail: fyang@chem.ecnu.edu.cn

ABBREVIATIONS USED:

TB, tuberculosis; Pks, polyketide synthase; MDR, multidrug-resistant; XDR, extensively drug-resistant; TE, thioesterase; SARs, structure-activity relationships; SIT, serum inhibition titration; Mtb, *Mycobacterium tuberculosis*; WHO, World Health Organization; TE, thioesterase; RR-TB, rifampicin-resistant tuberculosis; INH, Isoniazid; ETH, ethionamide; ABPP, activity-based protein profiling; EDC·HCl, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride; HOBt, 1-hydroxybenzotriazole; MABA, microplate alamar blue assay; PAINS, pan assay interference compounds; EDG, electron-donating group; EWG, electron-withdrawing group; SI, selectivity index; CC, column chromatography; TLC, thin layer chromatography; LCMS, liquid chromatography mass spectrometry;

TMS, tetramethylsilane; HRMS, high-resolution mass spectra; THF, tetrahydrofuran; DMF, dimethylformamide; CMC, carboxymethyl cellulose; PG, propylene glycol; CFU, colony forming units; MBC, minimum bactericidal concentration.

Supporting Information. ^{1}H and ^{13}C NMR spectra and analytical HPLC purities of all final compounds, HPLC traces for compounds **48** and **50**, melting points for all solid compounds (PDF); molecular formula strings (CSV). This material is available free of charge via the Internet at http://pubs.acs.org.

REFERENCES

1. WHO. Global Tuberculosis Report 2018. World Health Organization. **2018**.

2. Gunosewoyo, H.; Lun, S.; Bishai, W. R.; Kozikowski, A. P. Targeting the number two infectious disease killer--tuberculosis. *ACS Med. Chem. Rev.* **2015**, *50*, 283-296.

3. Mitnick, C. D.; Shin, S. S.; Seung, K. J.; Rich, M. L.; Atwood, S. S.; Furin, J. J.; Fitzmaurice, G. M.; Alcantara Viru, F. A.; Appleton, S. C.; Bayona, J. N.; Bonilla, C. A.; Chalco, K.; Choi, S.; Franke, M. F.; Fraser, H. S.; Guerra, D.; Hurtado, R. M.; Jazayeri, D.; Joseph, K.; Llaro, K.; Mestanza, L.; Mukherjee, J. S.; Munoz, M.; Palacios, E.; Sanchez, E.; Sloutsky, A.; Becerra, M. C. Comprehensive treatment of extensively drug-resistant tuberculosis. *N. Engl. J. Med.* **2008**, *359*, 563-574.

4. Zumla, A. I.; Gillespie, S. H.; Hoelscher, M.; Philips, P. P.; Cole, S. T.; Abubakar, I.; McHugh, T. D.; Schito, M.; Maeurer, M.; Nunn, A. J. New antituberculosis drugs, regimens, and adjunct therapies: needs, advances, and future prospects. *Lancet Infect. Dis.* **2014**, *14*, 327-340.

5. Zumla, A.; Raviglione, M.; Hafner, R.; von Reyn, C. F. Tuberculosis. *N. Engl. J. Med.* **2013**, *368*, 745-755.

6. Chahine, E. B.; Karaoui, L. R.; Mansour, H. Bedaquiline: a novel diarylquinoline for multidrug-resistant tuberculosis. *Ann. Pharmacother.* **2014**, *48*, 107-115.

7. Gler, M. T.; Skripconoka, V.; Sanchez-Garavito, E.; Xiao, H.; Cabrera-Rivero, J. L.; Vargas-Vasquez, D. E.; Gao, M.; Awad, M.; Park, S. K.; Shim, T. S.; Suh, G. Y.; Danilovits, M.; Ogata, H.; Kurve, A.; Chang, J.; Suzuki, K.; Tupasi, T.; Koh, W. J.; Seaworth, B.; Geiter, L. J.; Wells, C. D. Delamanid for multidrug-resistant pulmonary tuberculosis. *N. Engl. J. Med.* **2012**, *366*, 2151-2160.

8. Esposito, S.; Bianchini, S.; Blasi, F. Bedaquiline and delamanid in tuberculosis. *Expert Opin. Pharmacother.* **2015**, *16*, 2319-2330.

9. Ferlazzo, G.; Mohr, E.; Laxmeshwar, C.; Hewison, C.; Hughes, J.; Jonckheere, S.; Khachatryan, N.; De Avezedo, V.; Egazaryan, L.; Shroufi, A.; Kalon, S.; Cox, H.; Furin, J.; Isaakidis, P. Early safety and efficacy of the combination of bedaquiline and delamanid for the treatment of patients with drug-resistant tuberculosis in Armenia, India, and South Africa: a retrospective cohort study. *Lancet Infect. Dis.* **2018**, *18*, 536-544.

10. Lachatre, M.; Rioux, C.; Le Du, D.; Frechet-Jachym, M.; Veziris, N.; Bouvet, E.; Yazdanpanah, Y. Bedaquiline plus delamanid for XDR tuberculosis. *Lancet Infect. Dis.* **2016**, *16*, 294.

11. Marrakchi, H.; Laneelle, M. A.; Daffe, M. Mycolic acids: structures, biosynthesis, and beyond. *Chem. Biol.* **2014**, *21*, 67-85.

12. Takayama, K.; Wang, C.; Besra, G. S. Pathway to synthesis and processing of mycolic acids in Mycobacterium tuberculosis. *Clin. Microbiol. Rev.* **2005**, *18*, 81-101.

13. Alsayed, S. S. R.; Beh, C. C.; Foster, N. R.; Payne, A. D.; Yu, Y.; Gunosewoyo, H. Kinase targets for mycolic acid biosynthesis in Mycobacterium tuberculosis. *Curr. Mol. Pharmacol.* **2019**, *12*, 27-49.

14. Lun, S.; Guo, H.; Onajole, O. K.; Pieroni, M.; Gunosewoyo, H.; Chen, G.; Tipparaju, S. K.; Ammerman, N. C.; Kozikowski, A. P.; Bishai, W. R. Indoleamides are active against drug-resistant Mycobacterium tuberculosis. *Nat. Commun.* **2013**, *4*, 2907.

15. Morlock, G. P.; Metchock, B.; Sikes, D.; Crawford, J. T.; Cooksey, R. C. ethA, inhA, and katG loci of ethionamide-resistant clinical Mycobacterium tuberculosis isolates. *Antimicrob. Agents Chemother.* **2003**, *47*, 3799-3805.

16. Vilcheze, C.; Wang, F.; Arai, M.; Hazbon, M. H.; Colangeli, R.; Kremer, L.; Weisbrod, T. R.; Alland, D.; Sacchettini, J. C.; Jacobs, W. R., Jr. Transfer of a point mutation in Mycobacterium tuberculosis inhA resolves the target of isoniazid. *Nat. Med.* **2006**, *12*, 1027-1029.

17. Bhatt, A.; Molle, V.; Besra, G. S.; Jacobs, W. R., Jr.; Kremer, L. The Mycobacterium tuberculosis FAS-II condensing enzymes: their role in mycolic acid biosynthesis, acid-fastness, pathogenesis and in future drug development. *Mol. Microbiol.* **2007**, *64*, 1442-1454.

18. Portevin, D.; de Sousa-D'Auria, C.; Montrozier, H.; Houssin, C.; Stella, A.; Laneelle, M. A.; Bardou, F.; Guilhot, C.; Daffe, M. The acyl-AMP ligase FadD32 and AccD4-containing acyl-CoA carboxylase are required for the synthesis of mycolic acids and essential for

mycobacterial growth: identification of the carboxylation product and determination of the acyl-CoA carboxylase components. *J. Biol. Chem.* **2005**, *280*, 8862-8874.

19. Li, W.; Yazidi, A.; Pandya, A. N.; Hegde, P.; Tong, W. W.; de Moura, V. C. N.; North, E. J.; Sygusch, J.; Jackson, M. MmpL3 as a target for the treatment of drug-resistant nontuberculous mycobacterial infections. *Frontiers in Microbiology* **2018**, *9*, 1547.

20. Xu, Z.; Meshcheryakov, V. A.; Poce, G.; Chng, S. S. MmpL3 is the flippase for mycolic acids in mycobacteria. *Proc. Natl. Acad. Sci. U S A* **2017**, *114*, 7993-7998.

21. Portevin, D.; De Sousa-D'Auria, C.; Houssin, C.; Grimaldi, C.; Chami, M.; Daffe, M.; Guilhot, C. A polyketide synthase catalyzes the last condensation step of mycolic acid biosynthesis in mycobacteria and related organisms. *Proc. Natl. Acad. Sci. U S A* **2004**, *101*, 314-319.

22. Gavalda, S.; Bardou, F.; Laval, F.; Bon, C.; Malaga, W.; Chalut, C.; Guilhot, C.; Mourey, L.; Daffe, M.; Quemard, A. The polyketide synthase Pks13 catalyzes a novel mechanism of lipid transfer in mycobacteria. *Chem. Biol.* **2014**, *21*, 1660-1669.

23. Gavalda, S.; Leger, M.; van der Rest, B.; Stella, A.; Bardou, F.; Montrozier, H.; Chalut, C.; Burlet-Schiltz, O.; Marrakchi, H.; Daffe, M.; Quemard, A. The Pks13/FadD32 crosstalk for

the biosynthesis of mycolic acids in Mycobacterium tuberculosis. *J. Biol. Chem.* **2009**, *284*, 19255-19264.

24. Wilson, R.; Kumar, P.; Parashar, V.; Vilcheze, C.; Veyron-Churlet, R.; Freundlich, J. S.; Barnes, S. W.; Walker, J. R.; Szymonifka, M. J.; Marchiano, E.; Shenai, S.; Colangeli, R.; Jacobs, W. R., Jr.; Neiditch, M. B.; Kremer, L.; Alland, D. Antituberculosis thiophenes define a requirement for Pks13 in mycolic acid biosynthesis. *Nat. Chem. Biol.* **2013**, *9*, 499-506.

25. Thanna, S.; Knudson, S. E.; Grzegorzewicz, A.; Kapil, S.; Goins, C. M.; Ronning, D. R.; Jackson, M.; Slayden, R. A.; Sucheck, S. J. Synthesis and evaluation of new 2-aminothiophenes against Mycobacterium tuberculosis. *Org. Biomol. Chem.* **2016**, *14*, 6119-6133.

26. Lehmann, J.; Cheng, T. Y.; Aggarwal, A.; Park, A. S.; Zeiler, E.; Raju, R. M.; Akopian, T.; Kandror, O.; Sacchettini, J. C.; Moody, D. B.; Rubin, E. J.; Sieber, S. A. An antibacterial beta-lactone kills Mycobacterium tuberculosis by disrupting mycolic acid biosynthesis. *Angew. Chem. Int. Ed. Engl.* **2018**, *57*, 348-353.

27. Ioerger, T. R.; O'Malley, T.; Liao, R.; Guinn, K. M.; Hickey, M. J.; Mohaideen, N.; Murphy, K. C.; Boshoff, H. I.; Mizrahi, V.; Rubin, E. J.; Sassetti, C. M.; Barry, C. E., 3rd;

Sherman, D. R.; Parish, T.; Sacchettini, J. C. Identification of new drug targets and resistance mechanisms in Mycobacterium tuberculosis. *PLoS One* **2013**, *8*, e75245.

28. Aggarwal, A.; Parai, M. K.; Shetty, N.; Wallis, D.; Woolhiser, L.; Hastings, C.; Dutta, N. K.; Galaviz, S.; Dhakal, R. C.; Shrestha, R.; Wakabayashi, S.; Walpole, C.; Matthews, D.; Floyd, D.; Scullion, P.; Riley, J.; Epemolu, O.; Norval, S.; Snavely, T.; Robertson, G. T.; Rubin, E. J.; Ioerger, T. R.; Sirgel, F. A.; van der Merwe, R.; van Helden, P. D.; Keller, P.; Bottger, E. C.; Karakousis, P. C.; Lenaerts, A. J.; Sacchettini, J. C. Development of a novel lead that targets M. tuberculosis polyketide synthase 13. *Cell* **2017**, *170*, 249-259 e25.

29. Zhang, W.; Lun, S.; Wang, S. H.; Jiang, X. W.; Yang, F.; Tang, J.; Manson, A. L.; Earl, A. M.; Gunosewoyo, H.; Bishai, W. R.; Yu, L. F. Identification of novel coumestan derivatives as polyketide synthase 13 inhibitors against Mycobacterium tuberculosis. *J. Med. Chem.* **2018**, *61*, 791-803.

30. Gracia, S.; Marion, C.; Rey, J.; Popowycz, F.; Pellet-Rostaing, S.; Lemaire, M. Enantioselective straightforward access to benzo[b]thiophene analogs of Azatoxin. *Tetrahedron Lett.* **2012**, *53*, 3165-3168.

31. Chen, K.; Kuo, S. C.; Hsieh, M. C.; Mauger, A.; Lin, C. M.; Hamel, E.; Lee, K. H. Antitumor agents .174. 2',3',4',5,6,7-substituted 2-phenyl-1,8-naphthyridin-4-ones: Their synthesis, cytotoxicity, and inhibition of tubulin polymerization. *J. Med. Chem.* **1997**, *40*, 2266-2275.

32. Collins, L.; Franzblau, S. G. Microplate alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against Mycobacterium tuberculosis and Mycobacterium avium. *Antimicrob. Agents Chemother.* **1997**, *41*, 1004-1009.

33. Onajole, O. K.; Pieroni, M.; Tipparaju, S. K.; Lun, S.; Stec, J.; Chen, G.; Gunosewoyo, H.; Guo, H.; Ammerman, N. C.; Bishai, W. R.; Kozikowski, A. P. Preliminary structure-activity relationships and biological evaluation of novel antitubercular indolecarboxamide derivatives against drug-susceptible and drug-resistant Mycobacterium tuberculosis strains. *J. Med. Chem.* **2013**, *56*, 4093-4103.

34. Baell, J. B.; Holloway, G. A. New substructure filters for removal of pan assay interference compounds (PAINS) from screening libraries and for their exclusion in bioassays. *J. Med. Chem.* **2010**, *53*, 2719-2740.

35. Govindachari, T. R.; Nagarajan, K.; Pai, B. R. 126. Chemical examination of Wedelia calendulacea. Part I. Structure of wedelolactone. *J. Chem. Soc.* **1956**, 629-632.

36. Micheli, R. A.; Booth, A. N.; Livingston, A. L.; Bickoff, E. M. Coumestrol, plant phenolics, and synthetic estrogens: a correlation of structure and activity. *J. Med. Pharm. Chem.* **1962**, *5*, 321-335.

37. Rasool, N.; Khan, A. Q.; Ahmad, V. U.; Malik, A. A benzoquinone and a coumestan from Psoralea-Plicata. *Phytochemistry* **1991**, *30*, 2800-2803.

38. Ioerger, T. R.; Koo, S.; No, E. G.; Chen, X.; Larsen, M. H.; Jacobs, W. R., Jr.; Pillay, M.; Sturm, A. W.; Sacchettini, J. C. Genome analysis of multi- and extensively-drug-resistant tuberculosis from KwaZulu-Natal, South Africa. *PLoS One* **2009**, *4*, e7778.

39. Pieroni, M.; Tipparaju, S. K.; Lun, S.; Song, Y.; Sturm, A. W.; Bishai, W. R.; Kozikowski, A. P. Pyrido[1,2-a]benzimidazole-based agents active against tuberculosis (TB), multidrug-resistant (MDR) TB and extensively drug-resistant (XDR) TB. *ChemMedChem* **2011**, *6*, 334-342.

40. Nehybova, T.; Smarda, J.; Daniel, L.; Stiborek, M.; Kanicky, V.; Spasojevic, I.; Preisler, J.; Damborsky, J.; Benes, P. Wedelolactone acts as proteasome inhibitor in breast cancer cells. *Int. J. Mol. Sci.* **2017**, *18*, 729.

41. Nehybova, T.; Smarda, J.; Benes, P. Plant coumestans: recent advances and future perspectives in cancer therapy. *Anticancer Agents Med. Chem.* **2014**, *14*, 1351-1362.

Table of Contents Graphic