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DOI: 10.1002/cmdc.201100309

4-Substituted Thioquinolines and Thiazoloquinolines: Potent, Selective, and Tween-80 in vitro Dependent Families of Antitubercular Agents with Moderate in vivo Activity

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Two new families of closely related selective, non-cytotoxic, and potent antitubercular agents were discovered: thioquinolines and thiazoloquinolines. The compounds were found to possess potent antitubercular properties in vitro, an activity that is dependent on experimental conditions of MIC determination (resazurin test and the presence or absence of Tween-80). To clarify the therapeutic potential of these compound families, a medicinal chemistry effort was undertaken to generate a lead-like structure that would enable murine efficacy studies and help elucidate the in vivo implications of the in vitro observations. Although the final compounds showed only limited levels of systemic exposure in mice, modest levels of efficacy in vivo at nontoxic doses were observed.

Introduction

Despite the availability of treatments for tuberculosis (TB), the threat this disease represents is still a painful reality for the ten million people infected and the two million that die each year.[1] TB also represents an escalating threat to global health, with an increased prevalence of multidrug-resistant (MDR) TB strains, which are resistant to at least the two main first-line TB drugs isoniazid and rifampicin, and extremely drug-resistant (XDR) TB strains, which are also resistant to three or more of the six classes of second-line drugs.

In some eastern European/central Asian countries (EECAC) such as Azerbaijan, MDR/XDR strains can account for up to 22% of infections,[2] with mortality rates for XDR reaching up to 100% of those infected.[3] While these numbers are partially attributable to the misuse of current antitubercular agents, they are also a direct consequence of the nature of the treatment: a combination of at least three different drugs that must be taken for six months or longer. Due to side effects and the length of treatment, patients often discontinue therapy, with a consequent rise in drug-resistant strains and infection relapse cases. This has lead to a call by the World Health Organization (WHO) for the widespread implementation of directly observed treatment short-course (DOTS), in which treatment compliance is monitored by healthcare workers. Although this approach has been successful where appropriately implemented (cure rates of $>90\%$), the development of a new drug for the treatment of TB could still be the most cost-effective way of tackling the potential pandemic. Specifically, any new drug should be able to: 1) shorten the duration of treatment, 2) avoid any significant drug–drug interactions with current regimens, 3) treat MDR as well as XDR TB patients (new mode of action), and 4) be competitive in terms of cost with current drugs.

We and others have previously reported the antitubercular activity of various purine,[4] pyrazolopyrimidine,[5] and benzimidazole thiol ethers[6] (Figure 1). Herein we report our expansion into a similar and as yet unreported new family of quinoline thiol ethers endowed with potent antitubercular activity, pronounced selectivity for *Mycobacterium tuberculosis* $H_{37}Rv$ versus

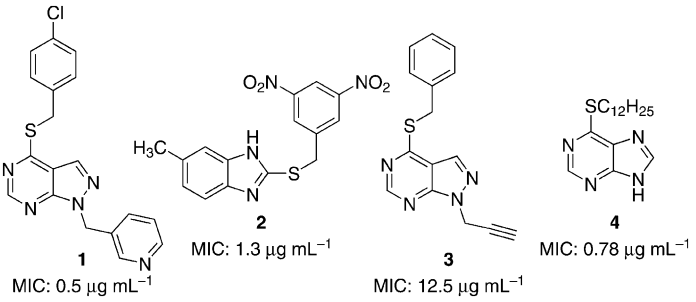

Figure 1. Previously reported thiol ethers with antitubercular activity.

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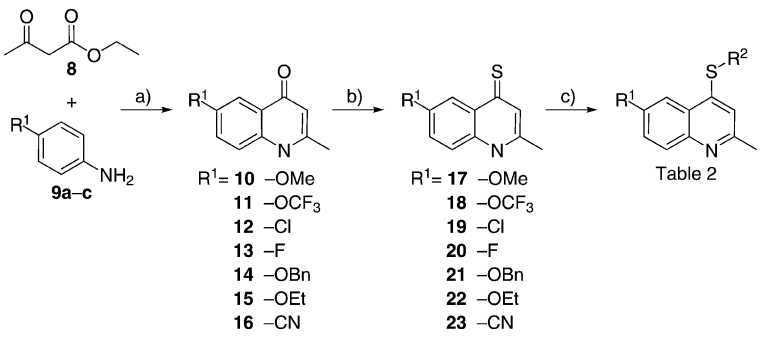
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cmdc.201100309.

other bacteria, and ample safety windows in terms of cytotoxic potential (compounds **5**, **6**, and **7**).

Results and Discussion

As part of our continuous anti-mycobacterial screening activities, three new antitubercular compounds were identified (Table 1). The antibacterial profiles of these compounds were established by determining the minimum inhibitory concentration (MIC_{80}) against *M. tuberculosis* $H_{37}Rv$, other members of the *Mycobacterium* genus (*M. bovis BCG* and *M. smegmatis*), as well as a panel of other bacterial species (*Staphylococcus aureus Oxford*, *Enterococcus faecalis*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Streptococcus pneumoniae*, and *Escherichia coli*). Additionally, both the cytotoxicity potential of the compounds in HepG2, MDCK, L6, and CHO cell lines as well as the possible genotoxicity liabilities toward *S. typhimurium* were determined.

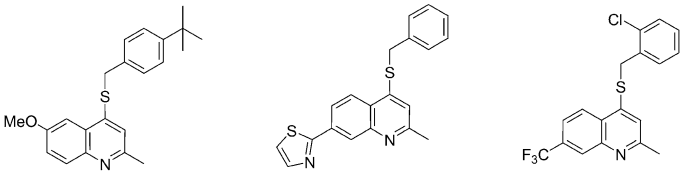
The compounds were shown to be selective anti-mycobacterials and were found to be endowed with the capacity to differentiate between fast-growing (*M. smegmatis*) and slow-growing (*M. tuberculosis* $H_{37}Rv$ and *M. bovis BCG*) mycobacteria. This evidence prompted us to initiate a medicinal chemistry effort to further explore and optimize the antitubercular potential of the hit structures. With this aim in mind and a focus on compound **5**, a straightforward synthetic sequence was devised to gain access to a number of analogues (Scheme 1).



Scheme 1. Synthetic sequence to 4-substituted thioquinolines. *Reagents and conditions:* a) AcOH, toluene, reflux, 2 h, then Ph_2O, 240 °C, 1 h; b) P_4S_{10}, pyridine, reflux, 5 h; c) R^2Br, K_2CO_3, DMF, RT, 2 h.

The acid-catalyzed cyclization–condensation between anilines **9a**–**c** and acetoacetate **8** yielded a range of 2-methyl 6-substituted quinolones **10**–**16**. Phosphorus pentasulfide mediated thionation of the cyclized compounds yielded thioquinolones **17**–**23**. Further S-alkylation with a range of alkyl halides yielded the final 4-thioquinoline products (Table 2). The choice of R^2 group was directly related to our previous work on thiopyrazolo[3,4-*d*]pyrimidines,[5] while only R groups that tolerate the harsh cyclization conditions were introduced. SAR analysis showed how a range of lipophilic *para*-substituted benzylic groups were tolerated, with a preference for methyl (**25**), *tert*-butyl (**5**), trifluoromethyl (**24**), and chloro (**26**) substitution patterns. With regard to the R^1 position, only methoxy

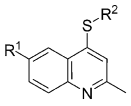
Table 1. New antitubercular quinoline thiol ether screening hits.[a,b]

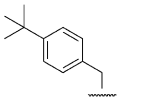
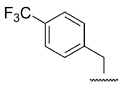
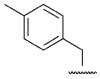
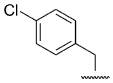
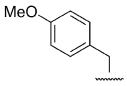
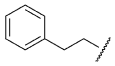
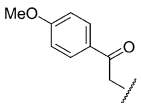
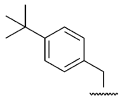
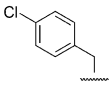
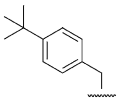
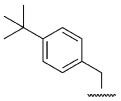
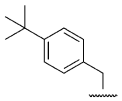
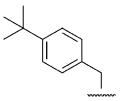
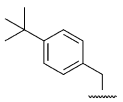


Isolate	Amoxicilin	Mupirocin	**5**	**6**	**7**
Anti-mycobacterial activity [MIC_{80}, $\mu g\,mL^{-1}$]:					
M. tuberculosis $H_{37}Rv$	–	–	<0.1	0.6	1.2
M. bovis BCG	–	–	<0.1	0.3	0.6
M. smegmatis	–	–	>32	>32	>32
Cytotoxicity [IC_{50}, $\mu g\,mL^{-1}$]:[c]					
HepG2	–	–	>31	>64	>31
MDCK	–	–	>31	>64	>31
L6	–	–	>31	>64	>31
CHO	–	–	>31	>64	>31
Antibacterial activity [MIC_{90}, $\mu g\,mL^{-1}$]:					
S. aureus Oxford	0.125	0.125	>64	>64	>64
E. faecalis	0.25	32	>64	>64	>64
H. influenzae	0.25	≤ 0.06	>64	>64	>64
M. catarrhalis	≤ 0.06	0.25	16	8	4
S. pneumoniae	2	0.125	>64	>64	>64
E. coli	4	1	>64	>64	>64
Genotoxicity:					
S. typhimurium	None at 16 μM	None at 16 μM	None at 16 μM	None at 16 μM	None at 16 μM
plus S9 preparation	None at 16 μM	None at 16 μM	None at 16 μM	None at 16 μM	None at 16 μM

[a] Data represent the mean values of at least three independent experiments. [b] MIC determinations for reference compounds rifampicin and isoniazid were performed. [c] Selected cell lines: HepG2, human caucasian hepatocyte carcinoma; MDCK, canine kidney; L6, rat skeletal muscle myoblast; CHO, Chinese hamster ovary.

Table 2. Antitubercular activity of a 4-thioquinoline substituted array.[a]



Compd	R^1	R^2	MIC_{80} [μg mL^{-1}] *M. tub. $H_{37}Rv$*	MIC_{80} [μg mL^{-1}] *M. smegmatis*	*Cli* [mL min^{-1} g^{-1}] Mouse	*Cli* [mL min^{-1} g^{-1}] Human	CHI log *D*[b]	Albumin binding [%]
5	OMe		0.01	>32	>30	1.3	5.1	98.5
24	OMe		0.06	>5	>30	–	4.3	–
25	OMe		0.03	>5	>30	–	4.3	97.7
26	OMe		0.1	>5	>30	–	4.3	97.8
27	OMe		0.1	>5	>30	–	3.7	97.3
28	OMe		0.3	>5	>30	–	3.8	97.5
29	OMe		0.6	>5	>30	–	4.1	97.7
30	OMe		>5	>5	>30	–	–	–
31	OMe		1.25	>5	>30	14.7	–	–
32	OCF_3		>5	>5	11	–	5.3	98.7
33	OCF_3		2.5	>5	23.9	–	5.1	98.2
34	Cl		0.1	>5	>30	3	5.94	98.5
35	F		0.3	>5	>30	1.1	5.3	98.3
36	OBn		>5	>5	–	–	5.8	98.7
37	OEt		2.5	>5	–	–	–	–
38	CN		>5	>5	–	–	–	–
INH[c]		–	0.25	–	–	–	–	–
RIF[d]		–	0.016	–	–	–	–	–

[a] Data represent mean values of at least three different experiments. [b] At pH 7.4. [c] Isoniazid. [d] Rifampicin.

and chloro groups were tolerated, with dramatic differences in terms of antitubercular activity stemming from subtle modifications (compounds **5** versus **32**; Table 2).

While the exquisite anti-mycobacterial selectivity profile of the compounds was maintained (MIC against *M. smegmatis*: $>5\ \mu g\,mL^{-1}$), and slow clearance was observed in human microsomal fractions, the nature of the available in vivo murine infectious TB model[7] made us focus our efforts toward improving the rapid clearance observed in mouse microsomal fractions (*Cli* $>30\ mL\,min^{-1}\,g^{-1}$, Table 2). Mapping the possible structural sources of the instability indicated that the 6-methoxy substitution makes an important contribution to fast microsomal clearance (compound **32** versus **5**). Compound **30** shows how the hypothetical alkylating potential of the α-S-benzylic position does not appear to contribute greatly to the instability, owing to the protective nature of the α-S-methyl substitution toward nucleophilic attack; compound **31** confirms this observation. Similarly, some contribution from the *para*-benzylic position was envisaged when comparing compounds **33** and **32**.

The presence of the thioether 4-quinoline substitution pattern proved to be a prerequisite for anti-mycobacterial activity. Both ether **39**, prepared from **10** by reaction with 4-*tert*-butylbenzyl bromide, and sulfoxide **40**, prepared by *meta*-chloroperoxybenzoic acid (MCPBA)-mediated oxidation of thioether **5**, were devoid of antitubercular properties (Table 3).

Further evaluation of other important drug discovery parameters such as lipophilicity and protein binding indicated that the molecules lie in what is generally considered to be borderline drug-like space.[8] This highlights the need to direct medicinal chemistry activities toward decreasing the lipophilic character of lead molecules in order to avoid the already known associated poor bioavailability and solubility implications of working with drug leads with high clog *P* values.

As part of the lead confirmation process, antitubercular activity was evaluated under a variety of conditions, including growth inhibition studies in solid *$H_{37}Rv$* 7H10-OADC media in the absence and presence of Tween-80, a surfactant commonly employed in *M. tuberculosis* *$H_{37}Rv$* cultures to avoid bacterial aggregation, which affects estimation of bacterial growth. MIC determinations with compound **5** showed a remarkable dependence on the presence of Tween-80, with shifts in MIC values from $<0.1\ \mu g\,mL^{-1}$ to $>8\ \mu g\,mL^{-1}$ in both *M. tuberculosis* and *M. bovis BCG* (Figure 2). Although the simplest possible explanation for this variation in MIC values could be attributed to the permeability-enhancing properties of Tween-80,[9] only modest increases have been shown previously,[10] and this would be the first report in which a shift of two orders of magnitude was found in comparing MIC values in the absence and presence of Tween-80. Additionally, compound solubility was shown to be independent of the presence of Tween-80 (data not shown).

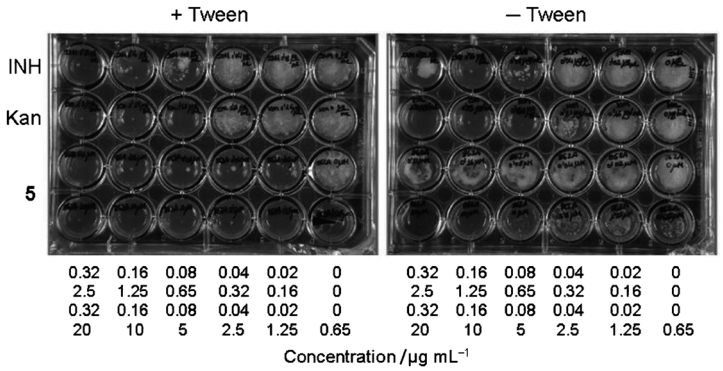


Figure 2. *M. bovis BCG* MIC values for compound **5** in solid media with or without Tween-80, with isoniazid (INH) and kanamycin (Kan) as references.

Alternatively, recent reports have shown how *Mycobacterium* spp. can degrade Tween-80, the by-products of which can be used downstream in lipid metabolism as carbon sources to support growth of actively growing and non-replicating mycobacteria.[11] In fact, a number of lipases and cutinases have been found in the *M. tuberculosis* genome that could potentially employ Tween-80 as a substrate. This hypothesis has already been confirmed for a number of cutinases and mycobacterial phospholipases.[12] This inherent adaptability of *M. tuberculosis* to a variety of carbon sources could therefore be related to the observed impact of Tween-80 on MIC determination. Moreover, on closer inspection and after having carefully analyzed the growth inhibition curves (Figure 3), the compounds showed a concentration-dependent biphasic growth inhibition behavior in the presence of Tween-80. Although effective bacterial inhibition at low concentrations ($0.05\ \mu g\,mL^{-1}$) of compound **5** was observed up to ~80% inhibition, much higher concentrations ($>1\ \mu g\,mL^{-1}$) were required to achieve >95% growth inhibition. This observation is a consequence of the method employed for MIC determination (resazurin reduction, see Supporting Information), in which an erratic color change

Table 3. Antitubercular activity of O, S, and S=O 4-substituted quinolines.

Isolate	**5**	**39**	**40**
Anti-mycobacterial activity [MIC_{80}, $\mu g\,mL^{-1}$]:[a]			
M. tuberculosis *$H_{37}Rv$*	0.01	3.7	>10

[a] Data represent the mean values of at least three independent experiments.

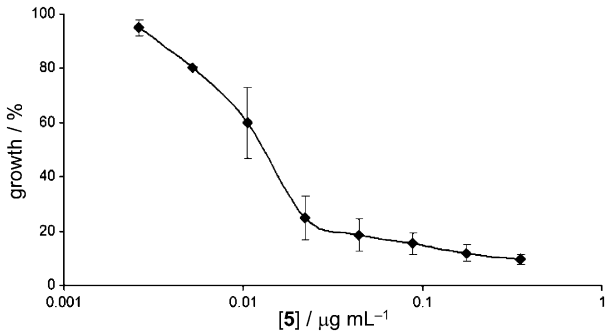
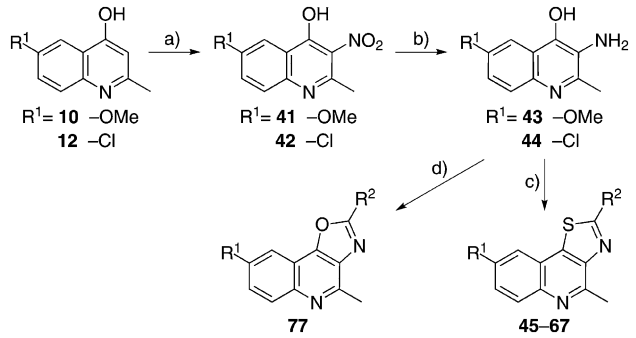


Figure 3. Grown inhibition curve for compound **5**.



Scheme 2. Synthetic sequence to thiazoloquinoline analogues **45**–**67** and oxazoloquinoline **77**. *Reagents and conditions:* a) HNO_3, propionic acid, 110 °C; b) Fe, NH_4Cl, $EtOH/H_2O$, reflux; c) R^2CO_2H, DIPEA, PyBOP, DMF, then P_4S_{10}, pyridine, 80 °C; d) R^2CO_2H, DIPEA, PyBOP, DMF, then P_2O_5, pyridine, reflux.

indicating 80–90% inhibition can occur over a number of inhibitor dilutions, masking the MIC determination.

Although this inhibitor-concentration-dependent phenotype has been previously shown for fluconazole (also known as the *trailing* effect),[13] this is the first report in which such behavior was observed for a novel family of antitubercular agents, and it remains unclear what the implications might be for the therapeutic value of such an observation for the compound family involved. To address this issue, compound **5** was progressed to in vivo murine pharmacokinetic (PK) evaluation (Table 4). The compound showed high clearance and insufficient exposure levels, making it unsuitable for an in vivo proof-of-concept evaluation.

Table 4. PK parameters determined for compound **5**.[a]

$t_{1/2}$ [h]	c_{max} [μg mL^{-1}][b]	c_{last} [μg mL^{-1}][c]	$AUC_{(0-t\,h)}$ [μg h mL^{-1}]	V_d [L kg^{-1}]	*Cli* [mL min^{-1} g^{-1}]
0.90	0.35	0.004	0.26	9.66	0.124

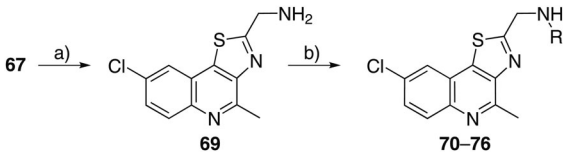
[a] Data represent the mean values of at least four independent experiments after intravenous administration to C57BL/6 mice at a dose of 2 mg kg^{-1} as a solution in encapsine 20%. [b] t_{max} = 15 min. [c] t_{last} = 4 h.

In light of this evidence, further medicinal chemistry efforts led to the discovery of the 4-methyl[1,3]thiazolo[4,5-*c*]quinoline core structure as an opportunity to overcome the rapid clearance observed in mice microsomal fractions. The thiazoloquinoline potentially stabilizes the most obvious metabolic liability, that is, the thioether functionality, in theory rendering an improved PK profile over the previous scaffold.

Compounds were prepared by following the synthetic sequence shown in Scheme 2. Nitration and subsequent iron/ammonium chloride reduction of 2-methyl-6-substituted quinolones **10** and **12** yielded the corresponding 3-aminoquinolones **43**–**44**. PyBOP-assisted coupling of 3-aminoquinolones with an array of carboxylic acids followed by thionation/dehydration with phosphorus pentasulfide led to assembly of the thiazole ring and formation of the required substituted thiazoloquinolines **45**–**67**.

The anti-mycobacterial activities of these compounds were evaluated and are listed in Table 5; the MIC values showed the same dependence on Tween-80. With regard to the R^1 position, chloro substitution was slightly preferred over methoxy substitution (compounds **45** versus **46**). An exploration of the chemical space around R^2 seems to indicate the preference of benzyl groups (**45**–**55**) over other functional groups such as pyridinylmethyl (**57**–**59**), pyrimidinylmethyl (**60**), indolylmethyl (**61**), 3-oxo-1,2,3,4-tetrahydroquinoxalinylmethyl (**62**), benzofuran (**64**), and phenoxymethyl (**65**–**66**) groups. In addition, SAR analysis of the *para* position of the benzyl ring confirms the preference toward more lipophilic substituents such as methyl, *tert*-butyl, trifluoromethyl, and chloro groups (**46**–**51**) in contrast to more hydrophilic moieties, such as acetamido, methylsulfonyl, and amino functional groups (**52**–**54**). The presence of a methylene group spacer in R^2 seems to be essential for anti-mycobacterial activity (compounds **47** versus **63**). Furthermore, compound **56**, which has a hydroxy group at the benzylic position, and compound **68**, in which in the methylene is replaced by an amino group, lack the activity found in their previous analogous, **49** and **51**, respectively.

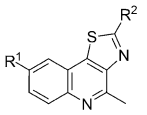
A small subset of N-substituted 8-aminomethyl thiazoloquinolines was then prepared by cleavage of *N*-Boc-protected thiazoloquinoline **67** and consecutive reductive amination with several aromatic and heteroaromatic aldehydes (Scheme 3). None of these secondary amines **70**–**76**, or the parent compound **69** showed any significant activity against *M. tuberculosis* $H_{37}Rv$ (Table 5).

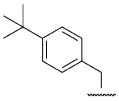
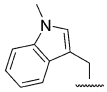
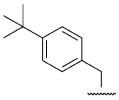
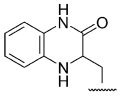
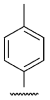
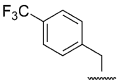
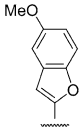
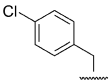
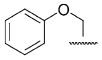
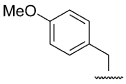
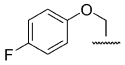
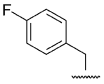
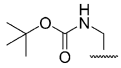
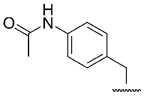
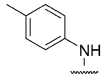
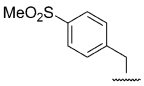
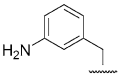
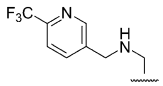
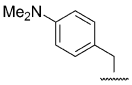
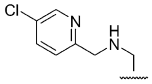
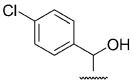
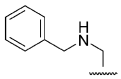
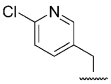
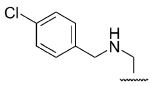
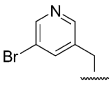
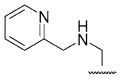
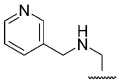
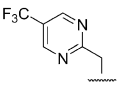
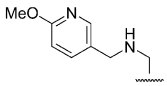


Scheme 3. Synthetic sequence to **71**–**78**. *Reagents and conditions:* a) HCl (4 M), dioxane, RT; b) aldehyde, $NaBH(OAc)_3$, RT.

Next, we focused our attention on the thiazoloquinoline ring. Several modifications in the core structure were envisaged to discern if this particular configuration is essential for

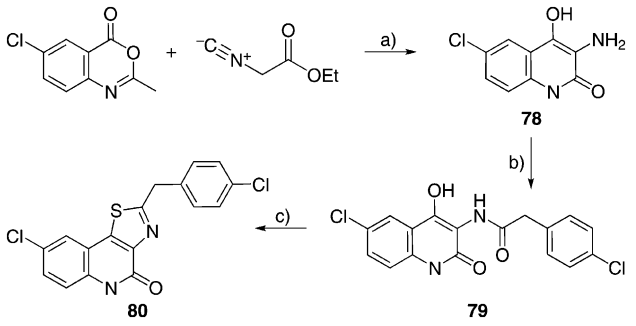
Table 5. Antitubercular activity of a 4-methyl[1,3]thiazolo[4,5-*c*]quinoline substituted array.



Compd	R^1	R^2	MIC_{80} [$\mu g\,mL^{-1}$][a]	Compd	R^1	R^2	MIC_{80} [$\mu g\,mL^{-1}$][a]
45	OMe		0.024	**61**	Cl		>4
46	Cl		0.035	**62**	Cl		>4
47	Cl		0.055	**63**	Cl		>30
48	Cl		0.035	**64**	Cl		>4
49	Cl		0.035	**65**	Cl		>4
50	Cl		0.035	**66**	Cl		>4
51	Cl		0.022	**67**	Cl		>2
52	Cl		>4	**68**	Cl		>2
53	Cl		>4	**69**	Cl		>1
54	Cl		3.4	**70**	Cl		>2
55	Cl		0.1	**71**	Cl		>4
56	Cl		>4	**72**	Cl		3.5
57	Cl		0.2	**73**	Cl		2.9
58	Cl		2	**74**	Cl		>4
59	Cl		1.6	**75**	Cl		>4
60	Cl		>4	**76**	Cl		>4

[a] Determined toward *M. tuberculosis* $H_{37}Rv$; data represent the mean values of at least three independent experiments.

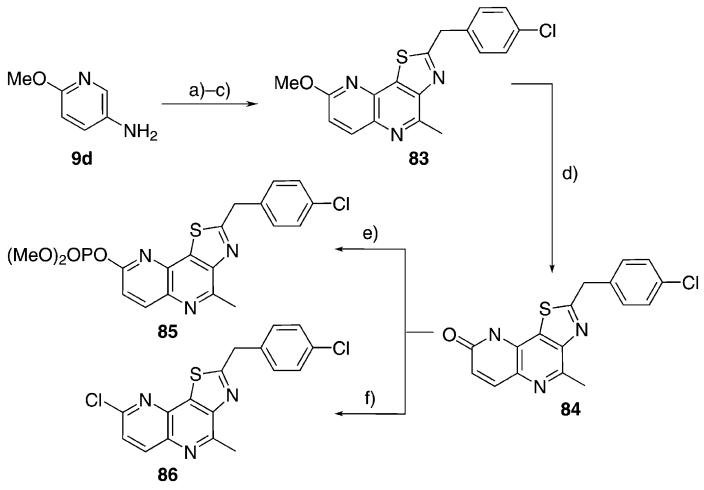
anti-mycobacterial activity. Thus, oxazoloquinoline **77** was prepared from **44** through a similar approach as its parent compound **49**, but in this case with phosphorus pentoxide as the cyclization/dehydration agent (Scheme 2). Moreover, thiazoloquinolinone **80** was prepared from commercially available 6-chloro-2-methyl-4*H*-3,1-benzoxazin-4-one in three consecutive steps (Scheme 4).



Scheme 4. Synthetic sequence to thiazoloquinolinone **80**. *Reagents and conditions:* a) DBU, THF, RT, overnight, then HCl, MeOH, reflux, 3 h; b) 4-ClPhCH$_2$CO$_2$H, DIPEA, PyBOP, DMF, RT, 2 h; c) KOH, Me$_2$NCSCl, MeOH, RT, 1 h, then BF3·OEt, CH$_2$Cl$_2$, MW, 110 °C, 10 min.

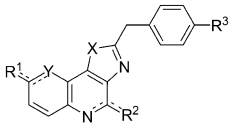
Several thiazolonaphthyridines were then synthesized by the sequence illustrated in Scheme 5. Briefly, compound **83** was prepared by cyclization–condensation between 5-amino-2-methoxypyridine (**9d**) and acetoacetate, subsequent nitration and reduction with iron/ammonium chloride, coupling with the corresponding carboxylic acid, and thionation/cyclization with phosphorus pentasulfide. Treatment with hydrogen bromide gave thiazolonaphthyridinone **84**, which could be then phosphorylated to **85** or halogenated to **86** with phosphoryl chloride using different conditions. The antitubercular activity of these compounds is listed in Table 6.

As is the case for 4-thioquinolines, subtle substitutions in the core structure translate into dramatic changes in anti-



Scheme 5. Synthetic sequence to thiazolonaphthyridines **83**–**86**. *Reagents and conditions:* a) **8**, AcOH, toluene, reflux, 2 h, then Ph_2O, 240 °C; b) HNO_3, propionic acid, 110 °C; c) Fe, NH_4Cl, EtOH/H_2O, reflux, then 4-ClPhCH$_2$CO$_2$H, DIPEA, PyBOP, DMF, then P_4S_{10}, pyridine, 80 °C; d) HBr, AcOH, 100 °C; e) $POCl_3$, 90 °C, then MeOH; f) $POCl_3$, MW, 160 °C.

Table 6. Antitubercular activity of compounds **77**, **80**, and **83**–**86**.



Compd	X	Y	R^1	R^2	R^3	MIC_{80} [μg mL^{-1}][a]
77	O	CH	Cl	Me	Me	>4
80	S	CH	Cl	O	Cl	>4
83	S	N	MeO	Me	Cl	0.9
84	S	N	O	Me	Cl	>4
85	S	N	$(MeO)_2OPO$	Me	Cl	>4
86	S	N	Cl	Me	Cl	>4

[a] Determined toward *M. tuberculosis* $H_{37}Rv$; data represent the mean values of at least three independent experiments.

mycobacterial activity. Thus, sulfur atom replacement for oxygen in oxazoloquinoline **77** led to a significant decrease in potency (compound **77** versus **47**) as previously observed for the thioquinoline family, or the presence of naphthyridine instead of quinoline ring (compound **83** versus **49**).

Compound **48** was selected as a lead candidate for in vivo murine efficacy studies. Although compound **45**, with a methoxy group, was found to have higher potency than compound **46**, the murine microsomal fraction clearance value strongly favors the chloro substitution pattern found in compound **48** (data not shown). Under our experimental conditions, the oral administration of **48** does not exhibit acute toxicity >1000 mg kg^{-1} for 1 day in mice. No significant variations in body weight, necropsy, clinical chemistry, or hematology values were observed. With this data in hand, the oral PK profile of the compound at 100 and 300 mg kg^{-1} was then established. Despite the poor absorption (Figure 4), an event most likely linked to low solubility in aqueous media, the compound was further progressed to evaluation for in vivo murine anti-TB efficacy.[7] The compound showed a 1.5-log decrease in mycobacterial burden (Figure 5) with respect to untreated controls. We believe this result, when put in the context of the very modest exposure levels achieved in mice and of the fact that AUC values at 100 and 300 mg kg^{-1} were found to be similar, leaves room for further optimization. Future synthetic efforts will be focused on the optimization of physicochemical parameters (clog *P* and solubility).

Conclusions

In summary, two new families of closely related compounds (thioquinolines and thiazoloquinolines) with potent antitubercular activity have been presented, and further work is necessary to optimize the physicochemical properties of the compounds in order to obtain leads with improved in vivo DMPK properties. The compounds are endowed with Tween-80 in vitro dependent anti-mycobacterial activity, an observation that does not translate into a complete lack of anti-

target dose [mg kg^{-1}]	t_{max} [h]	c_{max} [mg mL^{-1}]	c_{last} [mg mL^{-1}]	$AUC_{(0-t\,h)}$ [μg h mL^{-1}]
100	1	0.64	0.014	7.54
300	1	0.84	0.016	9.99

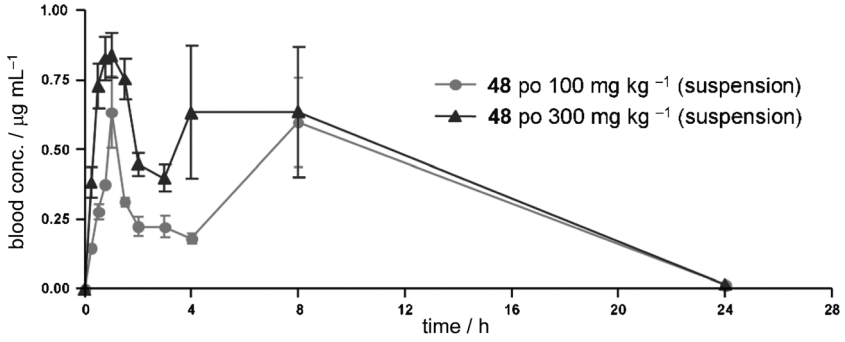


Figure 4. Peripheral blood levels of compound **48** after oral administration to C57BL/6 mice at doses of 100 and 300 mg kg^{-1}, as a suspension in 1% methyl cellulose ($n=4$); $t_{last}=24$ h.

compd	target dose [mg kg^{-1}]	exptl dose [mg kg^{-1}]	admin.	route	log decrease in lung CFU counting	p
INH	25	27	once a day	oral	>4.754	<0.001
48	300	270	once a day	oral	1.396	<0.001
48	300	270	twice a day	oral	1.533	<0.001

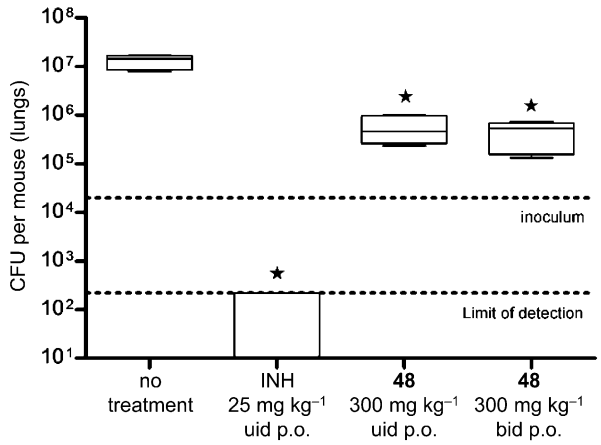


Figure 5. Antitubercular activity of **48** in a murine model of acute infection, with isoniazid (INH) as reference. Mice were infected with 10^5 CFU, and lung homogenates were obtained 9 days after infection ($n=5$ mice per group). Data were analyzed by one-factor ANOVA and Tukey's HSD post hoc multiple comparison test; $p<0.05$ was considered significant. The boxes extend from the 25th to the 75th percentile, with a line at the median; error bars indicate the highest and lowest values observed.

tubercular efficacy in vivo; the compounds present an in vitro biphasic concentration-dependent antitubercular behavior. Further studies are currently ongoing that will help elucidate the therapeutic potential of this compound family.

Experimental Section

Chemistry

General: All commercially available reagents and solvents were used without further purification unless otherwise stated. Automated flash chromatography was performed on a Biotage FlashMaster II system with peak detection at 254 nm. All products were obtained as amorphous solids, and melting points were not measured. ^{1}H NMR spectra were recorded at 300 MHz on a Varian spectrometer. Chemical shifts (δ) are given in ppm relative to the solvent reference as an internal standard ($CDCl_3$, $\delta=7.24$ ppm; $[D_6]DMSO$, $\delta=2.50$ ppm; CD_3OD, $\delta=3.31$ ppm). Data are reported as follows: chemical shift (multiplicity (s for singlet, d for doublet, t for triplet, q for quartet, sept for septet, m for multiplet, br broad), coupling constant [Hz], integration). HPLC–MS analyses were conducted on an Agilent 1100 instrument equipped with a Sunfire C_{18} column (30 mm × 2.1 mm i.d., 3.5 μm packing diameter) at 40 °C coupled with a Waters ZMD2000 mass spectrometer; the method of ionization was alternate-scan positive and negative electrospray. Most of the compounds used in biological testing had purity of >98%, as determined by HPLC analysis. *Method A:* flow: 1.5 mL min^{-1}, eluents: 1% aq. HCO_2H/CH_3CN, ramp 0–0.2 min 90:10, 0.2–3.5 min 90:10→10:90, 3.5–4.0 min 10:90, 4.0–5.0 min 90:10; *Method B:* flow: 1.5 mL min^{-1}, eluents: 1% aq. HCO_2H/CH_3CN, ramp 0–0.2 min 70:30, 0.2–3.5 min 70:30→10:90, 3.5–4.0 min 10:90, 4.0–5.0 min 70:30; *Method C:* flow: 1.5 or 1.0 mL min^{-1}, eluents: aq. 0.05 M NH_4OAc/CH_3CN, ramp 0–0.2 min 90:10, 0.2–3.5 min 90:10→10:90, 3.5–4.0 min 10:90, 4.0–5.0 min 90:10. 6-Benzyloxy-2-methyl-4(1*H*)-quinolinone (**13**), 6-fluoro-2-methyl-4(1*H*)-quinolinone (**14**), 6-ethoxy-2-methyl-4(1*H*)-quinolinone (**15**), and 2-methyl-4-oxo-1,4-dihydro-6-quinolinecarbonitrile (**16**) were purchased from UkrOrgSynthesis Building Blocks; 2-[(2-methyl-6-methoxy-4-quinolinyl)thio]-1-(4-methoxyphenyl)ethanone (**31**) was purchased from Asinex. All commercially available compounds were used as received, without further purification. Representative procedures and physical properties for examples of characterized analogues are described. Characterization data for all compounds are detailed in the Supporting Information.

General procedure for the preparation of 2-methyl-4-quinolinone derivatives 10–12 and 81: AcOH (6 mL) was added to a suspension of aniline derivative (**9a–c**; 80 mmol) and ethyl acetylacetate (9.63 g, 94 mmol) in toluene (150 mL). The reaction mixture was held at reflux for 2 h with azeotropic removal of H_2O by means of a Dean–Stark apparatus. Solvent was evaporated under vacuum, the formed solid was suspended in Ph_2O (20 mL) and heated at 240 °C for 1 h. The reaction mixture was cooled to 50 °C, and warm hexanes (100 mL) was added. The formed precipitate was filtered off, washed with hexanes (3×5 mL) and dried under vacuum to yield **10–12**, which were used without further purification.

6-Methoxy-2-methyl-4(1*H*)-quinolinone (10): Yield: 12.5 g, 83%; ^{1}H NMR (300 MHz, CD_3OD): $\delta=7.61$ (d, $J=2.8$ Hz, 1H, Ar*H*), 7.48 (d, $J=9.1$ Hz, 1H, Ar*H*), 7.29 (dd, $J=9.1$ and 2.8 Hz, 1H, Ar*H*), 6.19 (s, 1H, Ar*H*), 3.88 (s, 3H, CH_3), 2.44 ppm (s, 3H, CH_3); MS (ES^+): *m/z* (%): 109.1 (100) $[M+H]^+$.

General procedure for the thionation of 2-methyl-4-quinolinone derivatives 10–16: P_4S_{10} (888 mg, 2 mmol) was added in one portion to a suspension of 2-methyl-4-quinolinone (**10**–**16**; 2 mmol) in dry pyridine (25 mL). The reaction mixture was heated for 5 h at reflux with stirring. The resulting solution was cooled in an ice bath, and H_2O (5 mL) was added. The mixture was concentrated to 10 mL, and H_2O (10 mL) was added to the resulting crude. The formed precipitate was filtered off, washed with H_2O (3×3 mL), and dried under vacuum to yield pure products **17**–**19**, **22**, and **23**.

6-Methoxy-2-methyl-4(1*H*)-quinolinethione (17): Yield: 400 mg, 98%; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 12.03 (br s, 1H, N*H*), 7.40 (d, J = 2.7 Hz, 1H, Ar*H*), 6.75 (d, J = 9.1 Hz, 1H, Ar*H*), 6.63 (s, 1H, Ar*H*), 6.57 (dd, J = 9.1 and 2.8 Hz, 1H, Ar*H*), 3.13 (s, 3H, CH_3), 1.68 ppm (s, 3H, CH$_3$); MS (ES$^+$): *m/z* (%): 205.1 (100) $[M+H]^+$.

General procedure for the alkylation of 2-methyl-4-quinolinethione derivatives 17–23: The appropriate alkyl bromide (1.5 equiv) was added to a suspension of 2-methyl-4(1*H*)-quinolinethione (**17**–**23**; 1 equiv) and K_2CO_3 (5 equiv) in DMF (2 mL mmol^{-1}), and the reaction was stirred for 2 h at room temperature. The reaction mixture was quenched by the addition of aqueous NH_3 (28%, 3 mL). An aqueous solution of NH_4Cl (1 N, 75 mL) was added, and the formed precipitate was filtered off and washed with H_2O (3× 10 mL). The solid was purified by column chromatography to obtain pure products **5**, **24**–**30**, **32**–**34**, and **37**–**38**.

4-{[(4-*tert*-Butyl)phenyl]methylthio}-6-methoxy-2-methylquinoline (5): Yield: 1.1 g, 68%; ^{1}H NMR (300 MHz, CDCl$_3$): δ = 7.88 (d, J = 8.8 Hz, 1H, Ar*H*), 7.41–7.27 (m, 6H, Ar*H*), 7.12 (s, 1H, Ar*H*), 4.28 (s, 2H, CH_2), 3.89 (s, 3H, CH_3), 2.66 (s, 3H, CH_3), 1.32 ppm (s, 9H, 3× CH$_3$); MS (ES$^+$): *m/z* (%): 352.1 (100) $[M+H]^+$; LC–MS, t_R = 2.78 min, Method A.

Procedure for the synthesis of 2-methyl-4-thioquinoline derivatives 35–36: P_4S_{10} (222 mg, 0.5 mmol) was added in one portion to a suspension of 2-methyl-4-quinolinone (**13**, **14**; 0.5 mmol) in dry pyridine (5 mL). The reaction mixture was heated for 5 h at reflux with stirring. The solvent was removed, and the remaining residue was poured into H_2O (5 mL) and extracted with CH_2Cl_2 (3×10 mL). The combined organic layers were dried over $MgSO_4$. The solvent was evaporated, and LC–MS analysis of the crude was completed, confirming the presence of quinolinethiones (**20**, **21**). The resulting solid was suspended in DMF (3 mL), and K_2CO_3 (345 mg, 2.5 mmol) and the corresponding alkyl bromide (0.75 mmol) were added, and the reaction was stirred for 2 h at room temperature. The reaction mixture was quenched by the addition of aqueous NH_3 (28%, 1 mL). An aqueous solution of NH_4Cl (1 N, 10 mL) was added, and the formed precipitate was filtered off. The solid was purified by column chromatography to obtain pure products **35** and **36**.

4-[(4-*tert*-Butylphenyl)methylthio]-6-fluoro-2-methylquinoline (35): Yield: 48 mg, 28%; ^{1}H NMR (300 MHz, CDCl$_3$): δ = 7.97 (dd, J = 9.2 and 5.4 Hz, 1H, Ar*H*), 7.70 (dd, J = 10.0 and 2.8 Hz, 1H, Ar*H*), 7.44 (ddd, J = 9.1, 2.8 and 1.2 Hz, 1H, Ar*H*), 7.41–7.31 (m, 4H, Ar*H*), 7.14 (s, 1H, Ar*H*), 4.28 (s, 2H, CH_2), 2.67 (s, 3H, CH_3), 1.32 ppm (s, 9H, 3×CH_3); MS (ES$^+$): *m/z* (%): 340.1 (100) $[M+H]^+$; LC–MS, t_R = 3.13 min, Method A.

Procedure for the preparation of quinoline 39: 4-*tert*-Butylbenzyl bromide (340 mg, 1.5 mmol) was added to a suspension of 6-methoxy-2-methyl-4(1*H*)-quinolinethione (**10**; 200 mg, 1.05 mmol) and K_2CO_3 (724 mg, 5.25 mmol) in DMF (5 mL), and the reaction was stirred for 5 h at room temperature. The reaction mixture was filtered off, and the organic solution was concentrated to dryness. The solid was purified by column chromatography to obtain 4-{[(4-*tert*-butyl)phenyl]methoxy}-6-methoxy-2-methylquinoline (**39**). Yield: 60 mg, 17%; ^{1}H NMR (300 MHz, CDCl$_3$): δ = 8.74 (d, J = 8.9 Hz, 1H, Ar*H*), 7.55–7.46 (m, 4H, Ar*H*), 7.45–7.39 (m, 2H, Ar*H*), 6.85 (s, 1H, Ar*H*), 5.42 (s, 2H, CH_2), 3.94 (s, 3H, CH_3), 3.04 (s, 3H, CH_3), 1.36 ppm (s, 9H, 3×CH$_3$); MS (ES$^+$), *m/z* (%): 336.2 (74) $[M+H]^+$, 147.1 (100); LC–MS, t_R = 2.61 min, Method A.

Procedure for the preparation of sulfoxide 40: MCPBA (16 mg, 0.07 mmol) was added to a stirred suspension of compound **5** (50 mg, 0.14 mmol) in CH_2Cl_2 (5 mL), and the reaction was stirred for 1 h at 0 °C. The reaction mixture was concentrated to dryness. The solid was purified by column chromatography to obtain 4-{[(4-*tert*-butyl)phenyl]methylsulfinyl}-6-methoxy-2-methylquinoline (**40**). Yield: 12 mg, 46%; ^{1}H NMR (300 MHz, CDCl$_3$): δ = 8.03 (d, J = 9.1 Hz, 1H, Ar*H*), 7.49 (s, 1H, Ar*H*), 7.36 (d, J = 9.2 and 2.8 Hz, 1H, Ar*H*), 7.17 (d, J = 8.3 Hz, 2H, Ar*H*), 6.84 (d, J = 2.8 Hz, 1H, Ar*H*), 6.76 (d, J = 8.3 Hz, 2H, Ar*H*), 4.21 (d, J = 12.7 Hz, 1H, C*H*H), 4.13 (d, J = 12.7 Hz, 1H, CH*H*), 3.84 (s, 3H, CH_3), 2.68 (s, 3H, CH_3), 1.25 ppm (s, 9H, 3×CH$_3$); MS (ES$^+$), *m/z* (%): 368.2 (100) $[M+H]^+$; LC–MS, t_R = 2.90 min, Method A.

General procedure for the nitration of 2-methyl-6-substituted 4-quinolones 10, 12, and 81: A suspension of 2-methyl-4-quinolinol derivative (**10**, **12**, **81**; 50 mmol) in propionic acid (100 mL) was heated at 110 °C with stirring. HNO_3 (6.1 mL) was then added dropwise over 20 min. The reaction mixture was heated for 2 h at 110 °C with vigorous stirring. The resulting suspension was cooled to room temperature and filtered off, the solids were washed with cold EtOH (3×5 mL) and dried under vacuum to yield the products **41**–**42** and **82**.

6-Methoxy-2-methyl-3-nitro-4-quinolinol (41): Yield: 8.53 g, 73%; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 12.42 (br s, 1H, N*H*), 7.58 (d, J = 9.1 Hz, 1H, Ar*H*), 7.53 (d, J = 2.3 Hz, 1H, Ar*H*), 7.39 (dd, J = 9.1 and 2.9 Hz, 1H, Ar*H*), 3.85 (s, 3H, CH_3), 2.49 ppm (s, 3H, CH$_3$); MS (ES$^+$), *m/z* (%): 234.1 (100) $[M+H]^+$.

Procedure for the reduction of 2-methyl-3-nitro-4-quinolinol derivatives 41–42: A suspension of 2-methyl-3-nitro-4-quinolinol (**41**–**42**; 15 mmol) in EtOH (200 mL) was heated at reflux. Fe^0 (8.37 g, 150 mmol) and an aqueous solution of NH_4Cl (60 mL, 150 mmol, 2.5 M) were added, and the reaction was stirred at reflux to completeness. The warm mixture was filtered through a Celite patch, and the remaining solids were washed with warm EtOH (60 mL). The filtrate was concentrated to dryness and was resuspended with trituration in H_2O (40 mL). The formed precipitate was filtered off and washed with H_2O (2×10 mL) and hexanes (2× 10 mL) to yield 3-amino-2-methyl-4-quinolinol derivatives **43**–**44**.

3-Amino-6-methoxy-2-methyl-4-quinolinol (43): Yield: 1.76 g, 58%; ^{1}H NMR (300 MHz, CD$_3$OD): δ = 7.65–7.43 (m, 2H, Ar*H*), 7.28–7.18 (m, 1H, Ar*H*), 3.90 (s, 3H, CH_3), 2.50 ppm (s, 3H, CH$_3$); MS (ES$^+$), *m/z* (%): 204.1 (100) $[M+H]^+$.

General procedure for the formation of thiazoloquinoline derivatives 45–67: 3-Amino-2-methyl-4-quinolinol (**43**–**44**) and the corresponding carboxylic acid (1 equiv) were suspended in DMF (2 mL), and benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP; 1.5 equiv) and ethyldiisopropylamine (DIPEA; 3 equiv) were added. The reaction mixture was stirred for 2 h at room temperature and left to cool at 4 °C for 1 h. The resulting suspension was filtered off and washed with H_2O (3×2 mL) and hexanes (2×2 mL). The dried solid was suspended in pyridine (2 mL), and P_4S_{10} (1 equiv) was added in one portion. The mixture was heated at 80 °C for 2 h. H_2O (4 mL) was then added, and the mixture was allowed to cool to room temperature. The white pre-

cipitate was filtered off, washed with H_2O (2×2 mL) and hexane (2×2 mL) to yield the corresponding tiazoloquinoline derivatives **45**–**67**.

2-[(4-*tert*-Butylphenyl)methyl]-4-methyl-8-methoxy[1,3]thiazolo-[4,5-*c*]quinoline (45): Yield: 2.52 g, 59%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.03 (d, J = 9.1 Hz, 1H, Ar*H*), 7.45–7.34 (m, 4H, Ar*H*), 7.31 (dd, J = 9.1 and 2.8 Hz, 1H, Ar*H*), 7.06 (d, J = 2.8 Hz, 1H, Ar*H*), 4.52 (s, 2H, CH_2), 3.92 (s, 3H, CH_3), 3.08 (s, 3H, CH_3), 1.35 ppm (s, 9H, 3× CH_3); MS (ES$^+$), *m/z* (%): 377.2 (100) $[M+H]^+$; LC–MS, t_R = 3.47 min, Method B.

8-Chloro-4-methyl-2-[(4-trifluoromethylphenyl)methyl][1,3]thiazolo[4,5-*c*]quinoline (48): Yield: 372 mg, 65%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.08 (d, J = 8.6 Hz, 1H, Ar*H*), 7.81 (d, J = 2.0 Hz, 1H, Ar*H*), 7.70–7.58 (m, 3H, Ar*H*), 7.57–7.50 (m, 2H, Ar*H*), 4.60 (s, 2H, CH_2), 3.09 ppm (s, 3H, CH_3); ^{13}C NMR (100.6 MHz, [D$_6$]DMSO): δ = 191.9 (C), 171.1(C), 154.5 (C), 147.0 (C), 142.1 (C), 141.9 (C), 138.9 (C), 131.1 (CH), 131.1 (C), 130.2 (CH), 129.3 (CH), 125.7 (CH), 124.0 (CH), 123.1 (C), 67.7 (CH_2), 22.1 ppm (CH_3);MS (ES$^+$), *m/z* (%): 394.9 (43) $[M+2H]^+$, 393.0 (100) $[M+H]^+$; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{19}H_{12}ClF_3N_2S$: 393.0435, found: 393.0436; LC–MS, t_R = 3.29 min, Method C.

Procedure for the synthesis of thiazoloquinoline derivative 68: 1-Isocyanate-4-methylbenzene (96 mg, 0.71 mmol) and Et_3N (0.134 mL, 0.96 mmol) were added to a solution of 3-amino-6-chloro-2-methyl-4-quinolinol (**44**; 100 mg, 0.48 mmol) in EtOH (6 mL). The reaction mixture was stirred for 2 days at 70 °C. The solvent was partially evaporated (1 mL), and the resulting suspension was filtered off and washed with hexanes (3×4 mL). The dried solid was suspended in pyridine (4 mL), and P_4S_{10} (197 mg, 0.44 mmol) was added in one portion. The mixture was heated at reflux for 2 h. H_2O (4 mL) was then added, and the mixture was allowed to cool to room temperature. The white precipitate was filtered off, washed with H_2O (2×2 mL) and hexane (2×2 mL). The solid was purified by preparative HPLC to yield 8-chloro-4-methyl-*N*-(4-methylphenyl)[1,3]thiazolo[4,5-*c*]quinolin-2-amine (**68**; 10 mg, 6%); ^{1}H NMR (300 MHz, CD_3OD): δ = 10.87 (s, 1H, N*H*), 8.07 (d, J = 2.0 Hz, 1H, Ar*H*), 8.00 (d, J = 8.9 Hz, 1H, Ar*H*), 7.70 (d, J = 8.3 Hz, 2H, Ar*H*), 7.65 (dd, J = 9.0 and 2.2 Hz, 1H, Ar*H*), 7.22 (d, J = 8.3 Hz, 1H, Ar*H*), 2.89 (s, 3H, CH_3), 2.30 ppm (s, 3H, CH_3); MS (ES$^+$), *m/z* (%): 342.0 (48) $[M+2H]^+$, 340.0 (100) $[M+H]^+$; LC–MS, t_R = 3.53 min, Method A.

Preparation of compound 69: 1-*tert*-Butyl [(8-chloro-4-methyl[1,3]thiazolo[4,5-*c*]quinolin-2-yl)methyl]carbamate (970 mg, 2.67 mmol) was suspended in 4 M HCl (6.67 mL, 26.7 mmol) in dioxane, and the mixture was stirred for 2 h at room temperature. The mixture was concentrated to dryness and resuspended in satd. Na_2CO_3 (10 mL). The aqueous mixture was extracted with CH_2Cl_2/MeOH 4:1 (4×15 mL). The organic layer was dried over $MgSO_4$, filtered off, concentrated to dryness, and purified by column chromatography to give [(8-chloro-4-methyl[1,3]thiazolo[4,5-*c*]quinolin-2-yl)methyl]-amine (**69**; 420 mg, 60%); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.46 (d, J = 2.2 Hz, 1H, Ar*H*), 8.23 (d, J = 8.9 Hz, 1H, Ar*H*), 7.89 (dd, J = 8.9 and 2.3 Hz, 1H, Ar*H*), 4.76 (s, 2H, CH_2), 4.56 (br s, 2H, NH_2), 3.07 ppm (s, 3H, CH_3); MS (ES$^+$), *m/z* (%): 266.0 (45) $[M+2H]^+$, 264.0 (100) $[M+H]^+$; LC–MS, t_R = 2.45 min, Method C.

General synthesis for the formation of thiazoloquinolines 70–76: [(8-Chloro-4-methyl[1,3]thiazolo[4,5-*c*]quinolin-2-yl)methyl]-amine (**69**; 50 mg, 0.19 mmol) and the corresponding aldehyde (0.38 mmol) were dissolved in a mixture of CH_2Cl_2/MeOH 4:1 (5 mL) and $NaBH(OAc)_3$ (121 mg, 0.57 mmol) was added portionwise every hour with stirring (8 h, eight portions). The mixture was quenched by the addition of satd. $NaHCO_3$ (10 mL) and the aqueous layer was extracted with CH_2Cl_2/MeOH (4:1; 3×15 mL). The organic fraction was dried over Na_2SO_4 and concentrated to dryness. The crude was dissolved in MeOH and purified by HPLC to give the thiazoloquinolinone derivatives **70**–**76**.

[(8-Chloro-4-methyl[1,3]thiazolo[4,5-*c*]quinolin-2-yl)methyl][(6-trifluoromethyl-3-pyridinyl)methyl]amine (70): Yield: 51 mg, 64%; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.89 (s, 1H, Ar*H*), 8.35 (d, J = 2.3 Hz, 1H, Ar*H*), 8.29 (s, 1H, N*H*), 8.23 (d, J = 7.9 Hz, 1H, Ar*H*), 8.12 (d, J = 8.9 Hz, 1H, Ar*H*), 7.98 (d, J = 8.0 Hz, 1H, Ar*H*), 7.80 (dd, J = 8.9 and 2.3 Hz, 1H, Ar*H*), 4.84 (s, 2H, CH_2), 4.46 (s, 2H, CH_2), 3.00 ppm (s, 3H, CH_3); MS (ES$^+$), *m/z* (%): 425.0 (42) $[M+2H]^+$, 423.0 (100) $[M+H]^+$; LC–MS, t_R = 3.71 min, Method C.

Procedure for the formation of oxazoloquinoline 77: 3-Amino-6-chloro-2-methyl-4-quinolinol (**44**; 208 mg, 1 mmol) and 4-methylphenylacetic acid (150 mg, 1 mmol) were suspended in DMF (2 mL), and PyBOP (520 mg, 1 mmol) and DIPEA (0.53 mL, 2 mmol) were added. The reaction mixture was stirred for 2 h at room temperature. The resulting suspension was filtered off and washed with DMF (2×3 mL), H_2O (3×3 mL), and hexanes (2×3 mL). The solid was dried, suspended in pyridine (10 mL), and P_2O_5 (142 mg, 1 mmol) was added in one portion. The mixture was heated at reflux for 5 h. H_2O (4 mL) was then added, and the mixture was allowed to cool to room temperature. The white precipitate was filtered off, washed with H_2O (2×2 mL) and hexane (2×2 mL) to yield 8-chloro-4-methyl-2-[(4-methylphenyl)methyl][1,3]oxazolo [4,5-*c*]quinoline (**77**; 129 mg, 40%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.08–8.03 (m, 2H, Ar*H*), 7.60 (dd, J = 9.1 and 2.3 Hz, 1H, Ar*H*), 7.34–7.28 (m, 2H, Ar*H*), 7.20–7.15 (m, 2H, Ar*H*), 4.52 (s, 2H, CH_2), 4.05 (s, 3H, CH_3), 3.09 ppm (s, 3H, CH_3); MS (ES$^+$), *m/z* (%): 325.1 (37) $[M+2H]^+$, 323.1 (100) $[M+H]^+$; LC–MS, t_R = 3.09 min, Method C.

Synthesis of quinolinone 78: 6-Chloro-2-methyl-4*H*-3,1-benzoxazin-4-one (8 g, 40.9 mmol) in THF (60 mL) was added dropwise to a solution of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU; 6.23 g, 40.9 mmol) and ethyl isocyanoacetate (4.63 g, 40.9 mmol) in THF (60 mL) at room temperature. The solution was stirred overnight. AcOH (20% in H_2O; 50 mL) was then added with ice bath cooling. H_2O (140 mL) was then added, and the organic solvent was removed under vacuum. The resulting suspension was filtered off, the solids were suspended in MeOH (70 mL), and HCl (16 mL, 37%) was added to the mixture. The suspension was heated at reflux for 3 h with formation of a new white precipitate. The mixture was allowed to cool to room temperature, and the solids were filtered off, washed with Et_2O (50 mL) and dried under vacuum to give 3-amino-6-chloro-4-hydroxy-2(1*H*)-quinolinone (**78**; 5.72 g, 66%); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 12.05 (s, 1H, N*H*), 8.02 (d, J = 2.2 Hz, 1H, Ar*H*), 7.53 (dd, J = 8.8 and 2.2 Hz, 1H, Ar*H*), 7.34 (d, J = 8.8 Hz, 1H, Ar*H*), 7.05 ppm (br s, 2H, NH_2); MS (ES$^+$), *m/z* (%): 213.0 (37) $[M+2H]^+$, 211.0 (100) $[M+H]^+$.

Synthesis of acetamide 79: PyBOP (2.47 g, 4.75 mmol) and DIPEA (1.658 mL, 9.50 mmol) were added to a suspension of 3-amino-6-chloro-4-hydroxy-2(1*H*)-quinolinone (**78**; 1 g, 4.75 mmol) and 4-chlorophenylacetic acid (0.810 g, 4.75 mmol) in DMF (4 mL). The clear solution was allowed to react for 2 h. H_2O (5 mL) was then added, and the reaction was evaporated to near dryness under vacuum. The resulting crude was resuspended in H_2O (20 mL) to give a white precipitate that was filtered off and purified by column chromatography to give *N*-(6-chloro-4-hydroxy-2-oxo-1,2-dihydro-3-quinolinyl)-2-(4-chlorophenyl)acetamide (**79**; 430 mg, 26%) as a clear white-blue powder. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 11.93 (s, 1H, N*H*), 9.92 (s, 1H, O*H*), 7.79 (d, J = 2.3 Hz, 1H, Ar*H*),

7.55 (dd, $J=8.8$ and 2.3 Hz, 1H, Ar*H*), 7.44–7.38 (m, 4H, Ar*H*), 7.30 (d, $J=8.7$ Hz, 1H, Ar*H*), 3.92 ppm (s, 2H, CH_2); MS (ES^+), *m/z* (%): 367 (11) $[M+4H]^+$, 365.0 (67) $[M+2H]^+$, 363.0 (100) $[M+H]^+$.

Preparation of compound 80: KOH (15.45 mg, 0.275 mmol) was suspended in MeOH (5 mL), and *N*-(6-chloro-4-hydroxy-2-oxo-1,2-dihydro-3-quinolinyl)-2-(4-chlorophenyl)acetamide (**79**; 100 mg, 0.275 mmol) was added to the mixture. After 5 min stirring, 2-chloro-*N*,*N*-dimethylethanethioamide (34.0 mg, 0.275 mmol) was added in one portion. The mixture was allowed to react for 1 h, and the white precipitate formed was filtered off, washed with H_2O (5 mL), and dried under vacuum. The white solid was suspended in CH_2Cl_2 (5 mL), and BF_3·OEt (117 mg, 0.826 mmol) was added to the mixture in one portion. The mixture was sealed and heated with stirring under microwave irradiation (10 min, 110 °C). The mixture was concentrated to dryness and purified by preparative HPLC to give 8-chloro-2-[(4-chlorophenyl)methyl][1,3]thiazolo[4,5-*c*]quinolin-4(5*H*)-one (**80**; 20 mg, 20%); ^{1}H NMR (300 MHz, [D_6]DMSO): $\delta=$ 12.05 (s, 1H, N*H*), 7.88 (d, $J=2.3$ Hz, 1H, Ar*H*), 7.54 (dd, $J=8.8$ and 2.3 Hz, 1H, Ar*H*), 7.46–7.44 (m, 4H, Ar*H*), 7.42 (d, $J=8.8$ Hz, 1H, Ar*H*), 4.52 ppm (s, 2H, CH_2); MS (ES^+), *m/z* (%): 365.0 (12) $[M+4H]^+$, 363.0 (74) $[M+2H]^+$, 361.0 (100) $[M+H]^+$; LC–MS, $t_R=$ 2.96 min, Method B.

Synthesis of naphthyridine 83: 2-Methyl-6-methoxy-3-nitro-1,5-naphthyridin-4-ol (**82**; 580 mg, 2.46 mmol) was suspended in EtOH (40 mL), and the mixture was heated at reflux. Fe^0 (1.377 g, 24.6 mmol) and an aqueous solution of NH_4Cl (2.5 M, 9.86 mL, 24.6 mmol) were added, and the mixture was heated at reflux for 2 h. The warm mixture was filtered through a Celite patch, and the remaining solids were washed with warm EtOH (5 mL). The resulting liquids were concentrated to dryness to give a dark-brown powder. The solids were suspended DMF (5 mL), and DIPEA (1.723 mL, 9.86 mmol) was added to the mixture followed by 4-chlorophenylacetic acid (547 mg, 3.21 mmol) and PyBOP (1.716 g, 3.3 mmol). The resulting mixture was stirred at room temperature for 4 h. H_2O (25 mL) was then added, and the mixture was allowed to precipitate for 15 min. The formed solids were filtered off, washed with H_2O, and dried under vacuum. The solid (420 mg) was suspended in pyridine (5 mL), and P_4S_{10} (522 mg, 1.17 mmol) was added in one portion. The mixture was heated at reflux for 2 h. H_2O (4 mL) was then added, and the mixture was allowed to cool to room temperature. The white precipitate was filtered off and purified by column chromatography to give 2-[(4-chlorophenyl)methyl]-4-methyl-8-methoxy[1,3]thiazolo[4,5-*c*]-1,5-naphthyridine (**83**; 320 mg, 38%). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=8.41$ (d, $J=9.1$ Hz, 1H, Ar*H*), 7.41–7.35 (m, 4H, Ar*H*), 7.13 (d, $J=9.1$ Hz, 1H, Ar*H*), 4.54 (s, 2H, CH_2), 4.07 (s, 3H, CH_3), 3.15 ppm (s, 3H, CH_3); MS (ES^+), *m/z* (%): 358.0 (37) $[M+2H]^+$, 356.0 (100) $[M+H]^+$; LC–MS, $t_R=3.60$ min, Method C.

Preparation of naphthyridinone 84: 2-[(4-chlorophenyl)methyl]-4-methyl-8-methoxy[1,3]thiazolo[4,5-*c*]-1,5-naphthyridine (**83**; 200 mg, 0.562 mmol) was suspended in a 33% solution of HBr in AcOH (2 g, 4.68 mmol), and the stirred mixture was heated at 100 °C for 1 h. The resulting residue was concentrated to dryness purified by HPLC to give 2-[(4-chlorophenyl)methyl]-4-methyl-[1,3]thiazolo[4,5-*c*]-1,5-naphthyridin-8(9*H*)-one (**84**; 78 mg, 41%); ^{1}H NMR (300 MHz, [D_6]DMSO): $\delta=12.32$ (s, 1H, N*H*), 8.01 (d, $J=$ 9.1 Hz, 1H, Ar*H*), 7.51–7.43 (m, 4H, Ar*H*), 6.70 (d, $J=9.1$ Hz, 1H, Ar*H*), 4.61 ppm (s, 2H, CH_2), 2.48 ppm (s, 3H, CH_3); MS (ES^+), *m/z* (%): 344.0 (35) $[M+2H]^+$, 342.0 (100) $[M+H]^+$; LC–MS, $t_R=$ 2.31 min, Method C.

Preparation of compound 85: 2-[(4-Chlorophenyl)methyl]-4-methyl[1,3]thiazolo[4,5-*c*]-1,5-naphthyridin-8(9*H*)-one (**84**; 20 mg, 0.058 mmol) was suspended in $POCl_3$ (1 g, 6.52 mmol), and the mixture was heated at 90 °C for 30 min. The mixture was concentrated to dryness and dissolved in MeOH for loading onto HPLC for purification to give 2-[(4-chlorophenyl)methyl]-4-methyl[1,3]thiazolo[4,5-*c*]-1,5-naphthyridin-8-yl dimethyl phosphate (**85**; 7 mg, 27%); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=8.55$ (d, $J=8.8$ Hz, 1H, Ar*H*), 7.40–7.30 (m, 5H, Ar*H*), 4.52 (s, 2H, CH_2), 4.05 (s, 3H, CH_3), 4.01 (s, 3H, CH_3), 3.14 ppm (s, 3H, CH_3); MS (ES^+), *m/z* (%): 452.1 (43) $[M+2H]^+$, 450.1 (100) $[M+H]^+$; LC–MS, $t_R=2.82$ min, Method C.

Synthesis of naphthyridine 86: 2-[(4-Chlorophenyl)methyl]-4-methyl[1,3]thiazolo[4,5-*c*]-1,5-naphthyridin-8(9*H*)-one (**84**; 20 mg, 0.058 mmol) was suspended in $POCl_3$ (1 g, 6.52 mmol), and the mixture was heated at 160 °C for 2 min under microwave irradiation. The mixture was concentrated to dryness and dissolved in MeOH for loading onto HPLC for purification to give 8-chloro-2-[(4-chlorophenyl)methyl]-4-methyl[1,3]thiazolo[4,5-*c*]-1,5-naphthyridine (**86**; 6 mg, 29%); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=8.39$ (d, $J=8.8$ Hz, 1H, Ar*H*), 8.39 (d, $J=8.8$ Hz, 1H, Ar*H*), 7.38–7.33 (m, 4H, Ar*H*), 4.53 (s, 2H, CH_2), 3.12 ppm (s, 3H, CH_3); MS (ES^+), *m/z* (%): 364.0 (9) $[M+4H]^+$, 362.0 (65) $[M+2H]^+$, 360.0 (100) $[M+H]^+$, LC–MS, $t_R=$ 3.53 min, Method C.

Biology

All experimental work with animals was performed by following the 3R principles: replacing, reducing, and refining animal testing, and took place in our Association for the Assessment of Laboratory Animal Care International (AAALAC-1)-accredited facilities under the control and approval of the Tres Cantos GSK local ethics committee.

General antimicrobial activity assay: Whole-cell antimicrobial activity was determined by broth microdilution using the Clinical and Laboratory Standards Institute (CLSI)-recommended procedure, Document M7-A7, *Methods for Dilution Susceptibility Tests for Bacteria that Grow Aerobically*. Some compounds were evaluated against a panel of Gram-positive and Gram-negative organisms, including *Staphylococcus aureus*, *Enterococcus faecalis*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Streptococcus pneumoniae*, and *Escherichia coli*. Minimum inhibitory concentration (MIC) values were determined as the lowest concentration of compound producing >80 or 90% decrease in fluorescence observed.

Mycobacterium tuberculosis $H_{37}Rv$ inhibition assay: MIC determinations for each tested compound were performed in 96-well flat-bottomed polystyrene microtiter plates. Ten twofold drug dilutions in neat DMSO starting at 400 μM were performed; these drug solutions (5 μL each) were added to 95 μL Middlebrook 7H9 medium (Difco cat. no. 271310; columns A–H, rows 1–10 of the plate layout). Isoniazid was used as a positive control; eight twofold dilutions of isoniazid starting at 160 μg mL^{-1} were prepared, and 5 μL of this control curve were added to 95 μL Middlebrook 7H9 medium (row 11, columns A–H). Neat DMSO (5 μL) was added to row 12 (growth and blank controls). The inoculum was standardized to ~1×10^7 CFU mL^{-1} and diluted 1:100 in Middlebrook 7H9 broth (Middlebrook ADC enrichment, a dehydrated culture medium that supports the growth of mycobacterial species; Becton–Dickinson cat. no. 211887) to produce the final inoculum of $H_{37}Rv$ strain (ATCC 25618). This inoculum (100 μL) was added to the entire plate except wells G-12 and H-12 (blank controls). All plates were placed in a sealed box to prevent drying of the peripheral wells, and were incubated at 37 °C without shaking for 6 days.

A resazurin solution was prepared by dissolving one tablet of resazurin (Resazurin Tablets for Milk Testing; VWR International Ltd. cat. no. 330884Y) in 30 mL sterile phosphate-buffered saline (PBS); this solution was added to each well (25 μL per well). Fluorescence was measured (Spectramax M5, Molecular Devices, $\lambda_{ex} = 530$ nm, $\lambda_{em} = 590$ nm) after 48 h to determine the MIC value.

MIC in solid media: MIC values for each tested compound were performed in 24-well flat-bottomed polystyrene microtiter plates; 11 twofold drug dilutions were carried out in 7H10-OADC (1 mL, 55 °C) and pooled in the plate (DMSO was used as positive control). The inoculum was standardized to 5×10^4 CFU mL^{-1} in Middlebrook 7H9 broth. This inoculum was placed in each well (10 μL per well). All plates were placed in a sealed box to prevent drying out and were incubated at 37 °C for 15 days. MIC in these assays was defined as the lower concentration of the compound where $< 5\%$ of starting CFUs were observed.

Cytotoxicity assays: The cytotoxic effect of the compounds was tested on various cell lines. Moreover, two different exposure times (4 and 48 h) at 37 °C in a humidified 5% CO_2 atmosphere and two methods for determining cell damage (resazurin reduction and ATP content) were done. The well-known toxic compound doxorubicin was used as cytotoxic control. For all assays, the cell culture medium used was EMEM supplemented with 5% fetal calf serum; the concentration of DMSO never exceeded 0.5%.[14] Selected cell lines: HepG2, human caucasian hepatocyte carcinoma; MDCK, canine kidney; L6, rat skeletal muscle myoblast; CHO, Chinese hamster ovary.

Genotoxicity assays: Genotoxicity was measured by the *Salmonella typhimurium* SOS/*umu*-assay,[15] and is based on the ability of DNA-damaging agents to induce the expression of the *umu* operon. The strains used were *S. typhimurium* TA1535/pSK1002 and NM2009. The pSK1002 plasmid carries a *umuC–lacZ* fusion gene that produces a hybrid protein with β-galactosidase activity, the expression of which is controlled by the *umu* regulatory region. The plasmid prepared from *E. coli* CSH26/pSK1002 was introduced into *S. typhimurium* TA1535 by DNA to create TA1535/pSK1002. The other strain, NM2009, possesses elevated *O*-acetyltransferase levels and was constructed by subcloning the corresponding gene into a plasmid vector pACYC184 and introducing this plasmid into the original strain TA1535/pSK1002. The criterion for positive response is a twofold increase in β-galactosidase activity over the mean control values, showing a clear dose-related response.

Pharmacokinetic studies: Species: C57BL/6 mouse, female, 18–20 g; route: p.o., oral gavage; dose level: 100 and 300 mg kg^{-1}, 20 mL kg^{-1} vol. admin.; feeding regimen: fed ad libitum; compartment analyzed: peripheral total blood; vehicle p.o. PK (suspension): 1% methyl cellulose (1% MC); sampling scheme: 15, 30 and 45 min, and 1, 1.5, 2, 3, 4, 8 and 24 h; $n = 4$ per time point; analytical method (sensitivity): LC–MS (LLQ = 1–5 ng mL^{-1} in 25 μL blood); data analysis: WinNonlin 5.0, non-compartmental analysis (NCA), supplementary analysis with GraphPad 4 software.

Tolerability studies: Compound **48** was administered at 1000, 300, and 100 mg kg^{-1} p.o. to five animals each, by gavage. After administration, Irwin tests were done to every group, and body weight was recorded 24 h after administration. All groups were sacrificed by CO_2 overdose, blood samples were taken by intracardiac puncture for further analysis, and necropsy was done. Species: C57BL/6J mice, 20 g ±10%; sex: female, $n = 5$ per group; protocol: CEEA nos. 4 and 19; AESOP: AP1937v3; study design: 1 dose, o.d. maximal dose without findings (MDwF); route: p.o. oral gavage 20 g; 20 mL kg^{-1} vol. admin.; dose level: 100, 300, and 1000 mg kg^{-1}; feeding regimen: fed ad libitum; control group: vehicle, 1% methyl cellulose; analytical method: Irwin test, body weight evolution, MDwF, clinical chemistry, hematology, reticulocytes, autopsy, PK study at first day and three points for remain of study; statistics: *Kruskal Wallis* and *Dunns* test (no parametric).

Acknowledgements

The authors are grateful to the TB Alliance for financial support. We thankfully acknowledge Fátima Ortega for pharmacokinetic evaluations, Joaquín Rullas for efficacy studies, Antonio Martínez for essential animal lab upkeep and daily maintenance, María Jesús Almela for cytotoxicity studies, María Teresa Fraile for formulation studies, José Fiandor for advice and guidance for medicinal chemistry activities, and Sophie Huss for in vitro clearance evaluation.

Keywords: antibiotics · antitubercular agents · medicinal chemistry · trailing effect · Tween-80

[1] *Global Tuberculosis Control: Surveillance, Planning, Financing*, WHO Report **2007**, World Health Organization, Geneva (WHO/HTM/TB/2007.376).
[2] A. Wright, M. Zignol, A. Van Deun, D. Falzon, S. Ruesch-Gerdes, K. Feldman, S. Hoffner, F. Drobniewski, L. Barrera, D. van Soolingen, F. Boulabhal, C. N. Paramasivan, K. M. Kam, S. Mitarai, P. Nunn, M. Raviglione, *Lancet* **2009**, *373*, 1861–1873.
[3] G. Sotgiu, G. Ferrara, A. Matteelli, M. D. Richardson, R. Centis, S. Ruesch-Gerdes, O. Toungoussova, J.-P. Zellweger, A. Spanevello, D. Cirillo, C. Lange, G. B. Migliori, *Eur. Respir. J.* **2009**, *33*, 871–881.
[4] a) A. K. Pathak, V. Pathak, L. E. Seitz, W. J. Suling, R. C. Reynolds, *Bioorg. J. Med. Chem.* **2004**, *47*, 273–276; b) O. Moukha-Chafiq, M. L. Taha, H. B. Lazrek, J.-L. Imbach, *C. R. Acad. Sci. Ser. II* **2000**, *3*, 639–641.
[5] L. Ballell, R. A. Field, G. A. C. Chung, R. J. Young, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1736–1740.
[6] V. Klimešová, J. Koči, K. Waisser, J. Kaustová, *Farmaco* **2002**, *57*, 259–265.
[7] J. Rullas, J. I. García, M. Beltrán, P.-J. Cardona, N. Cáceres, J. F. García-Bustos, I. Angulo-Barturen, *Antimicrob. Agents Chemother.* **2010**, *54*, 2262–2264.
[8] C. A. Lipinski, *Drug. Discov. Today Tech.* **2004**, *1*, 337–341.
[9] R. M. Van Boxtel, R. S. Lambrecht, M. T. Collins, *Antimicrob. Agents Chemother.* **1990**, *34*, 2300–2303.
[10] L. J. V. Piddock, K. J. Williams, V. Ricci, *J. Antimicrob. Chemother.* **2000**, *45*, 159–165.
[11] Y. J. Tang, W. Shui, S. Myers, X. Feng, C. Bertozzi, J. D. Keasling, *Biotechnol. Lett.* **2009**, *31*, 1233–1240.
[12] a) S. K. Parker, K. M. Curtin, M. L. Vasil, *J. Bacteriol.* **2007**, *189*, 4153–4160; b) N. P. West, K. M. Cergol, M. Xue, E. J. Randall, W. J. Britton, R. J. Payne, *Chem. Commun.* **2011**, *47*, 5166–5168.
[13] a) O. Marchetti, P. Moreillon, M. P. Glauser, J. Bille, D. Sanglard, *Antimicrob. Agents Chemother.* **2000**, *44*, 2373–2381; b) G. St-Germain, *Mycoses* **2001**, *44*, 37–45.
[14] S. P. M. Crouch, R. Kozlowski, K. J. Slater, J. Fletcher, *J. Immunol. Methods* **1993**, *160*, 81–88.
[15] Y. Oda, S. Nakamura, I. Oki, T. Kato, H. Shinagawa, *Mutat. Res.* **1985**, *147*, 219–229.

Received: June 24, 2011
Revised: August 24, 2011
Published online on September 16, 2011

