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Development of antitubercular compounds based on a 4-quinolylhydrazone scaffold. Further structure–activity relationship studies

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ABSTRACT

A series of 4-quinolylhydrazones was synthesized and tested in vitro against *Mycobacterium tuberculosis*. At a concentration of 6.25 μg/mL, most of the newly synthesized compounds displayed 100% inhibitory activity against *M. tuberculosis* in cellular assays. Further screening allowed the identification of very potent antitubercular agents. Compound **4c** was also tested in a time-course experiment and against *mtb* clinical isolates, displaying interesting results.

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1. Introduction

Tuberculosis (TB), a disease caused by the facultative intracellular bacterium *Mycobacterium tuberculosis* (*mtb*), is a global health problem and a leading cause of death among adults in the developing world. It is estimated that in 2007, the mortality rate due to TB among adults was 18%. Moreover, the vast majority of TB deaths are in the developing world, with more than half occurring in Asia.[1]

Recrudescence of TB in the last 30 years is due to several reasons, the most important being the emergence of *mtb* resistant strains, which are insensitive to one (Single Drug Resistant—SDR-) or more (Multi Drug Resistant—MDR-) of the first-line antitubercular drugs.[2,3] In the last two decades, this scenario has been further worsened by the co-infection with the human immunodeficiency virus (HIV), which hampered the efficacy of currently available antitubercular therapies.[4,5] First line drugs for TB control include streptomycin, isoniazid (**1**, INH, Fig. 1), rifampin (RMP), ethambutol and pyrazinamide, while second line treatments are based on the use of *p*-aminosalicylic acid, ethionamide, cycloserine, macrolide antibiotics such as azithromycin and clarithromycin, and fluoroquinolones.[3] However, the main problem associated with TB therapy is the poor compliance with the prolonged treatment, and especially with the regimens used to treat MDR-TB that are badly tolerated, expensive, relatively ineffective, and must be taken for up to two years. INH is one of the most used and effective antitubercular drugs and recently, due to the appearance of *mtb* resistant strains, a great effort has been made to uncover the mechanism of action of this drug and the origin of drug resistance. INH interferes with the biosynthesis of mycolic acids. They represent the major and most specific lipid components of the mycobacterial outer membrane and are covalently linked with D-arabinogalactan and peptidoglican.[6–11] The mycobacteria cell wall plays an important role in protecting this organism from its environment and represents an effective barrier contributing to drug resistance.[12,13] INH is a prodrug which needs to be activated within the cellular environment by the mycobacterial catalase-peroxidase KatG or by metal ions to form an isonicotinoyl radical intermediate.[14–18] Activity of INH has been explained through the formation of a covalent INH–NADH adduct, but a direct damage produced by the INH-derived oxygen- and carbon-centred free radicals is also likely to play a role in killing this pathogen.[19–23]

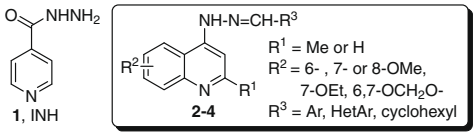

Figure 1. Structure of isoniazid (**1**) and general structure of target hydrazones **2–4**.

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E-mail address: savini@unisi.it (L. Savini).

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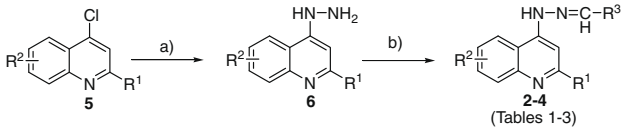
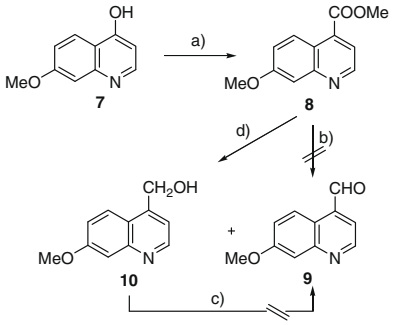
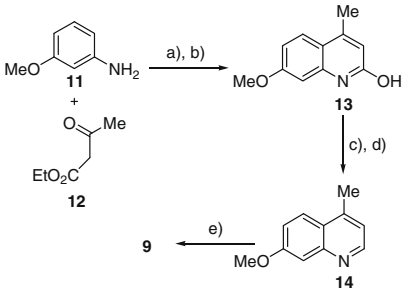


Scheme 1. Synthetic route to the target hydrazones **2–4**: (a) $NH_2NH_2{\cdot}H_2O$, EtOH, reflux, 4–6 h or $NH_2NH_2{\cdot}H_2O$, MW, 150 W, 5 min; (b) R^3CHO, EtOH, reflux.



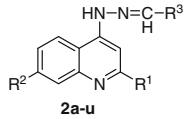
Scheme 2. Attempted synthetic routes to 7-methoxyquinoline-4-carboxaldehyde **9**; (a) (i) $(CF_3SO_2)O$, Na_2CO_3, CH_2Cl_2, 23 °C, 18 h; (ii) $Pd(OAc)_2$, dppp, CO (1 atm), Et_3N, MeOH, 24 h; (b) DIBAL-H, toluene, −78 °C, 1 h; (c) MnO_2, dioxane, 80 °C, 1 h; (d) $LiAlH_4$, −78 °C, THF, 2 h.



Scheme 3. Synthetic route to 7-methoxy-4-quinolincarboxaldehyde **9**; (a) 160 °C, 5 h; (b) H_2SO_4, steam bath; 3 h; (c) $POCl_3$, 135 °C, 45 min; (d) Pd/C, Et_3N, EtOH, H_2, 14 psi, 1 h; (e) SeO_2, dioxane, 100 °C, 2 h.

Furthermore, generation of metal ion-induced radical intermediates has been proposed as the mechanism of action of hydrazone antitumorals[24,25] and it is known that heteroaroyl hydrazones and Schiff base hydrazones are able to form complexes with different metals.[26–28] Recently, we developed a series of hydrazones endowed with antimalarial activity and we have been able to modulate hydrazone reactivity toward heme iron by exploiting specific substituents at the hydrazone scaffold.[29] In that study, we hypothesized that the efficacy of the compounds could be linked to the metal ion-mediated generation of specific radical intermediates. Furthermore, we described a series of quinolylhydrazones endowed with interesting antitubercular activity.[30] The antitubercular activity of some mefloquine-based hydrazones and of 2-/4-quinolinecarbaldehyde hydrazones has also been reported by others.[31–33] With the aim of developing low cost potential drugs and of further exploring the structure–activity relationships (SARs) of this promising hydrazone class of antitubercular agents, and encouraged by previous results, we synthesized and tested the quinolylhydrazones **2–4** as described in Figure 1 and Tables 1–3. Similarly to INH, the quinolylhydrazones herein described could be active by generating (metal ion-induced) toxic radical species.[24,25] Accordingly, steric and electronic effects of specific substituents at the quinoline ring and at the hydrazone's moieties are likely to be important in

Table 1
In vitro evaluation of antimicrobial activity as % inhibition versus *Mycobacterium tuberculosis H37Rv* and MIC (μM)



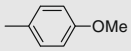
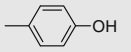
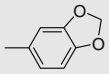
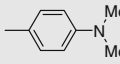
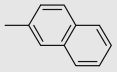
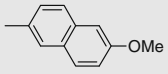
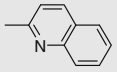
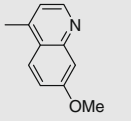
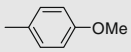
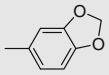
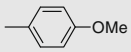
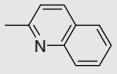
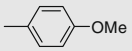
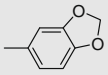
Compound	R^1	R^2	R^3	% Inh.[a]	MIC[b] (μM)
2a	H	OMe		100	16.4
2b	H	OMe		100	9.8
2c	H	OMe		100	5.3
2d	H	OMe		100	2.7
2e	H	OMe		100	9.7
2f	H	OMe		100	4.9
2g	H	OMe		99	11.8
2h	H	OMe		100	0.6
2i	H	OMe		100	0.6
2j	H	OMe		99	9.5
2k	H	OMe		100	9.5
2l	H	OMe		100	2.2
2m	H	OEt		100	9.7
2n	H	OEt		48	–
2o	H	OEt		26	–
2p	Me	OMe		99	19.5
2q	Me	OMe		100	4.6

Table 1 (*continued*)

Compound	R^1	R^2	R^3	% Inh.[a]	MIC[b] (μM)
2r	Me	OMe		100	4.6
2s	Me	OEt		20	–
2t	Me	OEt		98	9.0
2u	Me	OEt		78	–
RMP	–	–	–	–	0.037
INH	–	–	–	–	0.36

[a] % fluorescence inhibition at compound concentration of 6.25 μg/mL.
[b] MIC: lowest concentration effecting reduction in fluorescence of 90% relative to the controls.

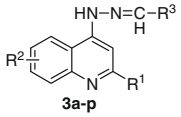
modulating reactivity toward metal ions and propensity to form radical species.[29]

2. Chemistry

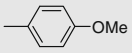
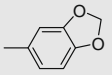
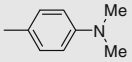
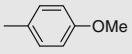
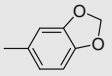
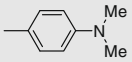
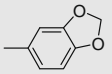
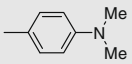
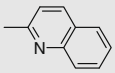
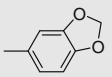
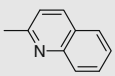
Hydrazones **2–4** (Fig. 1 and Tables 1–3) were prepared in 60–80% yield using the general synthetic method outlined in Scheme 1, and following previously described procedures.[34–37] Accordingly, the substituted hydrazines **6** were reacted with equimolar amounts of the suitable commercially available carboxaldehydes in refluxing EtOH. The hydrazine derivatives **6** were in turn prepared by reacting hydrazine hydrate with the opportunely substituted 4-chloroquinolines **5** by using our recently developed microwave-assisted procedure,[38] or according to our previously reported protocols.[34,35,39]

Aldehydes used to synthesize 4-quinolylhydrazones here described were commercially available with the only exception of 7-methoxy-4-quinolinecarboxaldehyde **9** (Scheme 2) employed for the synthesis of compound **2l**. Two different synthetic pathways were attempted to synthesize aldehyde **9**. In the first attempt, we started from 4-hydroxyquinoline derivative **7** that was treated with triflic anhydride and equimolar amounts of Na_2CO_3 in CH_2Cl_2 to get a triflate intermediate. This latter was immediately subjected to a palladium catalyzed carbonylation reaction. The triflate intermediate was treated with $Pd(OAc)_2$, 1,3-bis(diphenylphosphino)propane, Et_3N, and MeOH in DMSO under a carbon monoxide atmosphere[40] to afford ester **8** in satisfactory yield. An attempt to reduce the ester group to aldehyde by exposure to DIBAL in toluene at −78 °C was unsuccessful and resulted in the formation of an inseparable mixture of unreacted starting material **8**, aldehyde **9**, and alcohol **10**. To avoid tedious chromatographic purifications, **8** was converted to **10** using $LiAlH_4$. Subsequently, MnO_2 promoted oxidation of alcohol **10** was attempted. Unfortunately, the target aldehyde **9** could only be isolated in trace amounts. Several attempts to optimize the oxidation step failed to convey a substantial improvement of the reaction yield. A different synthetic route was planned as reported in Scheme 3. Accordingly, 3-methoxyaniline **11** was condensed with ethyl acetoacetate **12** and cyclized to the 6-methoxy-4-methyl-2-hydroxyquinoline **13** in good yield.[41] Subsequently, chlorination at C2 was quantitatively achieved by treatment with $POCl_3$ and the chlorine functionality was then removed by catalytic hydrogenolysis to afford the target intermediate **14**. Finally, the 4-methyl group of **14** was

Table 2
In vitro evaluation of antimicrobial activity as % inhibition versus *Mycobacyterium tuberculosis H37Rv* and MIC (μM)

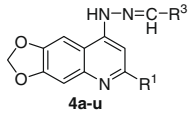


3a-p

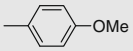
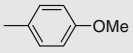
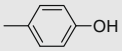
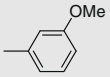
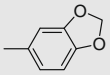
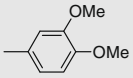
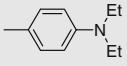
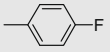
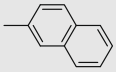
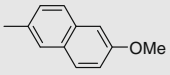
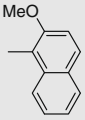
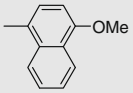
Compound	R^1	R^2	R^3	% Inh.[a]	MIC[b] (μM)
3a	H	6-OMe		100	10.2
3b	H	6-OMe		100	2.4
3c	H	6-OMe		100	19.5
3d	H	6-OMe		99	11.1
3e	Me	6-OMe		100	4.8
3f	Me	6-OMe		100	4.8
3g	Me	6-OMe		100	9.4
3h	H	8-OMe		99	11.3
3i	H	8-OMe		31	–
3j	H	8-OMe		100	19.5
3k	H	8-OMe		100	nd[c]
3l	H	8-OMe		99	4.8
3m	Me	8-OMe		95	nd
3n	Me	8-OMe		99	9.3
3o	Me	8-OMe		100	4.7
3p	Me	8-OMe		98	9.1
RMP	–	–	–	–	0.037
INH	–	–	–	–	0.36

[a] % fluorescence inhibition at compound concentration of 6.25 μg/mL.
[b] MIC: lowest concentration effecting reduction in fluorescence of 90% relative to the controls.
[c] nd: not determined.

Table 3
In vitro evaluation of antimicrobial activity as % inhibition versus *Mycobacterium tuberculosis H37Rv* and MIC (μM)



4a-u

Compound	R^1	R^3	% Inh.[a]	MIC[b] (μM)
4a	H		100	4.8
4b	H		74	–
4c	Me		100	2.6
4d	Me		9	–
4e	Me		100	9.3
4f	Me		100	nd
4g	Me		100	9.3
4h	Me		100	nd[c]
4i	Me		100	4.5
4j	Me		100	nd
4k	Me		100	8.3
4l	Me		100	19.3
4m	Me		100	nd
4n	Me		100	nd
4o	Me		100	nd
4p	Me		100	4.0
4q	Me		100	nd
4r	Me		87	–
4s	Me		74	–
4t	Me		100	10.6
4u	Me		100	5.3
RMP	–	–	–	0.037
INH	–	–	–	0.36

[a] % fluorescence inhibition at compound concentration of 6.25 μg/mL.
[b] MIC: lowest concentration effecting reduction in fluorescence of 90% relative to the controls.
[c] nd: not determined.

successfully oxidized using selenium oxide in refluxing dioxane which resulted in the clean and quantitative formation of the desired aldehyde **9**.

3. Results and discussion

Biological tests on *mtb* H37Rv were performed according to the method described in Refs. 42,43, and the results are illustrated in Tables 1–4. From the primary screening against *mtb* H37Rv, most of the tested compounds showed an inhibitory activity between 95 and 100% at a concentration of 6.25 μg/mL, therefore these compounds were submitted to a second level assay to evaluate the actual minimum inhibitory concentration (MIC) (Tables 1–3). Cytotoxicity, expressed as IC_{50} towards the VERO cell line, and selectivity indexes (SI) (Table 4) were also assessed for some of the most active compounds.

In our previous work, we explored the antitubercular properties of a range of quinolylhydrazones whose potency was significantly affected by substituents on both the quinoline system and the arylhydrazone moiety.[30] In particular, the antitubercular results on a restricted set of compounds have shown that alkoxy substituents at C7 were particularly favourable for the antitubercular activity. With the aim of expanding our SARs, we performed a thorough investigation of the effect of different alkoxy groups at the various positions of the quinoline nucleus. Accordingly, we synthesized and tested 7-alkoxy (Table 1, compounds **2a–u**), 6- or 8-OMe (Table 2, compounds **3a–p**), and 6,7-methylendioxy (Table 3, compounds **4a–u**) quinolylhydrazones. Moreover, the differently substituted quinoline rings were coupled to various aryl-, heteroaryl- or cyclohexylmethylene radicals.

In the 7-methoxyquinoline series (**2a**–**l**, Table 1), the presence of an unsubstituted phenyl ring at the hydrazone tether resulted

Table 4
In vitro cytotoxicity in the VERO cell line (IC_{50}) and selectivity index (SI) of selected compounds

Compound	IC_{50} (μg/mL)	SI[a]
2c	3.66	2.53
2d	1.09	1.59
2f	0.26	0.17
2h	0.454	2.27
2i	0.211	1.05
2q	0.12	0.08
3d	5.03	3.44
3l	1.68	1.08
3o	3.11	1.99
4i	0.22	0.14
4p	0.24	0.15
4u	>100	>66

[a] SI: IC_{50} (μg/mL)/MIC (μg/mL).

in a compound (**2a**) endowed with a MIC value of 16.4 μM. Introduction of electron-donor substituents (**2b–f**) increased the antitubercular potency with respect to the unsubstituted analogue (**2a** vs **2b–f**).

Among the various functionalities taken into consideration, introduction of an hydroxy group at meta position resulted particularly favourable for activity determining a sixfold increase in potency (**2d** vs **2a**). When the phenyl ring was expanded to a 2-naphthyl system (**2h**) the most potent derivative of the series was obtained (MIC = 0.6 μM) and the activity was also retained after introduction of a methoxy group at C6 of the naphthyl function (**2i**). Replacement of the naphthyl ring with a 2- or a 4-quinolyl system (**2j**,**k**) lowered the antitubercular potency (**2j** vs **2h**) although both compounds were still endowed with significant activity. In this case, introduction of a 7-OMe substituent at the distal quinoline ring resulted in a fourfold increase in potency (**2l** vs **2k**). When the 7-methoxyquinoline system was replaced by a 7-ethoxyquinoline, a drop of antitubercular activity was observed for **2n** (**2n** vs **2e**), while the activity was retained in the case of the 4-methoxybenzylidene derivative **2m** (**2m** vs **2b**). Introduction of a 2-Me at the quinoline nucleus only slightly influenced the potency of the resulting derivatives (**2p** vs **2b**, **2q** vs **2j**, **2u** vs **2o**).

On the other hand, with few exceptions, similar trends of activity were found when the methoxy group at C7 of **2a–l**, **p–r** was shifted to the 6- or 8-position of the quinoline nucleus (**3a–p**, Table 2). In general, antitubercular activity below 16 μM was observed for most of the tested compounds. When a 3,4-methylendioxyphenylmethylene hydrazone substituent was considered, the substitution pattern of the quinoline moiety became crucial to attain an optimal antitubercular activity. Indeed, this aryl system, coupled to a 6-methoxyquinoline as in **3b** gave rise to a fourfold increase of potency with respect to the corresponding 7-methoxyquinoline analogue **2e**, while the 8-methoxyquinoline derivative **3i** or the 7-ethoxyquinoline analogue **2n** experienced a drop in antitubercular activity at the concentration tested. In both cases, the activity was partially restored when a 2-Me substituent was introduced at the quinoline level (**2t** vs **2n** and **3m** vs **3i**).

Due to the favourable effect on antitubercular potency of the introduction of a methoxy group at C6 or C7 of the quinoline ring, we decided to introduce a 6,7-methylendioxy substituent (**4a**,**b**, Table 3) to evaluate if the simultaneous presence of both alkoxy functions could improve potency with respect to the C6 or C7 monosubstituted ethers. Most of the tested compounds in this series also presented a methyl at the quinoline C2 (**4c–u**). Preliminary evaluation of this set of compounds showed a 100% inhibition of *mtb* growth at a concentration of 6.25 μg/mL. From the MIC values reported in Table 3 we found out that, contrarily to the SAR trend observed for the 7-methoxyquinoline derivatives, the best results were obtained with the unsubstituted phenyl ring at the hydrazone level, since introduction of both electron-donating groups (*p*-MeO as in **4e**, *m*-MeO as in **4g**, 3,4-methylendioxy as in **4i**, or diethylamino as in **4k**) or electronwithdrawing groups (such as a *p*-F in **4l**) resulted in a decreased potency compared to **4c**. Moreover, 2- and 4-pyridyl substituents (**4r** and **4s**, respectively) were not tolerated at the hydrazone tether, confirming the trend observed for the 7-ethoxyquinoline derivatives **2o** and **2u**. On the contrary, introduction of a pyrrole (**4t**) or of a 2-imidazole (**4u**) resulted in active analogues, although less potent than the phenyl derivative **4c**. Cyclohexyl derivatives **3d** and **4b**,**d** were synthesized to assess the importance of an aromatic system at the hydrazone tether. While in the case of the 6,7-methylendioxy derivatives **4b**,**d**, introduction of the cyclohexyl ring resulted in a dramatic drop of activity (**4d**) or in a reduced potency (**4b**), in the 6-methoxyquinoline series, the hydrazone **3d** resulted endowed with an antitubercular activity comparable to that of the corresponding aryl-derivatives (e.g., **3a**).

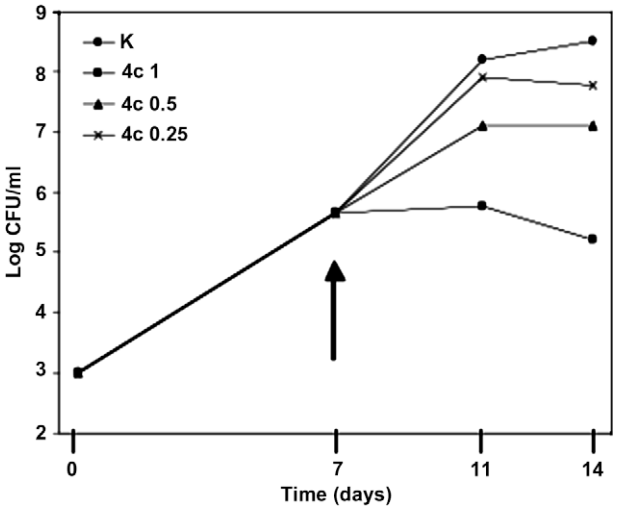


Figure 2. Activity of compound **4c** on *mtb* H37Rv. A growth curve of Mtb H37Rv was established in liquid culture (7H9/ADC). On day 7, the compound was added to a culture at three concentration and bacterial counts were determined on days 11 and 14.

Table 5
Activity of **4c** against clinical isolates

mtb Clinical isolate	MIC (μg/mL)	
	4c	INH
# UC0801	0.12	0.05
# UC0802	1.0	0.25
# UC0803	0.25	0.05
# UC0804	0.12	0.05
H37Rv	0.5	0.05

Preliminary evaluation of cytotoxicity of the most interesting analogues of the various series (Table 4) revealed that most of them were significantly cytotoxic. While compounds **2f**,**q** and **4i**,**p** presented a SI lower than 1, the remaining compounds presented a SI values between 1.08 and 3.44. Contrastingly, the imidazole derivative **4u** did not display cytotoxicity even at the higher concentration tested (IC_{50} >100 μg/mL) thus exhibiting an interesting SI value.

Among the active compounds, **4c** was selected as representative of the series for further biological studies. To assess the ability of **4c** to interfere with *mtb* growth, the compound was added to a culture of *mtb* H37Rv reference strain at the exponential phase of growth, at three concentration (the MIC, the sub-MIC and twice the MIC). As indicated in Figure 2, after adding the compound **4c** at day 7 a dose-dependent activity was observed, confirming that **4c** inhibits the growth of *mtb* in vitro. Compound **4c** displayed interesting data on clinical isolates superior to the one shown on *mtb* H37Rv reference strain. As reported in Table 5, the MIC of **4c** ranged from 0.12 to 1 μg/mL, indicating that **4c** is a promising new antitubercular agent. Cytotoxicity as assessed in VERO cells indicated that a concentration >2.5 μg/mL of compound is required in order to observe a toxic activity.

4. Conclusions

A series of novel antitubercular agents is reported, the synthesis of which is characterized by low cost of goods. During this study we identified a number of quinolylhydrazone derivatives endowed with interesting antitubercular properties. SAR studies revealed that substituents at both the quinoline and arylhydrazone moieties are able to modulate the antitubercular activity. Among the quinoline functionalities taken into consideration, the 7-OMe substituent resulted particularly favourable for anti-TB activity, especially

when coupled to a naphthyl ring at the hydrazone level, suggesting that electronic effects at the quinoline level and the overall lypophilicity of the molecule could both have a role in determining optimal activity. When the arylhydrazone moiety was examined, the naphthyl ring and the 3,4-methylendioxybenzene derivative displayed the most interesting anti-TB activity, even though the effect of the aryl hydrazone moiety is strongly dependent upon the quinoline substitution pattern. Further studies for assessing the mechanism of action of this class of antitubercular agents are underway and will also be useful for a clearer rationalization of the SAR trends here observed. Among the compounds synthesized and tested, **4c** revealed to have an interesting activity against clinical isolates.

5. Experimental

5.1. Chemistry

Reagents were purchased from Aldrich and were used as received. Reaction progress was monitored by TLC using Merck Silica Gel 60 F254 (0.040–0.063 mm) with detection by UV. Merck Silica Gel 60 (0.040–0.063 mm) was used for column chromatography. Melting points were determined in Pyrex capillary tubes using an Electrothermal 8103 apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian 300 MHz spectrometer with TMS as internal standard, ^{13}C NMR spectra were recorded on a Varian 300 MHz, or Bruker 400 MHz spectrometer. Splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), and broad (br); the value of chemical shifts (δ) are given in ppm and coupling constants (J) in hertz (Hz). ESI-MS spectra were performed by an Agilent 1100 Series LC/MSD spectrometer. Elemental analyses were performed in a Perkin-Elmer 240C elemental analyzer and the results were within ±0.4% of the theoretical values, unless otherwise noted. Yields refer to purified products and are not optimized. All moisture sensitive reactions were performed under argon atmosphere using oven-dried glassware and anhydrous solvents. All the organic layers were dried using anhydrous sodium sulfate. Compounds **13**, **2j**,**m**,**p**,**u**, **3a-c**,**e**,**f**,**h**,**j**,**o**, and **4i-l**,**p**,**q**,**s** were prepared as previously described.[29,37,41]

5.1.1. 7-Methoxyquinolin-4-carboxylic acid methyl ester (8)

Trifluoromethanesulfonic anhydride (240 μL, 1.4 mmol) was added to a mixture of 7-methoxy-4-hydroxyquinoline (**7**) (250 mg, 1.4 mmol), and sodium carbonate (152 mg, 1.4 mmol) in dry CH_2Cl_2 (4 mL), cooled to 0 °C. After stirring at 0–5 °C overnight, the reaction mixture was allowed to warm to 25 °C, water was added, the aqueous layer was extracted with CH_2Cl_2 (2 × 5 mL), the organic extracts were dried (Na_2SO_4), and the solvent was removed under vacuum. The resulting crude 7-methoxyquinolyl-4-trifluoromethanesulfonate was used in the next step without further purification. The above compound was dissolved in dry DMSO. Subsequently, $Pd(OAc)_2$ (7.5 mg, 0.03 mmol), Et_3N (0.46 mL, 3.3 mmol), 1,3-bis(diphenylphosphino)propane (37.0 mg, 0.07 mmol), and dry MeOH (0.90 mL, 22.1 mmol) were added to the above solution. The resulting mixture was heated at 70 °C under a carbon monoxide atmosphere for 40 h. Afterward it was allowed to warm to 25 °C and was poured into brine. The aqueous phase was extracted with Et_2O, the organic extracts were washed with brine, dried (Na_2SO_4) and the solvent was removed. The residue was purified by flash-chromatography (40:1 CH_2Cl_2/MeOH) and the title compound was obtained as a white solid in 49% yield. ^{1}H NMR ($CDCl_3$): δ 3.95 (s, 3H), 4.00 (s, 3H), 7.29 (dd, J = 2.3, 9.1 Hz, 1H), 7.46 (d, J = 2.4 Hz, 1H), 7.74 (d, J = 4.3 Hz, 1H), 8.65 (d, J = 9.3 Hz, 1H), 8.91 (d, J = 4.3 Hz, 1H); ESI-MS m/z: 218 $(M+H)^+$. Anal. Calcd for $C_{12}H_{11}NO_3$: C, 66.35; H, 5.10; N, 6.45. Found: C, 66.03; H, 5.44; N, 6.18.

5.1.2. 7-Methoxy-4-hydroxymethylquinoline (10)

A solution of **8** (100 mg, 0.46 mmol) in dry THF (2 mL) was cooled to −78 °C, $LiAlH_4$ (1.0 M in THF, 0.5 mL) was added dropwise within 45 min, and then the reaction mixture was slowly warmed to 25 °C (2 h). Excess $LiAlH_4$ was carefully quenched by adding a mixture of acetic acid (1 mL), H_2O (1 mL), and THF (2 mL). The resulting mixture was stirred in an ice bath for 1 h. Subsequently, water (3 mL) was added, the organic solvent was removed and the aqueous phase was extracted with CH_2Cl_2. The organic extracts were washed with a saturated solution of $NaHCO_3$, brine and finally dried (Na_2SO_4). The solvent was removed and the residue was purified by flash-chromatography (1:10 MeOH/$CHCl_3$) to afford alcohol **10** as a white solid (68.0 mg, 80%). ^{1}H NMR ($CDCl_3$): δ 2.14 (s, 3H), 5.28 (s, 2H), 7.15–7.25 (m, 1H), 7.36–7.39 (m, 2H), 7.83 (d, J = 9.1 Hz, 1H), 8.70 (d, J = 4.5 Hz, 1H). ESI-MS m/z: 190 $(M+H)^+$. Anal. Calcd for $C_{11}H_{11}NO_2$: C, 69.83; H, 5.86; N, 7.40. Found: C, 69.58; H, 6.15; N, 7.62.

5.1.3. 7-Methoxy-4-methylquinoline (14)

^{1}H NMR (DMSO-d_6): δ 2.62 (s, 3H), 3.93 (s, 3H), 7.06 (s, 1H), 7.19 (d, J = 9.1 Hz, 1H), 7.41 (s, 1H), 7.85 (d, J = 9.1 Hz, 1H), 8.66 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 160.5, 150.6, 149.9, 144.4, 125.2, 123.6, 120.3, 119.4, 108.0, 55.7, 18.8; ESI-MS m/z: 174 $(M+H)^+$. Anal. Calcd for $C_{11}H_{11}NO$: C, 76.28; H 6.40; N, 8.09, O, 9.24.

5.1.4. 7-Methoxyquinolin-4-carboxaldehyde (9)

^{1}H NMR ($CDCl_3$): δ 3.98 (s, 3H), 7.36–7.40 (m, 1H), 7.53 (d, J = 2.4 Hz, 1H), 7.64 (d, J = 4.3 Hz, 1H), 8.90 (d, J = 9.5 Hz, 1H), 9.11 (d, J = 3.9 Hz, 1H), 10.45 (s, 1H). ESI-MS m/z: 188 $(M+H)^+$. Anal. Calcd for $C_{11}H_9NO_2$: C, 70.58; H, 4.85; N, 7.48. Found: C, 70.96; H, 4.89; N, 7.16.

5.2. General procedure for the synthesis of hydrazones 2–4

A mixture of the appropriate carboxaldehyde (1 equiv) and 4-quinolylhydrazine derivative (1 equiv) was heated under reflux in ethanol for 2–3 h. After cooling and diluting with water, the respective hydrazones precipitated from the reaction mixture. They were filtered and washed with cold water. The purification was carried out by recrystallization and/or by flash column chromatography.[37]

5.2.1. 2-Benzylidene-1-(7-methoxyquinolin-4-yl)hydrazine (2a)

Yellow prisms, mp (EtOH) 278–280 °C. ^{1}H NMR (DMSO-d_6): δ 3.87 (s, 3H), 7.14 (t, J = 7.6 Hz, 1H), 7.18 (s, 1H), 7.19 (d, J = 8.8 Hz, 1H), 7.36–7.46 (m, 3H), 7.75 (d, J = 7.0 Hz, 2H), 8.22 (d, J = 8.8,1H), 8.32 (d, J = 5.7 Hz, 1H), 8.36 (s, 1H), 10.52 (br s, 1H). IR (Nujol) cm^{-1}: 3181, 1624, 1459, 1377; ESI-MS m/z: 382 $(M+H)^+$. Anal. Calcd for $C_{17}H_{15}N_3O$: C, 73.63; H, 5.45; N, 15.15. Found: C, 73.48; H, 5.38; N, 15.43.

5.2.2. 2-(4-Methoxybenzylidene)-1-(7-methoxyquinolin-4-yl)hydrazine (2b)

Yellow prisms, mp (EtOH) 212–215 °C. ^{1}H NMR (DMSO-d_6): δ 3.79 (s, 3H), 3.90 (s, 3H), 7.01 (d, J = 8.5 Hz, 2H), 7.33 (m, 1H), 7.36 (s, 1H), 7.40 (d, J = 6.9 Hz, 1H), 7.74 (d, J = 8.5 Hz, 2H), 8.45 (d, J = 6.9 Hz, 1H), 8.76 (d, J = 8.6 Hz, 1H), 8.78 (s, 1H), 11.50 (br s, 1H). IR (Nujol) cm^{-1}: 3448, 1626, 1603, 1245, 1231. ESI-MS m/z: 320 $(M+H)^+$. Anal. Calcd for $C_{18}H_{17}N_3O_2 \cdot 1/2HCl$: C, 66.40; H, 5.42; N, 12.91. Found: C, 66.75; H, 5.62; N, 12.73.

5.2.3. 2-(4-Hydroxybenzylidene)-1-(7-methoxyquinolin-4-yl)hydrazine (2c)

Yellow prisms, mp (EtOH) 239–242 °C. ^{1}H NMR (DMSO-d_6): δ 3.87 (s, 3H), 6.83 (d, J = 6.7 Hz, 2H), 7.09–7.15 (m, 3H), 7.59 (d, J = 7.9 Hz, 2H), 8.20 (d, J = 9.1 Hz, 1H), 8.26 (s, 2H), 9.83 (s, 1H), 10.83 (br s, 1H). IR (Nujol) cm^{-1}: 3102, 1461, 1376, 1157. ESI-MS

m/z: 294 $(M+H)^+$. Anal. Calcd for $C_{17}H_{15}N_3O_2$: C, 69.61; H, 5.15; N, 14.33. Found: C, 69.27; H, 5.44; N, 14.28.

5.2.4. 2-(3-Hydroxybenzylidene)-1-(7-methoxyquinolin-4-yl)hydrazine (2d)

Yellow prisms, mp (EtOH) 265–267 °C. ^{1}H NMR (DMSO-d_6): δ 3.88 (s, 3H), 6.78 (m, 2H), 7.12–7.26 (m, 5H), 8.18–8.27 (m, 2H), 8.48 (br s, 1H), 9.56 (s, 1H), 11.01 (br s, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 160.5, 158.4, 151.7, 151.2, 147.6, 143.3, 136.8, 130.5, 123.6, 118.7, 117.3, 117.2, 113.0, 112.1, 108.5, 100.3, 56.0. IR (Nujol) cm^{-1}: 3315, 1584, 1169. ESI-MS *m/z*: 294 $(M+H)^+$. Anal. Calcd for $C_{17}H_{15}N_3O_2$: C, 69.61; H, 5.15; N, 14.33. Found: C, 69.36; H, 5.38; N, 14.45.

5.2.5. 2-[(Benzo[*d*][1,3]dioxol-5-yl)methylene]-1-(7-methoxyquinolin-4-yl)hydrazine (2e)

Yellow prisms, mp (EtOH) 275–277 °C. ^{1}H NMR (DMSO-d_6): δ 3.94 (s, 3H), 6.11 (s, 2H), 7.02 (d, J = 7.9 Hz, 1H), 7.26 (d, J = 7.9 Hz, 1H), 7.36 (s, 1H), 7.37 (d, J = 7.4 Hz, 1H), 7.48 (s, 1H), 7.50 (d, J = 7.3 Hz, 1H), 8.49 (d, J = 7.4 Hz, 1H), 8.67 (s, 1H), 8.69 (d, J = 7.3 Hz, 1H), 11.01 (br s, 1H). IR (Nujol) cm^{-1}: 3280, 1638, 1597, 1243, 1112. ESI-MS *m/z*: 322 $(M+H)^+$. Anal. Calcd for $C_{18}H_{15}N_3O_3$·HCl: C, 60.42; H, 4.51; N, 11.74. Found: C, 60.72; H, 4.65; N, 11.86.

5.2.6. 2-(4-Dimethylaminobenzylidene)-1-(7-methoxyquinolin-4-yl)hydrazine (2f)

Yellow prisms, mp (EtOH) 258–262 °C. ^{1}H NMR (DMSO-d_6): δ 2.98 (s, 6H), 3.91 (s, 3H), 6.76 (d, J = 8.4 Hz, 2H), 7.28 (s, 1H), 7.29 (d, J = 6.6 Hz, 1H), 7.32 (d, J = 8.1 Hz, 1H), 7.62 (d, J = 8.4 Hz, 2H), 8.40 (d, J = 6.6 Hz, 1H), 8.55 (d, J = 8.1 Hz, 1H), 8.58 (s, 1H), 11.36 (br s, 1H). IR (Nujol) cm^{-1}: 3120, 1456, 1376, 1179. ESI-MS *m/z*: 321 $(M+H)^+$. Anal. Calcd for $C_{19}H_{20}N_4O$·3/4 HCl: C, 65.63; H, 6.01; N, 16.11. Found: C, 65.83; H, 6.25; N, 15.92.

5.2.7. 2-[(1*H*-Pyrrol-2-yl)methylene]-1-(7-methoxyquinolin-4-yl)hydrazine (2g)

Yellow prisms, mp (EtOH) 136–138 °C. ^{1}H NMR (DMSO-d_6): δ 3.86 (s, 3H), 6.10–6.14 (m, 1H), 6.40–6.43 (m, 1H), 6.91–6.93 (m, 1H), 7.07 (d, J = 8.7 Hz, 1H), 7.14 (s, 1H), 7.24 (d, J = 4.9 Hz, 1H), 8.14–8.18 (m, 3H), 10.73 (br s, 1H), 11.38 (s, 1H). IR (Nujol) cm^{-1}: 3341, 3197, 1582, 1231, 1122. ESI-MS *m/z*: 267 $(M+H)^+$. Anal. Calcd for $C_{15}H_{14}N_4O$: C, 67.65; H, 5.30; N, 21.04. Found: C, 67.47; H, 5.64; N, 21.31.

5.2.8. 2-[(Naphthalen-2-yl)methylene]-1-(7-methoxyquinolin-4-yl)hydrazine (2h)

Yellow prisms, mp (EtOH) 208–210 °C. ^{1}H NMR (DMSO-d_6): δ 3.87 (s, 3H), 7.11–7.35 (m, 3H), 7.52–7.57 (m, 2H), 7.91–7.98 (m, 3H), 8.02–8.12 (m, 2H), 8.26 (d, J = 8.2 Hz, 1H), 8.52 (m, 2H), 11.18 (br s, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 160.5, 151.8, 149.9, 147.7, 143.1, 134.1, 133.8, 133.4, 129.3, 129.1, 128.8, 128.4, 128.2, 127.4, 123.7, 123.3, 117.3, 112.1, 108.5, 100.5, 56.0; IR (Nujol) cm^{-1}: 3483, 1623, 1312, 1121. ESI-MS *m/z*: 328 $(M+H)^+$. Anal. Calcd for $C_{21}H_{17}N_3O$: C, 77.04; H, 5.23; N, 12.84. Found: C, 76.79; H, 5.01; N, 12.78.

5.2.9. 2-[(6-Methoxynaphthalen-2-yl)methylene]-1-(7-methoxyquinolin-4-yl)hydrazine (2i)

Yellow prisms, mp (EtOH) 238–240 °C. ^{1}H NMR (DMSO-d_6): δ 3.88 (s, 6H), 7.18–7.21 (m, 3H), 7.35 (d, J = 2.3 Hz, 1H), 7.84–7.91 (m, 2H), 7.92–8.01 (m, 2H), 8.02 (s, 1H), 8.03 (d, J = 8.2 Hz, 1H), 8.25 (d, J = 9.0 Hz, 1H), 8.48 (s, 1H), 11.10 (br s, 1H). IR (Nujol) cm^{-1}: 3327, 1622, 1458, 1376, 1227, 1191. ESI-MS *m/z*: 358 $(M+H)^+$. Anal. Calcd for $C_{22}H_{19}N_3O_2$: C, 73.93; H, 5.36; N, 11.76. Found: C, 74.05; H, 5.69; N, 11.51.

5.2.10. 2-[(Quinolin-4-yl)methylene]-1-(7-methoxyquinolin-4-yl)hydrazine (2k)

Yellow prisms, mp (EtOH) 252–254 °C. ^{1}H NMR (DMSO-d_6): δ 3.94 (s, 3H), 7.38–7.44 (m, 2H), 7.55 (d, J = 6.8 Hz, 1H), 7.75–7.84 (m, 2H), 7.99 (d, J = 4.5 Hz, 1H), 8.10 (d, J = 8.1 Hz, 1H), 8.53–8.63 (m, 2H), 8.86 (d, J = 9.0 Hz, 1H), 8.99 (d, J = 4.5 Hz, 1H), 9.69 (s, 1H), 10.71 (br s, 1H). IR (Nujol) cm^{-1}: 3366, 1620, 1239, 1050. ESI-MS *m/z*: 329 $(M+H)^+$. Anal. Calcd for $C_{20}H_{16}N_4O$: C, 73.15; H, 4.91; N, 17.06. Found: C, 72.82; H, 4.74; N, 17.18.

5.2.11. 2-[(7-Methoxyquinolin-4-yl)methylene]-1-(7-methoxyquinolin-4-yl)hydrazine (2l)

Yellow prisms, mp (EtOH) 274–275 °C. ^{1}H NMR (DMSO-d_6): δ 3.96 (s, 6H), 7.38–7.43 (m, 2H), 7.45 (d, J = 2.6 Hz, 1H), 7.49 (d, J = 2.5 Hz, 1H), 7.52 (d, J = 6.3 Hz, 1H), 7.87 (d, J = 4.6 Hz, 1H), 8.52 (d, J = 9.2 Hz, 1H), 8.58 (br s, 1H), 8.83 (d, J = 8.1 Hz, 1H), 8.93 (d, J = 4.6 Hz, 1H), 9.63 (s, 1H), 11.25 (br s, 1H). IR (Nujol) cm^{-1}: 3137, 3065, 1626, 1458, 1450, 1234, 1166. ESI-MS *m/z*: 359 $(M+H)^+$. Anal. Calcd for $C_{21}H_{18}N_4O_2$: C, 70.38; H, 5.06; N, 15.63. Found: C, 70.47; H, 5.41; N, 15.76.

5.2.12. 2-[(Benzo[*d*][1,3]dioxol-5-yl)methylene]-1-(7-ethoxyquinolin-4-yl)hydrazine (2n)

Yellow prisms, mp (EtOH) 257–259 °C. ^{1}H NMR (DMSO-d_6): δ 1.48 (t, J = 8.2 Hz, 3H), 4.32 (q, J = 8.2 Hz, 2H), 6.11 (s, 2H), 6.98 (d, J = 7.2 Hz, 1H), 7.21 (d, J = 7.2 Hz, 1H), 7.34 (d, J = 7.4 Hz, 1H), 7.38 (br s, 1H), 7.50 (br s, 1H), 7.58 (d, J = 6.9 Hz, 1H), 8.47 (d, J = 7.4 Hz, 1H), 8.59 (s, 1H), 8.62 (d, J = 6.9 Hz, 1H), 11.26 (br s, 1H). IR (Nujol) cm^{-1}: 3182, 1461, 1376, 1149, 1110, 1067. ESI-MS *m/z*: 336 $(M+H)^+$. Anal. Calcd for $C_{19}H_{17}N_3O_3$·HCl: C, 61.38; H, 4.88; N, 11.30. Found: C, 61.49; H, 4.56; N, 11.48.

5.2.13. 2-[(Pyridin-4-yl)methylene]-1-(7-ethoxyquinolin-4-yl)hydrazine (2o)

Yellow prisms, mp (EtOH) 211–213 °C. ^{1}H NMR (DMSO-d_6): δ 1.42 (t, J = 8.6 Hz, 3H), 4.18 (q, J = 8.6 Hz, 2H), 7.11 (d, J = 7.7 Hz, 1H), 7.26–7.34 (m, 2H), 7.69 (d, J = 5.6 Hz, 2H), 7.85 (d, J = 8.1 Hz, 1H), 8.36 (m, 2H), 8.59 (d, J = 5.2 Hz, 2H), 11.11 (br s, 1H). ESI-MS *m/z*: 293 $(M+H)^+$. IR (Nujol) cm^{-1}: 3216, 1578, 1555, 1465. Anal. Calcd for $C_{17}H_{16}N_4O$: C, 69.85; H, 5.52; N, 19.17. Found: C, 69.78; H, 5.76; N, 18.87.

5.2.14. 2-[(Quinolin-2-yl)methylene]-1-(2-methyl-7-methoxyquinolin-4-yl)hydrazine (2q)

Yellow prisms, mp (EtOH) 239–232 °C. ^{1}H NMR (DMSO-d_6): δ 2.75 (s, 3H), 3.95 (s, 3H), 7.36–7.43 (m, 2H), 7.59 (s, 1H), 7.70 (d, J = 8.1 Hz, 1H), 7.80 (t, J = 8.1 Hz, 1H), 8.00–8.10 (m, 2H), 8.35 (d, J = 8.8 Hz, 1H), 8.45 (d, J = 8.1 Hz, 1H), 8.74 (d, J = 8.8 Hz, 1H), 8.93 (s, 1H), 12.90 (br s, 1H). IR (Nujol) cm^{-1}: 3064, 1458, 1376, 1161, 1064. ESI-MS *m/z*: 343 $(M+H)^+$. Anal. Calcd for $C_{21}H_{19}N_4O$·HCl: C, 65.84; H, 4.70; N, 15.36. Found: C, 65.58; H, 5.06; N, 15.17.

5.2.15. 2-[(Quinolin-4-yl)methylene]-1-(2-methyl-7-methoxyquinolin-4-yl)hydrazine (2r)

Yellow prisms, mp (EtOH) 261–263 °C. ^{1}H NMR (DMSO-d_6): δ 2.59 (s, 3H), 3.89 (s, 3H), 7.18–7.28 (m, 3H), 7.71–7.86 (m, 2H), 8.01 (d, J = 4.6 Hz, 1H), 8.08 (d, J = 8.1 Hz, 1H), 8.39 (d, J = 9.0 Hz, 1H), 8.58 (d, J = 8.0 Hz, 1H), 8.96 (d, J = 4.4 Hz, 1H), 9.24 (s, 1H), 12.75 (br s, 1H). IR (Nujol) cm^{-1}: 3216, 1621, 1414, 1258, 1046. ESI-MS *m/z*: 343 $(M+H)^+$. Anal. Calcd for $C_{21}H_{18}N_4O$: C, 73.67; H, 5.30; N, 16.36. Found: C, 73.32; H, 5.12; N, 16.55.

5.2.16. 2-(4-Methoxybenzylidene)-1-(2-methyl-7-ethoxyquinolin-4-yl)hydrazine (2s)

Yellow prisms, mp (EtOH) 280–281 °C. ^{1}H NMR (DMSO-d_6): δ 1.39 (t, 3H), 2.63 (s, 3H), 3.81 (s, 3H), 4.18 (q, 2H), 7.02 (d,

J = 8.0 Hz, 2H), 7.22–7.29 (m, 3H), 7.77 (d, J = 8.0 Hz, 2H), 8.57 (d, J = 8.9 Hz, 1H), 8.65 (s, 1H), 10.73 (br s, 1H). IR (Nujol) cm^{-1}: 3422, 1617, 1582, 1232. ESI-MS m/z: 336 $(M+H)^+$. Anal. Calcd for $C_{20}H_{21}N_3O_2$: C, 71.61; H, 6.31; N, 12.53. Found: C, 61.76; H, 6.48; N, 12.54.

5.2.17. 2-[(Benzo[*d*][1,3]dioxol-5-yl)methylene]-1-(2-methyl-7-ethoxyquinolin-4-yl)hydrazine (2t)

Yellow prisms, mp (EtOH) 257–259 °C. ^1H NMR (DMSO-d_6): δ 1.41 (t, J = 8.3 Hz, 3H), 2.67 (s, 3H), 4.19 (q, J = 8.3 Hz, 2H), 6.11 (s, 2H), 7.01 (d, J = 7.9 Hz, 1H), 7.23 (d, J = 7.9 Hz, 1H), 7.28 (d, J = 9.0 Hz, 1H), 7.31 (s, 1H), 7.37 (s, 1H), 7.54 (m, 1H), 8.58 (d, J = 9.0 Hz, 1H), 8.62 (s, 1H), 10.88 (br s, 1H). IR (Nujol) cm^{-1}: 3178, 1584, 1505. ESI-MS m/z: 350 $(M+H)^+$. Anal. Calcd for $C_{20}H_{19}N_3O_3$·HCl: C, 62.26; H, 5.22; N, 10.89. Found: C, 62.13; H, 5.45; N, 10.73.

5.2.18. 2-(Cyclohexylmethylene)-1-(6-methoxyquinolin-4-yl)hydrazine (3d)

Yellow prisms, mp (EtOH) 191–193 °C. ^1H NMR (DMSO-d_6) δ 1.19–1.38 (m, 6H), 1.49–1.82 (m, 4H), 2.15–2.26 (m, 1H), 3.84 (s, 3H), 6.95 (d, J = 4.8 Hz, 1H,), 7.05 (d, J = 9.1 Hz, 1H), 7.15 (s, 1H), 7.56 (d, J = 4.8 Hz, 1H), 8.10 (d, J = 9.1 Hz, 1H), 8.33 (s, 1H), 11.87 (br s, 1H). IR (Nujol) cm^{-1}: 3307, 1615, 1587, 1408, 1222, 1064. ESI-MS m/z: 284 $(M+H)^+$. Anal. Calcd for $C_{17}H_{21}N_3O$: C, 72.06; H, 7.47; N, 14.83. Found: C, 72.00; H, 7.78; N, 14.55.

5.2.19. 2-(4-Dimethylaminobenzylidene)-1-(2-methyl-6-methoxyquinolin-4-yl)hydrazine (3g)

Yellow prisms, mp (EtOH) 147–149 °C. ^1H NMR (DMSO-d_6): δ 2.60 (s, 3H), 2.97 (s, 6H), 3.94 (s, 3H), 6.76 (d, J = 8.7 Hz, 2H), 7.24 (s, 1H), 7.43 (m, 1H), 7.62 (d, J = 8.5 Hz, 2H), 7.79 (d, 1H), 7.99 (m, 1H), 8.62 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 21.5, 21.9, 22.6, 56.8, 100.1, 103.0, 111.5, 112.3, 116.0, 121.9, 123.4, 124.3, 129.0, 137.3, 148.6, 149.6, 152.1, 153.3, 157.1. IR (Nujol) cm^{-1}: 3156, 3084, 1459, 1376, 1247, 1170. ESI-MS m/z: 335 $(M+H)^+$. Anal. Calcd for $C_{20}H_{22}N_4O$: C, 71.83; H, 6.63; N, 16.75. Found: C, 71.97; H, 6.27; N, 16.93.

5.2.20. 2-[(Benzo[*d*][1,3]dioxol-5-yl)methylene]-1-(8-methoxyquinolin-4-yl)hydrazine (3i)

Yellow prisms, mp (EtOH) 220–221 °C. ^1H NMR (DMSO-d_6): δ 3.91 (s, 3H), 6.07 (s, 2H), 6.96 (d, J = 7.8 Hz, 1H), 7.10 (d, J = 7.2 Hz, 1H), 7.17 (d, J = 7.8 Hz, 2H), 7.40 (m, 2H), 7.81 (d, J = 8.0 Hz, 1H), 8.31 (s, 1H), 8.47 (s, 1H), 10.88 (br s, 1H). IR (Nujol) cm^{-1}: 3181, 1577, 1503, 1251, 1100, 1037. ESI-MS m/z: 322 $(M+H)^+$. Anal. Calcd for $C_{18}H_{13}N_3O_3$: C, 67.28; H, 4.71; N, 13.08. Found: C, 67.19; H, 4.40; N, 12.85.

5.2.21. 2-[(Naphthalen-1-yl)methylene]-1-(8-methoxyquinolin-4-yl)hydrazine (3k)

Yellow prisms, mp (EtOH) 286–288 °C. ^1H NMR (DMSO-d_6): δ 3.99 (s, 3H), 7.30 (d, J = 7.8 Hz, 1H), 7.40 (d, J = 6.5 Hz, 1H), 7.50 (d, J = 8.2 Hz, 1H), 7.50–7.70 (m, 3H), 7.99–8.10 (m, 4H), 8.24 (d, J = 6.1 Hz, 1H), 8.80 (d, J = 8.2 Hz, 1H), 9.30 (s, 1H), 10.98 (br s, 1H). IR (Nujol) cm^{-1}: 3122, 1611, 1461, 1217. ESI-MS m/z: 328 $(M+H)^+$. Anal. Calcd for $C_{21}H_{17}N_3O$: C, 77.04; H, 5.23; N, 12.84. Found: C, 73.82; H, 4.92; N, 13.01.

5.2.22. 2-[(Quinolin-2-yl)methylene]-1-(8-methoxyquinolin-4-yl)hydrazine (3l)

Yellow prisms, mp (EtOH) 226–229 °C. ^1H NMR (DMSO-d_6): δ 3.95 (s, 3H), 7.17 (d, J = 7.7 Hz, 1H), 7.39–7.62 (m, 3H), 7.76 (t, J = 7.7 Hz, 2H), 7.89–8.03 (m, 3H), 8.25 (d, J = 8.5 Hz, 1H), 8.37 (d, J = 8.5 Hz, 1H), 8.54 (s, 1H), 11.28 (br s, 1H). IR (Nujol) cm^{-1}: 3436, 1627, 1485. ESI-MS m/z: 328 $(M+H)^+$. Anal. Calcd for $C_{20}H_{16}N_4O$: C, 73.15; H, 4.91; N, 17.06. Found: C, 72.75; H, 5.19; N, 17.22.

5.2.23. 2-[(Benzo[*d*][1,3]dioxol-5-yl)methylene]-1-(2-methyl-8-methoxyquinolin-4-yl)hydrazine (3m)

Yellow prisms, mp (EtOH) 290–292 °C. ^1H NMR (DMSO-d_6): δ 2.75 (s, 3H), 4.07 (s, 3H), 6.11 (s, 2H), 7.01 (d, J = 8.0 Hz, 1H), 7.21 (d, J = 8.0 Hz, 1H), 7.51 (d, J = 7.8 Hz, 1H), 7.56 (s, 1H), 7.57 (s, 1H), 7.62 (t, J = 8.0 Hz, 1H), 8.29 (d, J = 8.0 Hz, 1H), 8.76 (s, 1H), 12.61 (br s, 1H). IR (Nujol) cm^{-1}: 3437, 1610, 1455, 1262, 1098. ESI-MS m/z: 336 $(M+H)^+$. Anal. Calcd for $C_{19}H_{17}N_3O_3$: C, 68.05; H, 5.11; N, 12.53. Found: C, 68.01; H, 5.44; N, 12.82.

5.2.24. 2-[(Naphthalen-1-yl)methylene]-1-(2-methyl-8-methoxyquinolin-4-yl)hydrazine (3n)

Yellow prisms, mp (EtOH) 278–279 °C. ^1H NMR (DMSO-d_6): δ 2.51 (s, 3H), 3.92 (s, 3H), 7.12 (d, J = 7.9 Hz, 1H), 7.18 (br s, 1H), 7.32 (t, J = 8.0 Hz, 1H), 7.58–7.71 (m, 3H), 7.84 (d, J = 8.3 Hz, 1H), 7.94–8.06 (m, 3H), 8.70 (d, J = 8.5 Hz, 1H), 9.10 (s, 1H), 10.86 (br s, 1H). IR (Nujol) cm^{-1}: 1461, 1376. ESI-MS m/z: 342 $(M+H)^+$. Anal. Calcd for $C_{22}H_{19}N_3O$: C, 77.40; H, 5.61; N, 12.31. Found: C, 77.58; H, 5.38; N, 12.48.

5.2.25. 2-[(Quinolin-4-yl)methylene]-1-(2-methyl-8-methoxyquinolin-4-yl)hydrazine (3p)

Yellow prisms, mp (EtOH) 311–313 °C. ^1H NMR (DMSO-d_6): δ 2.79 (s, 3H), 4.10 (s, 3H), 7.56–7.88 (m, 5H), 8.08–8.15 (m, 3H), 8.55 (d, J = 8.0 Hz, 1H), 9.04 (d, J = 4.0 Hz, 1H), 9.47 (s, 1H), 12.62 (br s, 1H). IR (Nujol) cm^{-1}: 3298, 1596, 1459, 1376, 1172. ESI-MS m/z: 343 $(M+H)^+$. Anal. Calcd for $C_{21}H_{18}N_4O$: C, 73.67; H, 5.30; N, 16.36. Found: C, 73.48; H, 5.16; N, 16.11.

5.2.26. 2-(Cyclohexylmethylene)-1-([1,3]dioxolo[4,5-*g*]quinolin-8-yl)hydrazine (4b)

Yellow prisms, mp (EtOH) 223–225 °C. ^1H NMR (DMSO-d_6): δ 1.18–1.34 (m, 6H), 1.62–1.85 (m, 4H), 2.28–2.96 (m, 1H), 6.15 (s, 2H), 7.03 (d, J = 5.2 Hz, 1H), 7.16 (s, 1H), 7.55 (d, J = 4.9 Hz, 1H), 7.63 (s, 1H), 8.26 (d, J = 4.9 Hz, 1H), 10.32 (br s, 1H). IR (Nujol) cm^{-1}: 3261, 1616, 1589, 1408, 1398, 1272, 1036. ESI-MS m/z: 298 $(M+H)^+$. Anal. Calcd for $C_{17}H_{19}N_3O_2$: C, 68.67; H, 6.44; N, 14.13. Found: C, 68.87; H, 6.81; N, 13.85.

5.2.27. 2-Benzylidene-1-(6-methyl[1,3]dioxolo[4,5-*g*]quinolin-8-yl)hydrazine (4c)

Yellow prisms, mp (EtOH) 226–227 °C. ^1H NMR (DMSO-d_6): δ 2.41 (s, 3H), 6.08 (s, 2H), 7.05 (s, 1H), 7.10 (s, 1H), 7.28–7.32 (m, 1H), 7.37 (t, J = 7.3 Hz, 2H), 7.60 (s, 1H), 7.68 (d, J = 7.2 Hz, 2H), 8.24 (s, 1H), 10.71 (br s, 1H). IR (Nujol) cm^{-1}: 3328, 3181, 1462, 1455, 1377, 1246, 1140, 1040. ESI-MS m/z: 306 $(M+H)^+$. Anal. Calcd for $C_{18}H_{15}N_3O_2$: C, 70.81; H, 4.95; N, 13.76. Found: C, 70.68; H, 5.21; N, 13.81.

5.2.28. 2-(Cyclohexylmethylene)-1-(6-methyl[1,3]dioxolo[4,5-*g*]quinolin-8-yl)hydrazine (4d)

Yellow prisms, mp (EtOH) 155–158 °C; ^1H NMR (DMSO-d_6): δ 1.38–2.08 (m, 10H), 2.67 (m, 4H), 6.37 (s, 2H), 7.19 (s, 1H), 7.35 (s, 1H), 7.85 (d, J = 5.2 Hz, 1H), 7.91 (s, 1H), 10.88 (br s, 1H). IR (Nujol) cm^{-1}: 3349, 1626, 1560, 1508, 1478, 1405, 1272, 1036. ESI-MS m/z: 312 $(M+H)^+$. Anal. Calcd for $C_{18}H_{21}N_3O_2$: C, 69.43; H, 6.80; N, 13.49. Found: C, 69.68; H, 7.16; N, 13.38.

5.2.29. 2-(4-Methoxybenzylidene)-1-(6-methyl[1,3]dioxolo[4,5-*g*]quinolin-8-yl)hydrazine (4e)

Yellow prisms, mp (EtOH) 251–253 °C. ^1H NMR (DMSO-d_6): δ 2.39 (s, 3H), 3.72 (s, 3H), 6.06 (s, 2H), 6.92 (d, J = 8.8 Hz, 2H), 7.02–7.09 (m, 2H), 7.58 (s, 1H), 7.62 (d, J = 8.8 Hz, 2H), 8.17

(s, 1H), 10.48 (br s, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 25.5, 55.9, 98.1, 100.7, 102.3, 105.8, 111.2, 115.0, 128.4, 128.6, 142.5, 146.2, 147.1, 147.2, 150.3, 157.2, 160.8. IR (Nujol) cm^{-1}: 3119, 1461, 1377, 1207, 1131. ESI-MS *m/z*: 336 (M+H)$^+$. Anal. Calcd $C_{19}H_{17}N_3O_3$: C, 68.05; H 5.11; N 12.53. Found: C, 67.99; H 5.40; N 12.42.

5.2.30. 2-(4-Hydroxybenzylidene)-1-(6-methyl[1,3]dioxolo[4,5-*g*]quinolin-8-yl)hydrazine (4f)

Yellow prisms, mp (EtOH) 265–268 °C. ^{1}H NMR (DMSO-d_6): δ 2.47 (s, 3H), 6.15 (s, 2H), 6.82 (d, J = 8.3 Hz, 2H), 7.12 (d, J = 3.6 Hz, 2H), 7.59 (d, J = 8.3 Hz, 2H), 7.70 (s, 1H), 8.25 (s, 1H), 9.81 (br s, 1H), 10.98 (br s, 1H). IR (Nujol) cm^{-1}: 3351, 3122, 1609, 1458, 1251. ESI-MS *m/z*: 322 (M+H)$^+$. Anal. Calcd for $C_{18}H_{15}N_3O_3$: C, 67.28; H, 4.71; N, 13.08. Found: C, 67.37; H, 4.93; N, 13.23.

5.2.31. 2-(3-Hydroxybenzylidene)-1-(6-methyl[1,3]dioxolo[4,5-*g*]quinolin-8-yl)hydrazine (4h)

Yellow prisms, mp (EtOH) 182–185 °C. ^{1}H NMR (DMSO-d_6): δ 2.46 (s, 3H), 6.13 (s, 2H), 6.75 (d, J = 7.3 Hz, 1H), 7.08–7.25 (m, 5H), 7.64 (s, 1H), 8.19 (s, 1H), 9.52 (s, 1H), 10.62 (br s, 1H). IR (Nujol) cm^{-1}: 3637, 3446, 3254, 1461, 1229, 1134, 1040. ESI-MS *m/z*: 322 (M+H)$^+$. Anal. Calcd for $C_{18}H_{15}N_3O_3$: C, 67.28; H, 4.71; N, 13.08. Found: C, 67.44; H, 4.53; N, 13.42.

5.2.32. 2-[(Naphthalen-2-yl)methylene]-1-(6-methyl[1,3]dioxolo[4,5-*g*]quinolin-8-yl)hydrazine (4m)

Yellow prisms, mp (EtOH) 282–285 °C. ^{1}H NMR (DMSO-d_6): δ 2.49 (s, 3H), 6.14 (s, 2H), 7.12 (s, 1H), 7.23 (s, 1H), 7.47–7.57 (m, 2H), 7.69 (s, 1H), 7.88–7.98 (m, 3H), 8.06 (d, J = 6.5 Hz, 2H), 8.45 (s, 1H), 10.88 (br s, 1H). IR (Nujol) cm^{-1}: 3339, 1615, 1219. ESI-MS *m/z*: 356 (M+H)$^+$. Anal. Calcd for $C_{22}H_{17}N_3O_2$: C, 74.35; H, 4.82; N, 11.82. Found: C, 74.29; H, 4.47; N, 11.96.

5.2.33. 2-[(6-Methoxynaphthalen-2-yl)methylene]-1-(6-methyl-[1,3]dioxolo[4,5-*g*]quinolin-8-yl)hydrazine (4n)

Yellow prisms, mp (EtOH) 273–276 °C. ^{1}H NMR (DMSO-d_6): δ 2.52 (s, 3H), 3.88 (s, 3H), 6.18 (s, 2H), 7.14–7.35 (m, 4H), 7.74 (s, 1H), 7.87 (t, J = 5.6 Hz, 2H), 8.03 (d, J = 5.6 Hz, 2H), 8.46 (s, 1H), 11.08 (br s, 1H). IR (Nujol) cm^{-1}: 3357, 1606, 1248, 1108. ESI-MS *m/z*: 386 (M+H)$^+$. Anal. Calcd for $C_{23}H_{19}N_3O_3$: C, 71.67; H, 4.97; N, 10.90, C 71.55; H, 5.34; N, 10.98.

5.2.34. 2-[(2-Methoxynaphthalen-1-yl)methylene]-1-(6-methyl-[1,3]dioxolo[4,5-*g*]quinolin-8-yl)hydrazine (4o)

Yellow prisms, mp (EtOH) 287–290 °C. ^{1}H NMR (DMSO-d_6): δ 2.47 (s, 3H), 4.00 (s, 3H), 6.14 (s, 2H), 7.10 (d, J = 8.7 Hz, 2H), 7.38–7.53 (m, 2H), 7.63–7.67 (m, 1H), 7.76 (s, 1H), 7.90 (d, J = 8.1 Hz, 1H), 7.98 (d, J = 9.2 Hz, 1H), 9.03 (s, 1H), 9.26 (d, J = 8.6 Hz, 1H), 10.76 (br s, 1H). IR (Nujol) cm^{-1}: 3339, 1615, 1219. ESI-MS *m/z*: 386 (M+H)$^+$. Anal. Calcd for $C_{23}H_{19}N_3O_3$: C, 71.67; H, 4.97; N, 10.90. Found: C, 71.88; H, 4.65; N, 11.28.

5.2.35. 2-[(Pyridin-4-yl)methylene]-1-(6-methyl[1,3]dioxolo-[4,5-*g*]quinolin-8-yl)hydrazine (4r)

Yellow prisms, mp (EtOH) 289–291 °C. ^{1}H NMR (DMSO-d_6): δ 2.50 (s, 3H), 6.16 (s, 2H), 7.16 (s, 1H), 7.25 (s, 1H), 7.67 (s, 1H), 7.69 (d, J = 4.2 Hz, 2H), 8.25 (s, 1H), 8.61 (d, J = 4.2 Hz, 2H), 11.03 (br s, 1H). IR (Nujol) cm^{-1}: 3502, 3087, 1601, 1574, 1123, 1056. ESI-MS *m/z*: 307 (M+H)$^+$. Anal. Calcd for $C_{17}H_{14}N_4O_2$: C, 66.66; H, 4.61; N, 18.29. Found: C, 66.50; H, 4.94; N, 18.16.

5.2.36. 2-[(1*H*-Pyrrol-2-yl)methylene]-1-(6-methyl[1,3]dioxolo[4,5-*g*]quinolin-8-yl)hydrazine (4t)

Yellow prisms, mp (EtOH) 140–143 °C. ^{1}H NMR (DMSO-d_6): δ 2.43 (s, 3H), 6.11 (m, 4H), 6.37 (s, 1H), 6.89 (s, 1H), 7.08 (s, 1H), 7.20 (s, 1H), 7.59 (s, 1H), 8.12 (s, 1H), 11.32 (br s, 1H). IR (Nujol) cm^{-1}: 3400, 3301, 1560, 1212, 1137. ESI-MS *m/z*: 295 (M+H)$^+$. Anal. Calcd for $C_{16}H_{14}N_4O_2$: C, 65.30; H, 4.79; N, 19.04. Found: C, 65.59; H, 5.00; N, 18.86.

5.2.37. 2-[(Imidazol-2-yl)methylene]-1-(6-methyl[1,3]dioxolo[4,5-*g*]quinolin-8-yl)hydrazine (4u)

^{1}H NMR (DMSO-d_6): δ 2.46 (s, 3H), 6.13 (s, 2H), 7.10 (s, 3H), 7.30 (s, 1H), 7.56 (s, 1H), 8.16 (s, 1H), 10.70 (br s, 1H), 12.50 (br s, 1H). IR (Nujol) cm^{-1}: 3117, 3057, 1622, 1475, 1462. ESI-MS *m/z*: 296 (M+H)$^+$. Anal. Calcd for $C_{15}H_{13}N_5O_2$: C, 61.01; H, 4.44; N, 23.72. Found: C, 61.28; H, 4.49; N, 23.98.

5.3. In vitro pharmacology

5.3.1. In vitro evaluation of antitubercular activity against *M. tuberculosis* H37Rv and in vitro evaluation of cytotoxicity

The in vitro evaluation of antitubercular activity against *M. tuberculosis* H37Rv was carried out at the GWL Hansen's Disease Center within the TAACF (Tuberculosis Antimicrobial Acquisition Coordinating Facility) screening program for the discovery of novel drugs for treatment of TB under the direction of the US National Institute of Allergy and Infectious Diseases (NIAID), Southern Research Institute coordinates the overall program. The purpose of the screening program is to provide a resource whereby new experimental compounds can be tested for their capacity to inhibit the growth of virulent *M. tuberculosis*. Primary screening was conducted at 6.25 μg/mL against *M. tuberculosis* H37Rv in BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA).[42] Compounds exhibiting fluorescence were also tested in the BACTEC 460 radiometric system.[43] Compounds showing >90% inhibition in the primary screening were considered active and then retested at lower concentration against *M. tuberculosis* H37Rv to determine the actual minimum inhibitory concentration (MIC), using MABA. The MIC is defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to the controls.

Compounds were tested also for cytotoxicity (IC_{50}) in the VERO cell line. After 72 h exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega Cell Titer 96 Non-radioactive Cell Proliferation assay. Rifampin (RMP) was used as reference compound.

5.3.2. Time-course experiment and in vitro evaluation of antitubercular activity of 4c against clinical isolates

Activity of compound **4c** against *mtb* H37Rv was also assessed in a time-course experiment. Briefly, a growth curve of *mtb* H37Rv was established by seeding 30 mL of 7H9/ADC/Tween80 with 1000 CFU/mL. The culture was incubated at 37 °C and 7 d later the culture was split in four flasks and drugs added at the MIC, sub-MIC and twice the MIC. The fourth flask was used as a control and drug was not added. On days 11 and 14, serial dilutions of the culture were made in saline and 0.05 mL of these were plated in 7H11/OADC solid media. Fourteen days later colonies were counted to determine the colony forming units (CFU).

In vitro evaluation of antitubercular activity against clinical isolates was performed at Institute of Microbiology, Catholic University of the Sacred Heart using the Microplate blue alamar assay. Briefly, serial dilutions of the compound were added to a liquid media following and anti-tubercular activity assessed as indicated by Collins et al.[42]

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References and notes

1. http://www.who.int/tb/en/.
2. Migliori, G. B.; Ambrosetti, M.; Fattorini, L.; Penati, V.; Vaccarino, P.; Besozzi, G.; Ortona, L.; Saltini, C.; Orefici, G.; Moro, M. L.; Lona, E.; Cassone, A. *Int. J. Tuberc. Lung. Dis.* **2000**, *4*, 940.
3. Iseman, M. D. *Eur. Respir. J. Suppl.* **2002**, *36*, 87s.
4. Harries, A. D. M. D. *TB/HIV, A Clinical Manual*; World Health Organization: Geneve, 1996.
5. Nunn, P.; Williams, B.; Floyd, K.; Dye, C.; Elzinga, G.; Raviglione, M. *Nat. Rev. Immunol.* **2005**, *5*, 819.
6. Banerjee, A.; Dubnau, E.; Quemard, A.; Balasubramanian, V.; Um, K. S.; Wilson, T.; Collins, D.; de Lisle, G.; Jacobs, W. R., Jr. *Science* **1994**, *263*, 227.
7. Daffe, M.; Draper, P. *Adv. Microb. Physiol.* **1998**, *39*, 131.
8. Dessen, A.; Quemard, A.; Blanchard, J. S.; Jacobs, W. R., Jr.; Sacchettini, J. C. *Science* **1995**, *267*, 1638.
9. Mdluli, K.; Slayden, R. A.; Zhu, Y.; Ramaswamy, S.; Pan, X.; Mead, D.; Crane, D. D.; Musser, J. M.; Barry, C. E., 3rd *Science* **1998**, *280*, 1607.
10. Zuber, B.; Chami, M.; Houssin, C.; Dubochet, J.; Griffiths, G.; Daffe, M. *J. Bacteriol.* **2008**, *190*, 5672.
11. Hoffmann, C.; Leis, A.; Niederweis, M.; Plitzko, J. M.; Engelhardt, H. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 3963.
12. Fraser-Reid, B.; Tatsuta, K.; Thiem, J. In *Glycoscience: Chemistry and Chemical Biology*; Lowary, T. L., Ed.; Springer: Berlin, 1991; p 2005.
13. Brennan, P. J. *Tuberculosis (Edinb)* **2003**, *83*, 91.
14. Nguyen, M.; Claparols, C.; Bernadou, J.; Meunier, B. *Chembiochem.* **2001**, *2*, 877.
15. Rozwarski, D. A.; Grant, G. A.; Barton, D. H.; Jacobs, W. R., Jr.; Sacchettini, J. C. *Science* **1998**, *279*, 98.
16. Zhang, Y.; Heym, B.; Allen, B.; Young, D.; Cole, S. *Nature* **1992**, *358*, 591.
17. Bodiguel, J.; Nagy, J. M.; Brown, K. A.; Jamart-Gregoire, B. *J. Am. Chem. Soc.* **2001**, *123*, 3832.
18. Lei, B.; Wei, C. J.; Tu, S. C. *J. Biol. Chem.* **2000**, *275*, 2520.
19. Deretic, V.; Pagan-Ramos, E.; Zhang, Y.; Dhandayuthapani, S.; Via, L. E. *Nat. Biotechnol.* **1996**, *14*, 1557.
20. Shoeb, H. A.; Bowman, B. U., Jr.; Ottolenghi, A. C.; Merola, A. J. *Antimicrob. Agents Chemother.* **1985**, *27*, 399.
21. Shoeb, H. A.; Bowman, B. U., Jr.; Ottolenghi, A. C.; Merola, A. J. *Antimicrob. Agents Chemother.* **1985**, *27*, 404.
22. Shoeb, H. A.; Bowman, B. U., Jr.; Ottolenghi, A. C.; Merola, A. J. *Antimicrob. Agents Chemother.* **1985**, *27*, 408.
23. Sinha, B. K. *J. Biol. Chem.* **1983**, *258*, 796.
24. Richardson, D. R.; Sharpe, P. C.; Lovejoy, D. B.; Senaratne, D.; Kalinowski, D. S.; Islam, M.; Bernhardt, P. V. *J. Med. Chem.* **2006**, *49*, 6510.
25. Sarel, S.; Fizames, C.; Lavelle, F.; Avramovici-Grisaru, S. *J. Med. Chem.* **1999**, *42*, 242.
26. Al-Sha'alan, N. H. *Molecules* **2007**, *12*, 1080.
27. El-Behery, M.; El-Twigry, H. *Spectrochim. Acta. Mol. Biomol. Spectrosc.* **2007**, *66*, 28.
28. Tamasi, G.; Chiasserini, L.; Savini, L.; Sega, A.; Cini, R. *J. Inorg. Biochem.* **2005**, *99*, 1347.
29. Fattorusso, C.; Campiani, G.; Kukreja, G.; Persico, M.; Butini, S.; Romano, M. P.; Altarelli, M.; Ros, S.; Brindisi, M.; Savini, L.; Novellino, E.; Nacci, V.; Fattorusso, E.; Parapini, S.; Basilico, N.; Taramelli, D.; Yardley, V.; Croft, S.; Borriello, M.; Gemma, S. *J. Med. Chem.* **2008**, *51*, 1333.
30. Savini, L.; Chiasserini, L.; Gaeta, A.; Pellerano, C. *Bioorg. Med. Chem.* **2002**, *10*, 2193.
31. Jayaprakash, S.; Iso, Y.; Wan, B.; Franzblau, S. G.; Kozikowski, A. P. *ChemMedChem.* **2006**, *1*, 593.
32. Mao, J.; Wang, Y.; Wan, B.; Kozikowski, A. P.; Franzblau, S. G. *ChemMedChem.* **2007**, *2*, 1624.
33. Nayyar, A.; Malde, A.; Coutinho, E.; Jain, R. *Bioorg. Med. Chem.* **2006**, *14*, 7302.
34. Actor, P. P.; Pellerano, C. E. G. U.S. Patent 3,646,019, February 29, 1972.
35. Pellerano, C.; Savini, L. *Boll. Chim. Farm.* **1983**, *122*, 582.
36. Pellerano, C.; Savini, L. *Farmaco. [Sci.]* **1984**, *39*, 640.
37. Thomas, J.; Berkoff, C. E.; Flagg, W. B.; Gallo, J. J.; Haff, R. F.; Pinto, C. A. *J. Med. Chem.* **1975**, *18*, 245.
38. Gemma, S.; Kukreja, G.; Tripaldi, P.; Altarelli, M.; Bernetti, M.; Franceschini, S.; Savini, L.; Campiani, G.; Fattorusso, C.; Butini, S. *Tetrahedron Lett.* **2008**, *49*, 2074.
39. Pellerano, C.; Brizzi, V.; Savini, L. *Atti. Acc. Fisiocr. Siena.* **1971**, *3*, 253.
40. Dolle, R. E. S.; Stanley, J.; Kruse, I. L. *J. Chem. Soc., Chem. Commun.* **1987**, 904.
41. Pellerano, C.; Savini, L.; Brizzi, V. *Farmaco. [Sci]* **1985**, *40*, 486.
42. Collins, L.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **1997**, *41*, 1004.
43. Franzblau, S. G.; Witzig, R. S.; McLaughlin, J. C.; Torres, P.; Madico, G.; Hernandez, A.; Degnan, M. T.; Cook, M. B.; Quenzer, V. K.; Ferguson, R. M.; Gilman, R. H. *J. Clin. Microbiol.* **1998**, *36*, 362.