Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 12 (2004) 2501–2508
Bioorganic & Medicinal Chemistry

Ring-substituted quinolines as potential anti-tuberculosis agents ☆

Suryanarayana Vangapandu,[a,1] Meenakshi Jain,[a] Rahul Jain,[a,*] Sukhraj Kaur[b] and Prati Pal Singh[b]

[a]*Department of Medicinal Chemistry, National Institute of Pharmaceutical Education and Research, Sector 67, S.A.S. Nagar, Punjab 160 062, India*
[b]*Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research, Sector 67, S.A.S. Nagar, Punjab 160 062, India*

Received 8 March 2004; revised 19 March 2004; accepted 19 March 2004

Available online 10 April 2004

Abstract—We report in vitro antimycobacterial properties of ring-substituted quinolines (series 1–4) constituting 56 analogues against drug-sensitive and drug-resistant *M. tuberculosis H37Rv* strains. The most effective compounds **2h** ($R_1 = R_2 =$ *c*-C_6H_{11}, $R_3 = NO_2$, series 1) and **13g** ($R_1 = OC_7H_{15}$, $R_2 = NO_2$, series 4) have exhibited an MIC value of 1 μg/mL against drug-sensitive *M. tuberculosis H37Rv* strain that is comparable to first line anti-tuberculosis drug, isoniazid. Selected analogues (**2d**, **2g**, **2h**, **4e**, **6b**, **13b**, **13g**, and **14e**, MIC: ⩽6.25 μg/mL) upon further evaluation against single-drug-resistant (SDR) strains of *M. tuberculosis H37Rv* have produced potent efficacy in the range between 6.25 and 50 μg/mL.

1. Introduction

Development of resistance to existing drugs is a constant growing phenomenon that has concerned researchers throughout the world, and now has reached alarming levels for certain infectious diseases. This combined with the recent decline in the development of new drugs to combat them, can be anticipated to lead to infectious diseases lacking ready treatment regimens. The World Health Organization (WHO) estimates that there were 8.4 million new cases of tuberculosis in 1999, and that the annual global rate of increase in tuberculosis incidence was 3%.[1] Assuming this rate of increase persists, there will be over 10 million new cases of tuberculosis in 2005.[1] The emergence of multi-drug-resistant (MDR) strains of tuberculosis is of special concern due to the reported high death rates associated with MDR tuberculosis.[2–5] In addition, the current treatment regimens of MDR strains are approximately 100 times more expensive compared to drug-susceptible strains with only 50% survival rates.[2] Furthermore, the recent resurgence in the incidence of tuberculosis in association with human immunodeficiency virus (HIV) infection and AIDS warrants the development of new therapeutic strategies for the effective control of tuberculosis. In short, tuberculosis continues to be a public health threat, to the extent that the WHO has declared tuberculosis a global public health emergency in 1993.[1] Excellent drugs have been available for the treatment of tuberculosis in the past, but their clinical utility has been severely diminished due to the development of resistance to cheap and most effective agents such as isoniazid (INH).[6] Thus, there is an urgent need of new anti-tuberculosis agents with structural features different from that of existing drugs. However, no new chemotherapeutic agent specifically directed against tuberculosis has been introduced in the past 40 years.

2. Discussion

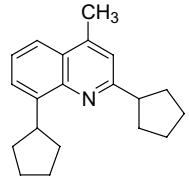
Our research efforts toward development of novel anti-tuberculosis agents are in the direction of discovering new classes of compounds, which are structurally different from known anti-tuberculosis drugs. It is expected that this approach will offer analogues, which may be effective against drug-resistant strains of *M. tuberculosis*.

Keywords: Ring-substituted quinolines; Tuberculosis; Drug-resistant tuberculosis; Homolytic free radical alkylation.
☆ NIPER communication no. 262.
* Corresponding author. Tel.: +91-0172-221-4682; fax: +91-0172-221-4692; e-mail: rahuljain@niper.ac.in
† Present address: PO Box 4356, University of Mississippi, University, MS 38677, USA.

0968-0896/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2004.03.045

Toward this end, through a structural-directed anti-tuberculosis drug-screening program, we have recently discovered that quinolines bearing various alkyl and cycloalkyl groups placed at the appropriate positions on the ring have potent anti-tuberculosis activities.[7] The most effective compound, 2,8-dicyclopentyl-4-methylquinoline (Fig. 1) of the series has exhibited potent anti-tuberculosis activities against both drug-sensitive and drug-resistant *M. tuberculosis*. Moreover, 2,8-dicyclopentyl-4-methylquinoline was synthesized in a single simplified step, and thus, in our opinion is an ideal lead to emulate when designing new analogues, and may be considered an excellent lead prototype for the development of congeners with potent antimycobacterial activities. We believe that the promising anti-tuberculosis activities demonstrated by this class of compounds has primarily emanated due to the presence of metabolically stable cyclic alkyl groups at various position in the quinoline ring. However, all of the compounds belonging to this class had the inherent drawback, chiefly, due to the absence of easily synthetically negotiable groups at the various positions of the quinoline ring.

Therefore, we focused our research efforts toward synthesis of cyclic/branched alkyl groups containing quinoline analogues that also have synthetically negotiable groups in the ring as the replacement of the unfavorable 4-methyl group. In our on-going pursuit toward development of novel anti-tuberculosis analogues, herein, we report synthesis and biological activities of ring-substituted-8-nitroquinolines and ring-substituted-8-quinolinamines (series 1–4) having various substitutions like alkyl, cycloalkyl, and alkoxy groups in the ring (Fig. 2). It is worthwhile to mention that many of these analogues were initially synthesized as the precursors for targeted antimalarials, and we were interested to screen them for their anti-tuberculosis activities.



2,8-Dicyclopentyl-4-methylquinoline

Figure 1.

3. Chemistry

All requisite ring-substituted-8-nitroquinolines (**2a**–**h**, series 1) were synthesized from commercially available 8-nitroquinoline (**1**) in a single step (Scheme 1). Thus, 8-nitroquinoline (**1**) upon reaction with various commercially available alkyl/cycloalkylcarboxylic acids in the presence of ammonium persulfate and catalytic amount of silver nitrate in 10% sulfuric acid and acetonitrile as solvent provided an easily separable mixture of 2-substituted and 2,4-disubstituted-8-nitroquinoline (**2a**–**h**) in good yield. The reaction, known to proceed through a homolytic free radical mechanism is highly efficient in introducing otherwise difficult, and previously unrealizable bulky alkyl/cycloalkyl groups directly into the quinoline ring.[7] Catalytic hydrogenation of ring-substituted-8-nitroquinolines (**2**) dissolved in 95% ethyl alcohol by raney-nickel (T_1 grade) in a Parr hydrogenator for 45 min at 45 psi and ambient temperature gave the corresponding ring-substituted-8-quinolinamines (**3a**–**c**) in quantitative yield. Finally, *N*-acetyl and *N*-trifluoroacetyl amides (**4a**–**e**) were obtained by the reaction of selected ring-substituted-8-quinolinamines (**3**) with acetic anhydride in pyridine or with trifluoroacetic anhydride at ambient temperature for 24 h (Scheme 1).

Similarly, 2-substituted/2,5-disubstituted-6-methoxy-8-nitroquinolines (**6a**–**i**, series 2, Scheme 2),[8] and 2-substituted-5,6-dimethoxy-8-nitroquinolines (**10a**–**b**, series 3, Scheme 3)[8] were synthesized from commercially available 6-methoxy-8-nitroquinoline (**5**), and 5,6-dimethoxy-8-nitroquinoline (**9**) in one step, and subse-

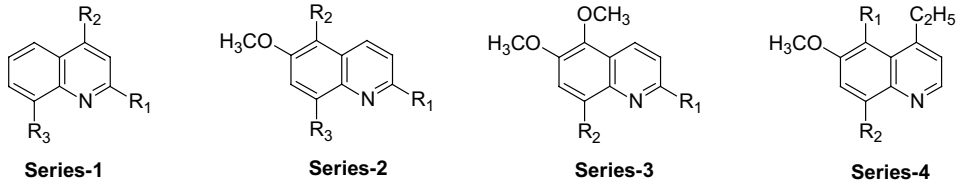
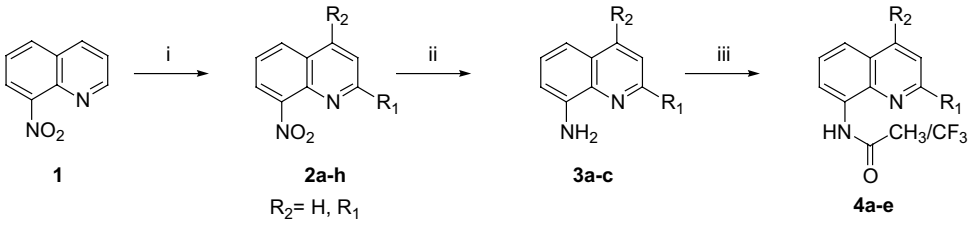
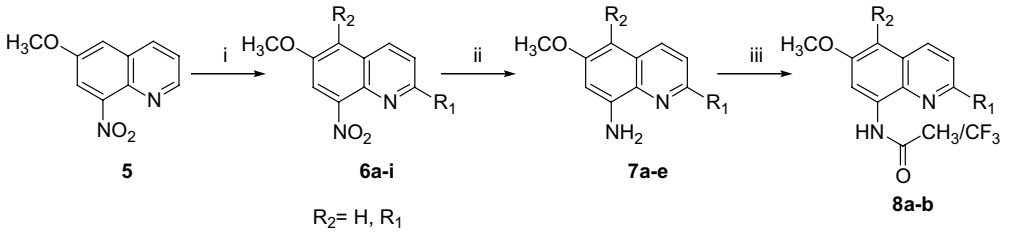


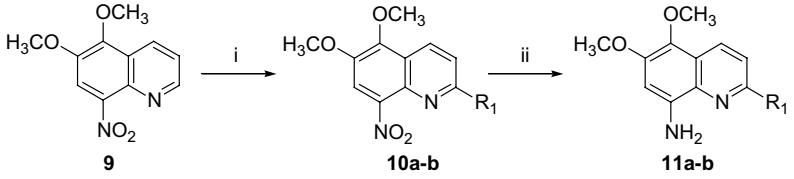
Figure 2.



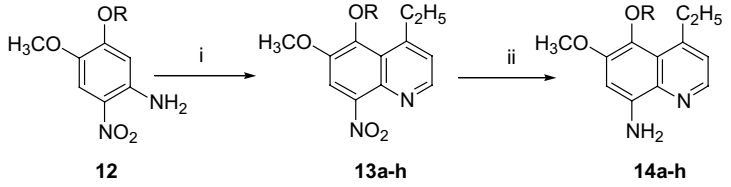
Scheme 1. Reagents and conditions: (i) R_1CO_2H, $AgNO_3$, $(NH_4)_2S_2O_8$, 10% H_2SO_4, CH_3CN, 70–80 °C; (ii) raney-Ni/H_2, EtOH, 45 psi, rt; (iii) $(CH_3CO)_2O/C_5H_5N$ or $(CF_3CO)_2O$, rt.



Scheme 2. Reagents and conditions: (i) R_1CO_2H, $AgNO_3$, $(NH_4)_2S_2O_8$, 10% H_2SO_4, CH_3CN, 70–80 °C; (ii) raney-Ni/H_2, EtOH, 45 psi, rt; (iii) $(CH_3CO)_2O/C_5H_5N$ or $(CF_3CO)_2O$, rt.



Scheme 3. Reagents and conditions: (i) R_1CO_2H, $AgNO_3$, $(NH_4)_2S_2O_8$, 10% H_2SO_4, CH_3CN, 70–80 °C; (ii) raney-Ni/H_2, EtOH, 45 psi, rt.



Scheme 4. Reagents and conditions: (i) 1-chloro-3-pentanone, 85% *o*-H_3PO_4, As_2O_3, 80 °C, 3 h; (ii) raney-Ni/H_2, EtOH, 45 psi, rt.

quently converted to the corresponding 8-quinolinamines (**7a**–**e**, **11a**–**c**), and 8-quinolinamides (**8a**–**b**) as described earlier for analogues **3**–**4**.

On the other hand, 5-alkoxy-4-ethyl-6-methoxy-8-nitroquinoline analogues (**13a**–**h**, series 4, Scheme 4) were synthesized by reacting corresponding 5-alkoxy-4-methoxy-2-nitroanilines (**12**)[9,10] with 1-chloro-3-pentanone via a Skraup synthesis in the presence of *o*-phosphoric acid and arsenic trioxide at 100 °C.[11] Catalytic hydrogenation of 5-alkoxy-4-ethyl-6-methoxy-8-nitroquinolines (**13a**–**h**) by raney-nickel in a Parr hydrogenator for 45 min at 45 psi and ambient temperature gave the corresponding 5-alkoxy-4-ethyl-6-methoxy-8-quinolinamines (**14a**–**h**) in quantitative yield.

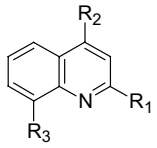
4. Biological activities

In vitro activities of the synthesized compounds (series 1–4) for tuberculosis inhibition against *M. tuberculosis H37Rv* strain (ATCC 27294, susceptible both to rifampin and isoniazid) were carried out using the microplate alamar blue assay (MABA).[12] Compounds exhibiting fluorescence were then tested in the BACTEC 460 radiometric system[13] and/or in broth microdilution assay, and activities expressed as minimum inhibitory concentration (MIC, μg/mL) are summarized in Tables 1–4. Compounds demonstrating at least 90% inhibition are re-tested at lower concentrations in the broth microdilution assay to determine the actual MIC, a value defined as the lowest concentration inhibiting $\geqslant$90% of the inoculum relative to controls.

Among the 8-nitroquinoline analogues (series 1), **2h** ($R_1 = R_2 = c\text{-}C_6H_{11}$, $R_3 = NO_2$) displayed the exceptional anti-tuberculosis activity (99% inhibition) at a concentration of 1 μg/mL (MIC). On the other hand, compounds **2d** ($R_1 = c\text{-}C_6H_{11}$, $R_2 = H$, $R_3 = NO_2$), **2g** ($R_1 = R_2 = c\text{-}C_5H_9$, $R_3 = NO_2$), and **4e** ($R_1 = R_2 = c\text{-}C_6H_{11}$, $R_3 = NHCOCF_3$) also produced excellent biological activity (with 99%, 96%, and 96% inhibition, respectively) at a concentration of 6.25 μg/mL (MIC) (Table 1). These results are in agreement with our earlier observation that emphasizes the importance of cycloalkyl group in enhancing the biological efficacy of the quinoline ring containing analogues.[7] Surprisingly, replacement of the nitro group (analogue, **2h**) with amino group (analogue, **3c**) resulted in complete loss of anti-tuberculosis activity. Furthermore, blocking of the amino group (analogue, **4e**) again affected substantial increase in bio-efficacy suggesting that the presence of cationic amino group in the quinoline framework is detrimental for anti-tuberculosis activity.

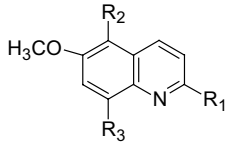
In the series 2, analogue **6b** [$R_1 = R_2 = CH(CH_3)$, $R_3 = NO_2$] produced highest inhibitory activity (96%) at a concentration of 6.25 μg/mL (MIC). Whereas, all remaining analogues from this series were found to be inactive (Table 2). Similarly, in vitro anti-tuberculosis activity evaluation against *M. tuberculosis H37Rv*, of

Table 1. In vitro antimycobacterial activity evaluation of ring-substituted-quinolines (series 1) against *M. tuberculosis H37Rv*



No.	R_1	R_2	R_3	% Inhibition	MIC (μg/mL)
1	H	H	NO_2	0	>6.25
2a	$CH(CH_3)_2$	H	NO_2	3	>6.25
2b	$C(CH_3)_3$	H	NO_2	12	>6.25
2c	*c*-C_5H_9	H	NO_2	20	>6.25
2d	*c*-C_6H_{11}	H	NO_2	99	6.25
2e	$CH(CH_3)_2$	$CH(CH_3)_2$	NO_2	22	>6.25
2f	$C(CH_3)_3$	$C(CH_3)_3$	NO_2	23	>6.25
2g	*c*-C_5H_9	*c*-C_5H_9	NO_2	96	6.25
2h	*c*-C_6H_{11}	*c*-C_6H_{11}	NO_2	99	1.00
3a	H	H	NH_2	0	>6.25
3b	*c*-C_6H_{11}	H	NH_2	9	>6.25
3c	*c*-C_6H_{11}	*c*-C_6H_{11}	NH_2	0	>6.25
4a	H	H	$NHCOCH_3$	0	>6.25
4b	H	H	$NHCOCF_3$	0	>6.25
4c	*c*-C_6H_{11}	H	$NHCOCH_3$	1	>6.25
4d	*c*-C_6H_{11}	H	$NHCOCF_3$	9	>6.25
4e	*c*-C_6H_{11}	*c*-C_6H_{11}	$NHCOCF_3$	96	6.25
Isoniazid				99	1.00

Table 2. In vitro antimycobacterial activity evaluation of ring-substituted-6-methoxyquinolines (series 2) against *M. tuberculosis H37Rv*



No.	R_1	R_2	R_3	% Inhibition	MIC (μg/mL)
5	H	H	NO_2	nd	nd
6a	H	$CH(CH_3)_2$	NO_2	0	>6.25
6b	$CH(CH_3)_2$	$CH(CH_3)_2$	NO_2	96	6.25
6c	$C(CH_3)_3$	H	NO_2	0	>6.25
6d	$C(CH_3)_3$	$C(CH_3)_3$	NO_2	15	>6.25
6e	1-Adamantyl	H	NO_2	0	>6.25
6f	H	*c*-C_5H_9	NO_2	0	>6.25
6g	*c*-C_5H_9	*c*-C_5H_9	NO_2	0	>6.25
6h	H	*c*-C_6H_{11}	NO_2	12	>6.25
6i	*c*-C_6H_{11}	*c*-C_6H_{11}	NO_2	0	>6.25
7a	H	H	NH_2	nd	nd
7b	$C(CH_3)_3$	H	NH_2	0	>6.25
7b	H	*c*-C_5H_9	NH_2	24	>6.25
7d	H	*c*-C_6H_{11}	NH_2	nd	nd
7e	1-Adamantyl	H	NH_2	0	>6.25
8a	H	H	$NHCOCH_3$	nd	nd
8b	H	H	$NHCOCF_3$	nd	nd
Isoniazid				99	1.00

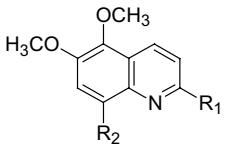
nd, not determined.

ring-substituted-5,6-dimethoxyquinolines (series 3) did not result in any efficacious compound (Table 3).

Analogue **13g** ($R_1 = OC_7H_{15}$, $R_2 = NO_2$) produced very pronounced anti-tuberculosis activity (99% inhibition) at a concentration of 1 μg/mL (MIC), and established to be most effective among 5-alkoxy-4-ethyl-6-methoxy-8-nitroquinolines tested (series 4). At the same time, analogues **13b** [$R_1 = OCH(CH_3)_3$, $R_2 = NO_2$], and **14e** ($R_1 = OC_5H_{11}$, $R_2 = NH_2$) also produced excellent inhibitory activities (97%, 99%, respectively) at a concentration of 6.25 μg/mL (Table 4).

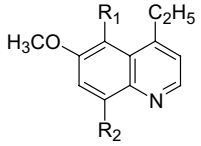
Compounds (**2d**, **2g**, **2h**, **4e**, **6b**, **13b**, **13g**, and **14e**) exhibiting MIC values of 6.25 μ g/mL or less were selected, and evaluated for activity against single-

Table 3. In vitro antimycobacterial activity evaluation of ring-substituted-5,6-dimethoxyquinolines (series 3) against *M. tuberculosis H37Rv*



No.	R_1	R_2	% Inhibition	MIC (μg/mL)
9	H	NO_2	0	>6.25
10a	$CH(CH_3)_2$	NO_2	0	>6.25
10b	$C(CH_3)_3$	NO_2	0	>6.25
11a	H	NH_2	0	>6.25
11b	$CH(CH_3)_2$	NH_2	0	>6.25
11c	$C(CH_3)_3$	NH_2	0	>6.25
Isoniazid			99	1.00

Table 4. In vitro antimycobacterial activity evaluation of 4-ethyl-5-alkoxy-6-methoxyquinolines (series 4) against *M. tuberculosis H37Rv*



No.	R_1	R_2	% Inhibition	MIC (μg/mL)
13a	OC_3H_7	NO_2	0	>6.25
13b	$OCH(CH_3)_2$	NO_2	97	6.25
13c	OC_4H_9	NO_2	0	>6.25
13d	OC_5H_9	NO_2	0	>6.25
13e	OC_5H_{11}	NO_2	48	>6.25
13f	OC_6H_{13}	NO_2	76	>6.25
13g	OC_7H_{15}	NO_2	99	1.00
13h	OC_8H_{17}	NO_2	2	>6.25
14a	OC_3H_7	NH_2	0	>6.25
14b	$OCH(CH_3)_2$	NH_2	0	>6.25
14c	OC_4H_9	NH_2	0	>6.25
14d	OC_5H_9	NH_2	47	>6.25
14e	OC_5H_{11}	NH_2	99	6.25
14f	OC_6H_{13}	NH_2	0	>6.25
14g	OC_7H_{15}	NH_2	73	>6.25
14h	OC_8H_{17}	NH_2	10	>6.25
Isoniazid			99	1.00

drug-resistant (SDR) *M. tuberculosis* strains and results are reported in Table 5. Analogues **2h** (series 1), **13g**, and **14e** (series 4) were found to be most effective and produced >90% inhibition at a concentration of 6.25 μg/mL (MIC) against rifampin (RMP-R), ethambutol (EMB-R), and isoniazid (INH-R) resistant *M. tuberculosis* strains. At the same time, analogue **6b** has also exhibited excellent efficacy with MIC of 12.5 μg/mL against rifampin (RMP-R), ethambutol (EMB-R), and isoniazid (INH-R) resistant *M. tuberculosis* strains; whereas, all remaining analogues have shown moderate activities against various drug-resistant strains of *M. tuberculosis* (Table 5). Concurrently, three of the most effective compounds (**2h**, **13g**, and **14e**) were also evaluated in vitro for cytotoxicity (IC_{50}) in VERO cells, along with safety index (SI) calculated as the ratio of IC_{50}/MIC_{H37Rv}, and results are reported in Table 5.

The results obtained, thus are indicating that in addition to the presence of essential cycloalkyl group, quinoline ring containing straight chain alkoxy and nitro group also exhibits potent anti-tuberculosis activities. On the other hand, compounds containing amino or amide groups have produced promising activities, and could serve as important leads for further extension toward anti-tuberculosis drug development.

5. Conclusions

The study described above has uncovered and established the discovery of new types of quinoline analogues with significant and promising anti-tuberculosis activity against both sensitive and various single-drug-resistant strains of *M. tuberculosis*. Biological activity studies clearly indicate that the presence of quinoline skeleton with 2,4-dicycloalkyl or 5-alkoxy groups, and a nitro group placed at C-8 position of the ring are essential for maximum efficacy. Without any doubt, it can be concluded that these compounds are important lead for the development of a new class of potential anti-tuberculosis drugs. In vivo anti-tuberculosis activity evaluation of most promising analogues is currently underway in our laboratory and results will be reported in due course of time.

6. Experimental

Melting points were recorded on Mettler DSC 851 or capillary melting point apparatus and are uncorrected.

Table 5. In vitro cytotoxicity (IC_{50}) and antimycobacterial activities evaluation of effective compounds (series 1–4) against single-drug-resistant *M. tuberculosis H37Rv* strain [minimum inhibitory concentration (MIC) in μg/mL]

No	IC_{50} (μg/mL)	SI	*M. tuberculosis* EMB-R	*M. tuberculosis* RMP-R	*M. tuberculosis* INH-R
2d	nd	nd	25.0	25.0	25.0
2g	nd	nd	25.0	25.0	12.5
2h	>100	>50	6.25	6.25	6.25
4e	nd	nd	nd	50.0	50.0
6b	nd	nd	12.5	12.5	12.5
13b	nd	nd	50.0	50.0	50.0
13g	>8	>1.38	6.25	6.25	6.25
14e	>8	>1.3	6.25	6.25	6.25

nd, not done.

^{1}H NMR spectra were recorded on 300 MHz Bruker FT-NMR (Avance DPX300) spectrometer using tetramethylsilane as internal standard and the chemical shifts are reported in δ units. Mass spectra were recorded on either GCMS (Shimadzu QP 5000 spectrometer) auto sampler/direct injection (EI) or HRMS (Finnigan Mat LCQ spectrometer) (APCI/ESI). Elemental analyses were recorded on Elementar Vario EL spectrometer. All chromatographic purification was performed with silica gel 60 (230–400 mesh), whereas all TLC (silica gel) development was performed on silica gel coated (Merck Kiesel 60 F_{254}, 0.2 mm thickness) sheets. All chemicals were purchased from Aldrich Chemical Ltd (Milwaukee, WI, USA). Solvents used for the chemical synthesis acquired from commercial sources were of analytical grade, and were used without further purification unless otherwise stated.

6.1. General method for the synthesis of 2-substituted/2,4-disubstituted-8-nitroquinolines (2a–h)

A solution of 8-nitroquinoline (**1**, 1 mmol) in CH_3CN (5 mL) was heated to 70–80 °C. Silver nitrate (0.6 mmol), requisite alkyl/cycloalkylcarboxylic acid (2.5 mmol), and 10% H_2SO_4 (15 mL) was added to the reaction mixture. A freshly prepared solution of ammonium persulfate (3 mmol) in water (10 mL) was added dropwise during 10 min. The heating source was removed and reaction proceeded with the evolution of carbon dioxide. After 15 min, reaction mixture was poured onto ice, and made alkaline by adding 25% NH_4OH solution. Extracted with $CHCl_3$ (5×50 mL), and combined extracts were washed with NaCl solution (2×15 mL). Dried over Na_2SO_4, and solvent removed in vacuo to afford oil, which upon column chromatography over silica gel (230–400 mesh) afforded 2-substituted/2,4-disubstituted-8-nitroquinolines (**2a**–**h**).

6.1.1. 2-Isopropyl-8-nitroquinoline (2a). Yield: 41%; mp 107–109 °C; ^{1}H NMR ($CDCl_3$) δ 8.14 (d, 1H, $J = 8.5$ Hz), 7.94 (m, 2H), 7.48 (m, 2H), 3.23 (m, 1H), 1.36 (d, 6H, $J = 6.9$ Hz); EIMS m/z 216 (M^+). Anal. Calcd for $C_{12}H_{12}N_2O_2$ (216.2): C, 66.65; H, 5.59; N, 12.96. Found: C, 66.68; H, 5.58; N, 12.88.

6.1.2. 2-*tert*-Butyl-8-nitroquinoline (2b). Yield: 45%; mp 104–105 °C; ^{1}H NMR ($CDCl_3$) δ 8.14 (d, 1H, $J = 8.7$ Hz), 7.92 (m, 2H), 7.63 (d, 1H, $J = 8.7$ Hz), 7.49 (m, 1H), 1.42 (s, 9H); EIMS m/z 230 (M^+). Anal. Calcd for $C_{13}H_{14}N_2O_2$ (230.3): C, 67.81; H, 6.13; N, 12.17. Found: C, 67.88; H, 6.12; N, 12.33.

6.1.3. 2-Cyclopentyl-8-nitroquinoline (2c). Yield: 38%; mp 76–78 °C; ^{1}H NMR ($CDCl_3$) δ 8.11 (d, 1H, $J = 8.5$ Hz), 7.93 (m, 2H), 7.50 (d, 1H, $J = 8.5$ Hz), 7.44 (m, 1H), 3.78 (m, 1H), 1.88 (m, 8H); EIMS m/z 242 (M^+). Anal. Calcd for $C_{14}H_{14}N_2O_2$ (242.3): C, 69.41; H, 5.82; N, 11.56. Found: C, 69.44; H, 5.87; N, 11.49.

6.1.4. 2-Cyclohexyl-8-nitroquinoline (2d). Yield: 37%; mp 68–69 °C; ^{1}H NMR ($CDCl_3$) δ 8.14 (d, 1H, $J = 8.6$ Hz), 7.97 (m, 2H), 7.53 (d, 1H, $J = 8.6$ Hz), 7.34 (m, 1H), 3.74 (m, 1H), 1.97 (m, 10H); APCIMS m/z 257 (M+1). Anal. Calcd for $C_{15}H_{16}N_2O_2$ (256.3): C, 70.29; H, 6.29; N, 10.93. Found: C, 70.37; H, 6.35; N, 10.98.

6.1.5. 2,4-Diisopropyl-8-nitroquinoline (2e). Yield: 22%; oil; ^{1}H NMR ($CDCl_3$) δ 8.23 (d, 1H, $J = 8.5$ Hz), 7.86 (d, 1H, $J = 8.5$ Hz), 7.51 (m, 1H), 7.32 (s, 1H), 3.69 (m, 1H), 3.23 (m, 1H), 1.38 (m, 12H); EIMS m/z 258 (M^+). Anal. Calcd for $C_{15}H_{18}N_2O_2$ (258.3): C, 69.74; H, 7.02; N, 10.84. Found: C, 69.99; H, 7.15; N, 10.88.

6.1.6. 2,4-Di-*tert*-butyl-8-nitroquinoline (2f). Yield: 20%; oil; ^{1}H NMR ($CDCl_3$) δ 8.21 (d, 1H, $J = 8.3$ Hz), 7.81 (d, 1H, $J = 8.3$ Hz), 7.47 (m, 1H), 7.30 (s, 1H), 1.42 (s, 9H), 1.40 (s, 9H); EIMS m/z 286 (M^+). Anal. Calcd for $C_{17}H_{22}N_2O_2$ (286.3): C, 71.30; H, 7.74; N, 9.78. Found: C, 71.21; H, 7.68; N, 9.73.

6.1.7. 2,4-Dicyclopentyl-8-nitroquinoline (2g). Yield: 21%; oil; ^{1}H NMR ($CDCl_3$) δ 8.22 (d, 1H, $J = 8.4$ Hz), 7.85 (d, 1H, $J = 8.4$ Hz), 7.49 (m, 1H), 7.30 (s, 1H), 3.73 (m, 1H), 3.32 (m, 1H), 1.86 (m, 16H); EIMS m/z 310 (M^+). Anal. Calcd for $C_{19}H_{22}N_2O_2$ (310.4): C, 73.52; H, 7.14; N, 9.03. Found: C, 73.58; H, 7.33; N, 9.17.

6.1.8. 2,4-Dicyclohexyl-8-nitroquinoline (2h). Yield: 20%; oil; ^{1}H NMR ($CDCl_3$) δ 8.20 (d, 1H, $J = 8.4$ Hz), 7.78 (d, 1H, $J = 8.4$ Hz), 7.44 (m, 1H), 7.20 (s, 1H), 3.26 (m, 1H), 2.85 (m, 1H), 1.57 (m, 20H); APCIMS m/z 339 (M+1). Anal. Calcd for $C_{21}H_{26}N_2O_2$ (338.4): C, 74.52; H, 7.74; N, 8.28. Found: C, 74.69; H, 7.75; N, 8.30.

6.2. General method for the synthesis of ring-substituted-8-quinolinamines (3a–c)

A solution of 8-nitroquinoline (**2a**, **2d**, or **2h**, 5 mmol) in 95% ethyl alcohol (20 mL) was hydrogenated over wet raney-nickel (T_1 grade) at 45 psi in a parr hydrogenator for 45 min. Catalyst was removed by filtration, and filtrate was evaporated under vacuum to afford the required 8-quinolinamine as dark colored oil.

6.2.1. 8-Quinolinamine (3a). Yield: 95%; oil; ^{1}H NMR ($CDCl_3$) δ 8.76 (m, 1H), 8.10 (m, 1H), 7.36 (m, 2H), 7.14 (m, 1H), 7.93 (m, 1H), 4.90 (br s, 2H); EIMS m/z 144 (M^+). Anal. Calcd for $C_9H_8N_2$ (144.2): C, 74.98; H, 5.59; N, 19.43. Found: C, 75.21; H, 5.77; N, 19.07.

6.2.2. 2-Cyclohexyl-8-quinolinamine (3b). Yield: 88%; oil; ^{1}H NMR ($CDCl_3$) δ 8.05 (m, 1H), 7.54 (m, 2H), 7.42 (m, 1H), 7.09 (m, 1H), 4.76 (br s, 2H), 3.03 (m, 1H), 1.70 (m, 10H); EIMS m/z 226 (M^+). Anal. Calcd for $C_{15}H_{18}N_2$

(226.3): C, 79.61; H, 8.02; N, 12.38. Found: C, 79.76; H, 7.95; N, 12.15.

6.2.3. 2,4-Dicyclohexyl-8-quinolinamine (3c). Yield: 89%; oil; ^{1}H NMR ($CDCl_3$) δ 7.44 (m, 2H), 7.32 (m, 1H), 6.99 (m, 1H), 4.88 (br s, 2H), 3.05 (m, 2H), 1.70 (m, 20H); APCIMS m/z 309 (M+1). Anal. Calcd for $C_{21}H_{28}N_2$ (308.5): C, 81.77; H, 9.15; N, 9.08. Found: C, 81.57; H, 9.05; N, 9.17.

6.3. General method for the synthesis of ring-substituted-8-quinolinamides (4a–e)

A solution of 8-quinolinamine (**3a**–**c**, 2 mmol) in acetic anhydride/pyridine (10:1 mL) or trifluoroacetic anhydride (10 mL) was stirred for 4 h at ambient temperature. The solvent was removed, and residue dissolved in $CHCl_3$ (50 mL). Washed with brine solution (2×15 mL), and organic layer dried over Na_2SO_4. Solvent removed in vacuo to afford 8-quinolinamides (**4a**–**e**) in excellent yield.

6.3.1. *N*-Quinolin-8-yl-acetamide (4a). Yield: 95%; mp 88–90 °C; ^{1}H NMR ($CDCl_3$) δ 9.79 (br s, 1H), 8.78 (m, 2H), 8.15 (m, 1H), 7.50 (m, 1H), 7.46 (m, 2H), 2.37 (s, 3H); EIMS m/z 186 (M^+). Anal. Calcd for $C_{11}H_{10}N_2O$ (186.2): C, 70.95; H, 5.41; N, 15.04. Found: C, 71.22; H, 5.47; N, 15.37.

6.3.2. 2,2,2-Trifluoro-*N*-quinolin-8-yl-acetamide (4b). Yield: 79%; mp 59–61 °C; ^{1}H NMR ($CDCl_3$) δ 9.91 (br s, 1H), 8.91 (m, 1H), 8.58 (m, 1H), 8.37 (m, 1H), 7.76 (m, 1H), 7.61 (m, 2H); EIMS m/z 240 (M^+). Anal. Calcd for $C_{11}H_7F_3N_2O$ (240.2): C, 55.01; H, 2.94; N, 11.66. Found: C, 55.35; H, 3.07; N, 11.59.

6.3.3. *N*-(2-Cyclohexyl-quinolin-8-yl)-acetamide (4c). Yield: 88%; oil; ^{1}H NMR ($CDCl_3$) δ 8.73 (br s, 1H), 8.12 (m, 1H), 7.95 (m, 2H), 7.72 (m, 1H), 7.53 (m, 1H), 3.24 (m, 1H), 2.12 (s, 3H), 1.74 (m, 10H); EIMS m/z 268 (M^+). Anal. Calcd for $C_{17}H_{20}N_2O$ (268.4): C, 76.09; H, 7.51; N, 10.44. Found: C, 76.17; H, 7.55; N, 10.38.

6.3.4. *N*-(2-Cyclohexyl-quinolin-8-yl)-2,2,2-trifluoro-acetamide (4d). Yield: 95%; oil; ^{1}H NMR ($CDCl_3$) δ 10.94 (br s, 1H), 8.19 (m, 1H), 7.99 (m, 2H), 7.70 (m, 1H), 7.51 (m, 1H), 3.24 (m, 1H), 1.58 (m, 10H); EIMS m/z 322 (M^+). Anal. Calcd for $C_{17}H_{17}F_3N_2O$ (322.3): C, 63.35; H, 5.32; N, 8.69. Found: C, 63.51; H, 5.44; N, 8.49.

6.3.5. *N*-(2,4-Dicyclohexyl-quinolin-8-yl)-2,2,2-trifluoro-acetamide (4e). Yield: 97%; oil; ^{1}H NMR ($CDCl_3$) δ 11.17 (br s, 1H), 8.63 (d, 1H, $J = 7.6$ Hz), 7.82 (d, 1H, $J = 7.6$ Hz), 7.50 (m, 1H), 7.27 (s, 1H), 3.28 (m, 1H), 2.86 (m, 1H), 1.62 (m, 20H); EIMS m/z 404 (M^+). Anal. Calcd for $C_{23}H_{27}F_3N_2O$ (404.5): C, 68.30; H, 6.73; N, 6.93. Found: C, 68.47; H, 6.73; N, 6.99.

6.4. Ring-substituted-6-methoxyquinoline (5–8)

Synthesis and spectral data of the synthesized analogues (series 2) are reported earlier.[8]

6.5. Ring-substituted-5,6-dimethoxyquinoline (9–11)

Synthesis and spectral data of the synthesized analogues (series 3) are reported earlier.[8]

6.6. General method for the synthesis of 5-alkoxy-4-ethyl-6-methoxy-8-nitroquinolines (13b and 13d)

A homogeneous mixture of appropriate 5-alkoxy-4-methoxy-2-nitroaniline (**12**, 0.046 mol), 1-chloro-3-pentanone (0.025 mol), and *o*-phosphoric acid (85%, 20 mL) was placed in a three-necked flask fitted with a thermometer and a dropping funnel. The reaction mixture was heated at 80 °C (internal) with mechanical stirring for 10 min. An additional quantity of 1-chloro-3-pentanone (0.025 mol) was added and stirring continued for another 10 min at 80 °C. Evolution of some hydrogen chloride gas was observed. Arsenic(III) oxide (0.036 mol) was then added at once to the reaction mixture and stirring continued for additional 2.5 h at 80 °C. The dark colored reaction mixture was cooled, diluted with water (125 mL), and filtered. The filtrate was basified with 25% NH_4OH solution, extracted with dichloromethane (3×125 mL). Combined organic extracts were washed with brine solution (3×20 mL) and water (3×10 mL), and dried over sodium sulfate. The solvent was removed under vacuum to afford brown colored crude product. Purified by flash column chromatography using EtOAc/hexanes (20:80) to provide 5-alkoxy-4-ethyl-6-methoxy-8-nitroquinolines as low melting solid.

6.6.1. 5-Isopropoxy-4-ethyl-6-methoxy-8-nitroquinoline (13b). Yield: 69%; mp 43–44 °C; ^{1}H NMR ($CDCl_3$) δ 8.76 (d, 1H, $J = 4.3$ Hz), 7.84 (s, 1H), 7.27 (d, 1H, $J = 4.3$ Hz), 4.14 (m, 1H), 4.02 (s, 3H), 3.32 (m, 2H), 1.40 (t, 6H), 1.06 (t, 3H, $J = 7.4$ Hz); ESIMS m/z 291 (M+1). Anal. Calcd for $C_{16}H_{18}N_2O_4$ (290.3): C, 62.06; H, 6.25; N, 9.65. Found: C, 62.09; H, 6.21; N, 9.66.

6.6.2. 5-Cyclopentyloxy-4-ethyl-6-methoxy-8-nitroquinoline (13d). Yield: 52%; mp 55–56 °C; ^{1}H NMR ($CDCl_3$) δ 8.85 (d, 1H, $J = 4.5$ Hz), 7.93 (s, 1H), 7.33 (d, 1H, $J = 4.5$ Hz), 3.91 (m, 1H), 3.85 (s, 3H), 2.70 (m, 2H), 1.65 (m, 8H), 1.28 (m, 3H); ESIMS m/z 317 (M+1). Anal. Calcd for $C_{17}H_{20}N_2O_4$ (316.4): C, 64.54; H, 6.37; N, 8.86. Found: C, 64.77; H, 6.42; N, 8.73.

Analytical data of all remaining 5-alkoxy-4-ethyl-6-methoxy-8-nitroquinolines are previously reported.[11b]

6.7. General method for the synthesis of 5-alkoxy-4-ethyl-6-methoxy-8-quinolinamines (14b and 14d)

These analogues were synthesized using procedure reported above for 8-quinolinamines (**3a–c**).

6.7.1. 5-Isopropoxy-4-ethyl-6-methoxy-8-quinolinamine (14b). Yield: 72%; oil; ^{1}H NMR ($CDCl_3$) δ 8.48 (d, 1H, $J = 4.2$ Hz), 7.13 (d, 1H, $J = 4.2$ Hz), 6.76 (s, 1H), 4.58 (br s, 2H), 3.94 (s, 3H), 3.87 (m, 1H), 3.25 (m, 2H), 1.35 (m, 6H), 0.95 (t, 3H, $J = 7.1$ Hz); APCIMS m/z 261 (M+1). Anal. Calcd for $C_{15}H_{20}N_2O_2$ (260.3): C, 69.20; H, 7.74; N, 10.76. Found: C, 69.57; H, 7.68; N, 10.65.

6.7.2. 5-Cyclopentyloxy-4-ethyl-6-methoxy-8-quinolinamine (14d). Yield: 90%; oil; ^{1}H NMR ($CDCl_3$) δ 8.55 (d, 1H, $J = 4.3$ Hz), 7.15 (d, 1H, $J = 4.3$ Hz), 6.78 (s, 1H), 4.72 (br s, 2H), 3.91 (s, 3H), 3.88 (m, 1H), 2.73 (m, 2H), 1.61 (m, 8H), 1.23 (m, 3H); APCIMS m/z 287 (M+1). Anal. Calcd for $C_{17}H_{22}N_2O_2$ (286.4): C, 71.30; H, 7.74; N, 9.78. Found: C, 71.42; H, 7.59; N, 9.85.

Analytical data of all remaining 5-alkoxy-4-ethyl-6-methoxy-8-quinolinamines are reported earlier.[11b]

Broth microdilution assay: A loop full of *M. tuberculosis H37Rv* from Lowenstein–Jensen slants was inoculated into 100 mL of 7H9 broth medium (7H9 medium supplemented with 10% ADC and 0.001% Tween 80), and incubated at 37 °C for two weeks. Two days before the susceptibility testing, the culture was diluted 1:10 in fresh 7H9 broth medium. After two days, the culture was ultrasonicated to make a single cell suspension, and further diluted 1:10 in 7H9 broth just prior to the inoculation of microdilution tubes. This procedure yielded an actively growing culture, which reproducibly contained 5×10^6 CFU/mL as determined by plating. The stock solutions of the compounds were prepared in DMSO diluted 1:3 times in 7H9 broth. Further, serial twofold dilutions of the compounds were prepared from the stock solutions in 7H9 broth medium to provide the final concentrations of 4.0, 2.0, 1.0, 0.5, and 0.25 μg/mL. The same concentrations were tested for isoniazid (INH), which was taken as the positive control. The autoclaved microdilution tubes contained 1600 μL 7H9 broth medium, 200 μL of drug dilution, and 200 μL of 1:10 times diluted and ultrasonicated *M. tuberculosis* inocula. All the test tubes were tightly screw-capped and incubated at 37 °C for 14 days. The tubes were checked for the surface growth layer. The MIC was defined as the lowest concentration of test compounds at which the surface growth layer could not be observed. The negative controls included 7H9 broth with no drug and with equivalent amounts of DMSO as the experimental tubes. DMSO did not inhibit the growth of *M. tuberculosis* in the concentrations used for dissolving the compounds.

Acknowledgements

Authors are thankful to the Tuberculosis Antimicrobial Acquisition and Coordination Facility (TAACF), which provided partial antimycobacterial data through a research and development contract with the U.S. National Institute of Allergy and Infectious Diseases.

References and notes

1. World Health Organization. Global Tuberculosis Control; WHO Report 2001; World Health Organization: Geneva, Switzerland; WHO/CDS/TB/2001.287.
2. (a) World Health Organization. Tuberculosis Fact Sheet, No. 104, 2000; see http://www.who.int/inf-fs/en/fact104.html; (b) Somoskkovi, A.; Parsons, L. M.; Salfinger, M. *Respir. Res.* **2001**, *2*, 164; (c) Snell, N. J. C. *Expert Opin. Invest. Drugs* **1998**, *7*, 545.
3. Haeym, B.; Honore, N.; Truffot-Pernot, C.; Banerjee, A.; Schurra, C.; Jacobs, W. R., Jr.; Van Embden, J. D. A.; Grosset, J. H.; Cole, S. T. *Lancet* **1994**, *344*, 293.
4. Snider, D. E.; Castro, K. G. *New Engl. J. Med.* **1998**, *338*, 1689.
5. Casal, M. J. *Drugs Today* **1999**, *35*, 1.
6. World Health Organization. In *Anti-tuberculosis Drug Resistance in the World: The WHO/IUATLD Global Project on Anti-tuberculosis Drug Resistance Surveillance 1994–1997*; 1997; WHO/TB/1997.229.
7. (a) Jain, R.; Balasubramanian, V.; Nayyar, A.; Plade, P. B. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1051, and references cited therein; For mechanistic aspects of the homolytic free radical reaction, please see: (b) Minisci, F.; Bernardi, R.; Bertini, F.; Galli, R.; Perchinummo, M. *Tetrahedron* **1971**, *27*, 3575.
8. (a) Jain, M.; Vangapandu, S.; Sachdeva, S.; Singh, S.; Singh, P. P.; Gena, G. B.; Tikoo, K.; Ramarao, P.; Kaul, C. L.; Jain, R. *J. Med. Chem.* **2004**, *47*, 285; (b) Jain, M.; Vangapandu, S.; Sachdeva, S.; Jain, R. *Bioorg. Med. Chem.* **2004**, *12*, 1003.
9. The starting material, 5-nitroguaiacol required for the synthesis of various 5-alkoxy-4-methoxy-2-nitroanilines was synthesized from guaiacol using literature procedure: Drake, N. L.; Harris, H. C.; Jaeger, C. B. *J. Am. Chem. Soc.* **1948**, *70*, 168.
10. Carroll, F. I.; Berrang, B.; Linn, C. P.; Twine, C. E., Jr. *J. Med. Chem.* **1979**, *22*, 694.
11. (a) Jain, R.; Jain, S.; Gupta, R. C.; Anand, N.; Dutta, G. P.; Puri, S. K. *Ind. J. Chem.* **1994**, *33B*, 251; (b) Vangapandu, S.; Sachdeva, S.; Jain, M.; Singh, S.; Singh, P. P.; Kaul, C. L.; Jain, R. *Bioorg. Med. Chem.* **2003**, *11*, 4557; (c) Vangapandu, S.; Sachdeva, S.; Jain, M.; Singh, S.; Singh, P. P.; Kaul, C. L.; Jain, R. *Bioorg. Med. Chem.* **2004**, *12*, 239.
12. Franzblau, S. G.; Witzig, R. S.; McLaughlin, J. C.; Torres, P.; Madico, G.; Herandez, A.; Degnan, M. T.; Cook, M. B.; Quenzer, V. K.; Ferguson, R. M.; Gilman, R. H. *J. Clin. Microb.* **1998**, *36*, 362.
13. Collins, L.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **1997**, *41*, 1004.