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Original article

Design, synthesis and evaluation of acridine and fused-quinoline derivatives as potential *anti*-tuberculosis agents

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ABSTRACT

The synthesis of twelve acridine and polycyclic acridine derivatives prepared *via* the Friedländer reaction is described. The one-pot reactions of 2-amino-5-chloro or 5-nitro-benzophenones and a variety of cyclanones and indanones were carried out in a MW oven under TFA catalysis in good yields. The products were designed according natural antituberculosis products and were evaluated for growth inhibitory activity towards *Mycobacterium tuberculosis* $H_{37}Rv$ (Mtb) through the National Institute of Allergy and Infectious Diseases (NIAID, USA). Three of them underwent additional testings. The cyclopenta[*b*]quinoline derivative **9** and the acridine derivative **13** showed remarkable MIC values against the rifampin resistant strain. The former exhibited bactericidal activity at 50 μg/mL, its intracellular activity is similar to rifampin and it was not cytotoxic at low concentrations so it can be considered a new lead compound.

1. Introduction

Currently, one third of the world's population is infected with *Mycobacterium tuberculosis* and 8.9–9.9 million new and relapse cases of tuberculosis (TB) are reported every year. The emergence of new cases, the increased incidence of multi-drug resistant strains of *M. tuberculosis*, the adverse effects of first- and second-line *anti*-tuberculosis drugs, and the incidence of TB associated with viral infections (Human Immunodeficiency Virus, HIV) have led to renewed research interest in natural products in the hope of discovering new antitubercular leads.

Natural products and some of their derivatives have been reported to exhibit remarkable growth inhibitory activity towards *M. tuberculosis* and some of them have been selected as prototype molecules for the development of new antitubercular agents. A large group of alkaloids with complex structures and potent inhibitory activity was described [1].

Investigations of extracts from the ascidian *Lissoclinum notti* and *Diplosoma* sp. led to the isolation of reported pyridoacridone alkaloids. Among them, ascididemin (**1**) exhibited potent antitubercular activity but it also showed high toxicity against Vero cells (SI values >1), Fig. 1. For this reason, a series of simplified tetracyclic-core analogous were prepared in an effort to decouple mammalian cell toxicity from anti-TB activity. The two most interesting compounds were found to inhibit the growth of *M. tuberculosis* $H_{37}Rv$ but negligible cytotoxicity towards Vero and P388 cells [2].

Acridine derivatives, atebrin and quinacrine, have been widely used in malaria chemotherapy during World War II in the absence of quinine and this skeleton is still being explored for betters antimalarials. In 2006, a series of 9-substituted tetrahydroacridines was synthesized and evaluated against *M. tuberculosis* $H_{37}Rv$ and $H_{37}Ra$ strains and exhibited potent activities comparable to the standard drugs [3]. In 2004, Jain et al. [4] reported a series of substituted quinolones with high anti-TB activity. The most effective compound, 2,8-dicyclopentyl-4-methylquinoline exhibited activity against both drug-sensitive and drug-resistant *M. tuberculosis*. It is worthwhile to mention that many of these analogs were initially synthesized as the precursors for targeted antimalarials.

More recently, three quinoline alkaloids (**2**–**4**) isolated from the leaves of *Lunasia amara* Blanco (Rutaceae) revealed interesting activity against *M. tuberculosis* $H_{37}Rv$, Fig. 2 [5].

For the above exposed, the chemotherapeutic properties of acridone and quinoline alkaloids and their synthetic analogs continue to attract interest.

In this regard, we have prepared the acridindione derivative **5** employing a MW-assisted three-component Hantzsch-type condensation of aniline, benzaldehyde and dimedone [6] but it showed no inhibition against *M. tuberculosis* (National Institute of Allergy and Infectious Diseases, NIAID, USA). 1,2,3,4-

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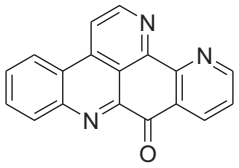

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Fig. 1. Structure of ascididemin.

Tetrahydroacridin-9-one and three fused 4-quinolone analogs were also synthesized under MW irradiation with shorter reaction time and from easily starting materials compared to the known methods in order to design potential *anti*-tuberculosis agents [7]. On the other hand, a series of novel acridine derivatives was reported as potent DNA-binding and apoptosis-inducing antitumor agents [8].

We have previously prepared a series of polysubstituted quinolines synthesized *via* the microwave-assisted Friedländer reaction and a catalytic amount of concentrated hydrochloric acid (Scheme 1). These products were obtained in good yields at short times and were tested against the parasites causing malaria, leishmaniasis and trypanosomiasis, other types of neglected diseases. Among them the tetrahydroacridine derivative **6** was also described [9], Scheme 2.

The aim of the present study was to prepare a number of analogs of **6** in order to explore preliminary aspects of the structure–antiTB activity relationship. The synthetic procedure involves the Friedländer reaction under MW irradiation and trifluoroacetic acid (TFA) catalysis and the targeted products resemble both quinoline and acridine nuclei from natural antitubercular compounds.

2. Results and discussion

2.1. Chemistry

Herein, the synthesis of twelve acridine and polyclyclic acridine derivatives (**6**–**17**) prepared *via* the Friedländer reaction is described (Table 1). The one-pot reactions of 2-amino-5-chloro or 5-nitro-benzophenones and a variety of cyclanones and indanones were carried out in a MW oven at constant power (400 W), Scheme 2. The reactions proceed to completion between 2 and 7 min in good to excellent yields, meanwhile when they were performed under conventional heating the reaction times extended to 2–3 h and the yields were lower or similar, as it was shown earlier [9]. The use of TFA as catalyst proved to be more efficient and milder than the hydrochloric acid catalysis in both experimental conditions. Cyclic ketones such as cyclohexanone, cycloheptanone, dimedone

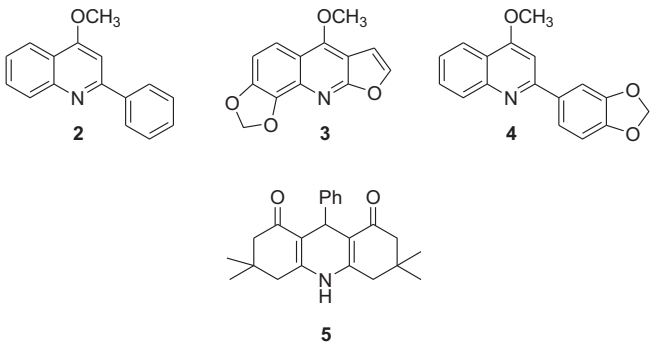
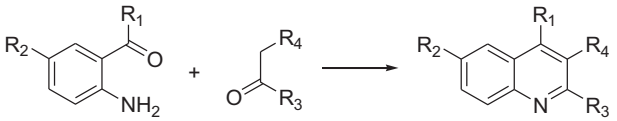


Fig. 2. Quinoline alkaloids isolated from *L. amara* B (**2**–**4**) and a synthetic acridindione derivative **5**.



Scheme 1. Synthesis of quinolines *via* the Friedländer reaction.

and cyclopentanone were used as starting materials to obtain compounds **6**–**9** and **13**–**15**, meanwhile 2-indanone produced **10**, 1-indanone **11** and **16**, and 1,3-indanodione yielded **12** and **17**. Certain substituted 1-indanones were employed in 2007 for achieving indenoquinolines also *via* Friedländer synthesis but in the presence of sodium ethoxide as catalyst at refluxing temperature of ethanol [10].

Although the features of the Friedländer reaction are generally well understood, its mechanism has not unambiguously established. Experimental reports support two different mechanistic proposals. One pathway involves the initial formation of the Schiff base from the 2-amino-substituted aromatic compound and the carbonyl partner followed by an intramolecular aldol reaction to give the hydroxyl imine adduct with subsequent loss of water to produce the quinoline. Alternatively, it has been proposed that the initial rate-limiting step is an intermolecular aldol reaction which in turns gives the quinoline [11]. However it is well established that MW-reactants interactions are increased with the polarity of the material. We could assume the formation of the Schiff base followed by cyclodehydration considering the possible MW activation effects by dipole–dipole interactions according to the literature [12].

2.2. Biological evaluation

2.2.1. In vitro activity against M. tuberculosis

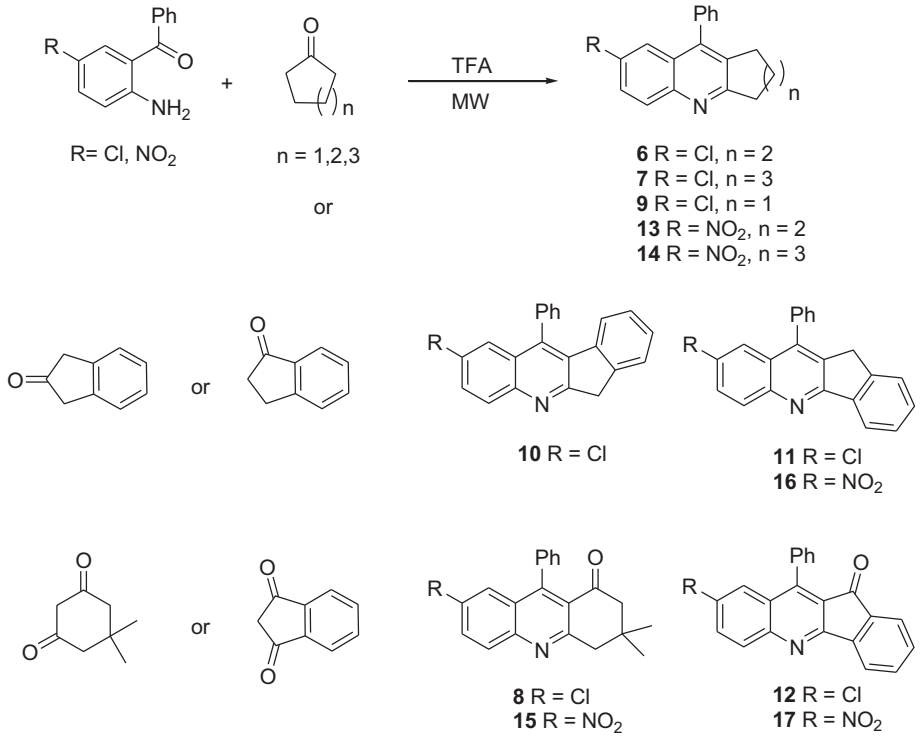
The synthesized products **6**–**17** were evaluated for growth inhibitory activity towards *M. tuberculosis* $H_{37}Rv$ (Mtb) through the National Institute of Allergy and Infectious Diseases (NIAID, USA). The structure of the named compounds and their IC_{50} and IC_{90} values are shown in Table 1. Amikacin, cycloserine, ethambutol, isoniazid, pyrimethamin, primethamine and rifampim were used as reference drugs.

Compounds **9**, **13** and **14** exhibited IC_{50} values of 73.41, 44.35 and 61.93 μM, respectively and underwent additional testing. This subset was determined by an algorithm that considered primarily activity and analytical quality of the samples but also considered other aspects such as chemotype series and solubility.

This testing includes *in vitro* testing of $H_{37}Rv$ under both anaerobic and aerobic conditions as well as minimal bactericidal concentration (MBC). Single drug resistant strain testing (isoniazid, rifampin and moxifloxacin resistant Mtb strains) and intracellular inhibition of Mtb $H_{37}Rv$ growth using murine macrophage cell line and cytotoxicity in this cell-line were also determined.

2.2.2. Minimal inhibitory concentration (MIC)

The MIC for each compound was determined by testing ten, two-fold dilutions in concentration ranges. The MIC is reported as the lowest concentration (μg/mL) of drug that visually inhibited growth of the organism. In addition, the percentage of inhibition at the MIC is provided (Table 2). Rifampin and isoniazid were used as positive controls. Although MIC values of compounds **9**, **13** and **14** were higher than the MIC values for the reference drugs, the tested compounds showed good percent inhibition values (83, 69 and 72% respectively) against the rifampin resistant strain (RMP-R). Compounds **9** and **13** exhibited the best results.



Scheme 2. Synthetic pathway for the preparation of compounds **6**–**17**.

2.2.3. Minimal bactericidal concentration (MBC)

The established rejection value of >40 colonies for the MBC assay was based on the calculated concentration of Mtb in the MIC plates. Results, reported as μg/mL concentration, are determined based on Colony Forming Units (CFUs) enumerated from agar plates. Only agar plates with countable colonies have reportable counts. If a compound lacks bactericidal activity, many times the CFUs are too numerous to count (TNTC) and are thus reported as such. Results are provided in Table 3. According to them, only compound **9** proved bactericidal activity (50 μg/mL) without observing any CFU at that concentration.

2.2.4. Low-oxygen recovery assay (LORA)

Traditional screening of drugs against *M. tuberculosis* only addresses or targets the organism in an active replicating state. It is well documented that Mtb can reside in a state of non-replicating persistence (NRP) which has not been adequately assessed in the development of new antimicrobials. Results for the LORA assay are reported as the lowest concentration (μg/mL) of drug that visually inhibited growth of the organism. This NRP state is considered an antimicrobial tolerance factor, so LORA may identify drugs that could reduce anti-tubercular treatment period. Compounds **9** and **13** exhibited higher MIC values under anaerobic than under aerobic conditions. Only compound **14** showed the same MIC value for both experimental conditions.

2.2.5. Intracellular drug activity

Intracellular drug activity is reported as log reduction values calculated as reduction in Mtb concentration from zero hour to 7 days post-infection. The three concentrations chosen were based on the MIC data generated in the HTS primary screen. The mid concentration bracketed the reported MIC with the lower concentration ten-fold below the mid and the higher concentration ten-fold above the mid. Drug cytotoxicity is reported as cell proliferation, macrophage toxicity (MTT) or percentage of viability (Table 4). Compound **9** showed a similar tendency as rifampin, so at higher drug concentrations lower UFC are obtained and the log reduction is higher. Meanwhile, compounds **13** and **14** did not follow this pattern. Concerning citotoxicity, compounds **9** and **14** exhibited percentages of viability comparable to rifampin at low and mid concentrations, so they are not toxic to macrophages; however at high concentrations the three tested compounds are citotoxic.

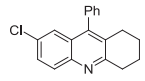
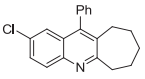
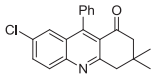
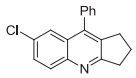
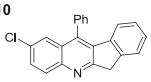
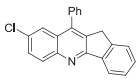
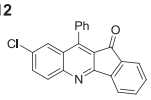
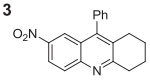
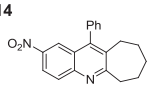
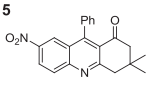
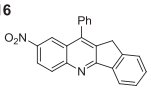
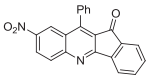
3. Conclusions

We herein show the syntheses of 12 new acridine derivatives inspired in natural occurring antitubercular and three of them exhibited inhibition activity against *M. tuberculosis* (Mtb) $H_{37}Rv$. This series could be obtained in good yields and short times in a one-pot MW-assisted Friedländer reaction with affordable starting materials and under TFA catalysis. The cyclopenta[*b*]quinoline derivative **9** and the acridine derivative **13** had remarkable MIC values against the rifampin resistant strain but only compound **9** exhibited bactericidal activity at 50 μg/mL Furthermore, its intracellular activity is similar to rifampin and it was not citotoxic at low concentrations. Summarizing, this derivative can be considered a new lead structure for designing new tricyclic analogs with lower toxicity towards macrophages and improved *anti*-tuberculosis activity.

4. Material and methods

The structures of the synthesized compounds were established through their ^{1}H and ^{13}C NMR, MS and IR spectra. Melting points were determined in a capillary Electrothermal 9100 SERIES-Digital apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were

Table 1
Acridine and polycyclic derivatives **6**–**17** and their *in vitro* activity against *M. tuberculosis* $H_{37}Rv$.

Compound	IC_{50} (μM)	IC_{90} (μM)
6 	>100.00	>100.00
7 	>100.00	>100.00
8 	>100.00	>100.00
9 	73.41	>100.00
10 	>100.00	>100.00
11 	>100.00	>100.00
12 	>100.00	>100.00
13 	44.35	>100.00
14 	61.93	95.41
15 	>100.00	>100.00
16 	>100.00	>100.00
17 	>100.00	>100.00
Amikacin	0.07	0.08
Cycloserine	12.47	13.49
Ethambutol	<1.56	32.79
Isoniazid	0.18	0.29
Pyrimethamin	37.35	74.96
Primethamine	>5.00	>5.00
Rifampin	0.02	0.02

recorded at room temperature using a Bruker 300 MHz spectrometer. The operating frequencies for protons and carbons were 300.13 and 75.46 MHz, respectively. The chemical shifts (δ) were given in ppm. IR spectra were recorded on an FT Perkin Elmer Spectrum One from KBr discs. Mass spectra were measured on MS/DSQ II. Elemental analysis (C, H and N) were performed on an Exeter CE 440 and the results were within ±0.4% of the calculated values. Analytical TLCs were performed on DC-Alufolien Kieselgel 60 F_{254} Merck. Microwave-assisted reactions were carried out in a CEM Discover oven.

4.1. Synthesis

4.1.1. General procedure for the synthesis of compounds **6**–**17**

A neat mixture of 1.00 mmol of 2-amino-5-chlorobenzo phenone or 2-amino-5-nitrobenzophenone and 1.50 mmol of the corresponding cyclanone with 0.15 mL TFA was subjected to MW irradiation, at 400 W and 250 °C. The completion of the reaction was determined by TLC.

4.1.1.1. 7-chloro-9-phenyl-1,2,3,4-tetrahydroacridine **6**. Reaction time 6 min, it was triturated at rt with cyclohexane, white solid, mp (225–227)°C, yield 80%. Pf lit. 225–227 °C, yield 52% [9]. ^{1}H NMR ($DCCl_3$): δ 1.82–1.85 (2H, m, CH_2), 1.98–2.03 (2H, m, CH_2), 2.65–2.69 (2H, m, CH_2), 3.64–3.72 (2H, m, CH_2), 7.21–8.98 (8H, m, Ph–H and Het–H). ^{13}C NMR ($DCCl_3$): δ 22.6, 25.7, 31.4, 122.6, 123.7, 124.3, 126.2, 127.8, 129.3, 129.4, 129.8, 134.7, 138.8, 139.7, 151.5, 162.4. IR (cm^{-1}): υ 3019, 2942, 1630, 1580, 1478, 1178, 717. Calcd analysis for $C_{19}H_{16}ClN$, %: C, 77.68; H, 5.49; N, 4.77. Found, %: C, 77.74; H, 5.85; N, 4.99.

4.1.1.2. 2-Chloro-11-phenyl-7,8,9,10-tetrahydro-6H-cyclohepta[b] quinoline **7**. Reaction time 7 min, it was crystallized from EtOH, white solid, mp (193–195)°C. Pf lit. 175 °C [13], yield 44%. ^{1}H NMR ($DCCl_3$): δ 1.5–1.7 (2H, m, CH_2), 1.75–1.95 (4H, m, CH_2), 2.65–2.75 (2H, m, CH_2), 3.2–3.4 (2H, m, CH_2), 7.19–7.25 (3H, m, Ph–H), 7.51–7.56 (2H, m, Ph–H), 7.58 (1H, s), 7.84 (1H, d, J = 9.0 Hz), 7.99 (1H, d, J = 9.0 Hz). ^{13}C NMR ($DCCl_3$): δ 23.8, 27.5, 31.0, 35.2, 39.1, 122.6, 123.6, 124.2, 126.2, 127.8, 129.4, 129.6, 129.9, 136.5, 138.4, 141.4, 151.4, 161.0. IR (cm^{-1}): υ 2955, 2889, 1489, 831, 707. Calcd analysis for $C_{20}H_{18}ClN$, %: C, 78.04; H, 5.89; N, 4.55. Found, %: C, 78.20; H, 6.06; N, 4.83.

4.1.1.3. 7-Chloro-3,3-dimethyl-9-phenyl-3,4-dihydroacridin-1(2H)-one **8**. Reaction time 3 min, it was crystallized from EtOH, yellow solid, mp (207–208)°C, yield: 85%. ^{1}H NMR ($CDCl_3$): δ 1.24 (6H, s, 2 × CH_3), 2.57 (2H, s, CH_2), 3.28 (2H, s, CH_2), 7.14–7.17 (2H, m, Ph–H), 7.49 (1H, d, J = 9.2 Hz, Het–H), 7.54–7.69 (3H, m, Ph–H), 7.72 (1H, dd, J = 2.2 and 9.2 Hz, Het–H), 8.06 (1H, d, J = 2.2 Hz, Het–H). ^{13}C NMR ($CDCl_3$): δ 27.3, 29.3, 30.4, 46.2, 53.2, 122.6, 127.7, 127.8, 128.2, 128.9, 130.4, 132.1, 133.7, 147.0, 152.5, 161.3, 198.6. IR (cm^{-1}): υ 3; 074; 1,757, 1,645, 1,277, 837, 754. Calcd analysis for $C_{21}H_{18}ClNO$, %: C, 75.11; H, 5.40; N, 4.17. Found, %: C, 75.20; H, 5.32; N, 4.10.

4.1.1.4. 7-Chloro-9-phenyl-2,3-dihydro-1H-cyclopenta[b]quinoline **9**. Reaction time 3.5 min, it was triturated at rt with cyclohexane, yellow solid, mp (129–131)°C, yield 60%. ^{1}H NMR (DMSO-d_6): δ 2.19–2.21 (2H, m, N=CH_2–CH_2–CH_2), 2.89 (2H, t, J = 7.5 Hz, N=CH_2–CH_2–CH_2), 3.20 (2H, t, J = 7.5 Hz, N=CH_2–CH_2–CH_2), 7.47 (2H, d, J = 7.1 Hz, Ph–H), 7.51 (1H, d, J = 2.3 Hz, Het–H), 7.57 (1H, t, J = 7.3 Hz, Ph–H), 7.62 (2H, t, J = 7.3 Hz, Ph–H), 7.76 (1H, dd, J = 2.3 and 8.9 Hz, Het–H), 8.06 (1H, d, J = 8.9 Hz, Het–H). ^{13}C NMR (DMSO-d_6): δ 23.4, 30.3, 34.5, 124.4, 127.0, 129.2, 129.4, 129.5, 129.9, 131.2, 135.3, 135.9, 142.9, 144.6, 168.0. MS (m/z): 279 (M^+, 100.0), 244 ([M^+ – Cl], 94.6), 202 ([M^+ – C_6H_5], 8.5), 167 ([M^+ – C_6H_5Cl], 7.2). Calcd analysis for $C_{18}H_{14}ClN$: C, 77.40; H, 5.15; N, 5.02. Found, %: C, 77.16; H, 5.07; N, 5.10.

4.1.1.5. 2-Chloro-11-phenyl-6H-indeno[2,1-b]quinoline **10**. Reaction time 6 min, it was triturated at rt with EtOH, orange solid, mp (254–255)°C, yield 81%. ^{1}H NMR ($DCCl_3$): δ 3.86 (2H, s, CH_2), 7.32–7.51 (5H, m, Ph–H), 7.53–7.58 (4H, m, Ph–H), 7.62 (1H, d, J = 9.0 Hz, Het–H), 8.05 (1H, d, J = 9.0 Hz, Het–H), 8.89 (1H, s, Het–H). ^{13}C NMR ($DCCl_3$): δ 40.9, 119.6, 122.6, 124.1, 126.9, 127.6, 127.8,

Table 2
MIC values of compounds **9**, **13** and **14**.

Compound	MIC $H_{37}Rv$ (μg/mL)	% Inhib.[a]	MIC INH-R[b] (μg/mL)	% Inhib.	MIC RMP-R[c] (μg/mL)	% Inhib.	MIC OFX-R[d] (μg/mL)	% Inhib.
9	12.5	88	6.25	69	6.25	83	12.5	81
13	12.5	52	6.25	41	3.125	69	3.125	50
14	75	76	37.5	52	37.5	72	37.5	70
Rifampin (pos control)	0.049	65	0.02	74	NA[e]	NA	1.56	71
Isoniazid (pos control)	NA	NA	NA	NA	0.02	85	NA	NA

[a] Percent inhibition at the MIC concentration.
[b] INH-R = isoniazid resistance.
[c] RMP-R = rifampin resistance.
[d] OFX-R = ofloxacin resistance.
[e] NA = not applicable: compound not used in assay.

128.7, 129.0, 130.6, 130.7, 134.3, 137.2, 139.2, 139.8, 140.1, 142.6, 146.1, 159.3. Calcd analysis for $C_{22}H_{14}ClN$, %: C, 80.61; H, 4.30; N, 4.27. Found, %: C, 80.58; H, 4.38; N, 4.33.

4.1.1.6. 8-Chloro-10-phenyl-11H-indeno[1,2-b]quinoline ***11***. Reaction time 2 min, it was triturated at rt with EtOH, white solid, mp (250–251)°C, yield 92%. ^{1}H NMR ($DCCl_3$): δ 4.09 (2H, s, CH_2), 7.51–7.84 (5H, m, Ph–H), 7.67–7.70 (3H, m, Ph–H), 7.76 (1H, d, J = 2.0 Hz, Het–H), 7.82 (1H, dd, J = 2.0 and 9.0 Hz, Het–H), 9.16 (1H, d, J = 9.0 Hz, Het–H), 9.34 (1H, d, J = 7.7 Hz, Ph–H). ^{13}C NMR ($DCCl_3$): δ 35.6, 124.3, 126.1, 127.7, 128.6, 129.5, 129.8, 130.2, 131.0, 133.4, 133.8, 134.2, 135.0, 135.6, 136.4, 137.4, 147.9, 151.7, 156.4. Calcd analysis for $C_{22}H_{14}ClN$, %: C, 80.61; H, 4.30; N, 4.27. Found, %: C, 80.63; H, 4.36; N, 4.30.

4.1.1.7. 8-Chloro-10-phenyl-11H-indeno[1,2-b]quinolin-11-one ***12***. Reaction time 4.5 min, it was crystallized from EtOH, yellow solid, mp (239–241)°C, yield 93%. ^{1}H NMR (DMSO-d_6): δ 7.40-7.43 (3H, m, Ph–H), 7.46–7.51 (6H, m, Ph–H), 8.45 (1H, d, J = 9.1 Hz, Het–H), 8.67 (1H, d, J = 9.1 Hz, Het–H), 8.82 (1H, s, Het–H). ^{13}C NMR (DMSO-d_6): δ 118.4, 121.0, 121.4, 123.4, 124.1, 125.8, 127.1, 128.4, 129.6, 130.1, 130.6, 131.1, 133.6, 136.2, 137.0, 142.3, 145.2, 148.1, 150.4, 194.4. Calcd analysis for $C_{22}H_{12}ClNO$, %: C, 77.31; H, 3.54; N, 4.10. Found, %: C, 77.38; H, 3.50; N, 4.15.

4.1.1.8. 7-Nitro-9-phenyl-1,2,3,4-tetrahydroacridine ***13***. Reaction time 6 min, it was crystallized from water, yellow solid, mp (156–158)°C, yield 52%. ^{1}H NMR ($DCCl_3$): δ 1.77–1.85 (2H, m, CH_2), 1.95–2.04 (2H, m, CH_2), 2.64 (2H, t, J = 6.4 Hz, CH_2), 3.23 (2H, t, J = 6.7 Hz, CH_2), 7.22–7.26 (2H, m, Ph–H), 7.53–7.58 (3H, m, Ph–H), 8.10 (1H, d, J = 9.2 Hz, Het–H), 8.29 (1H, d, J = 2.6 Hz, Het–H), 8.36 (1H, dd, J = 2.6 Hz and 9.2 Hz). ^{13}C NMR ($DCCl_3$): δ 22.5, 22.6, 28.1, 34.5, 121.9, 122.9, 125.7, 128.6, 128.9, 129.0, 130.1, 130.8, 135.3, 144.9, 148.3, 148.4, 163.5. MS (*m*/*z*): 304 (M^+, 100.0), 257 ([M^+ – NO_2], 33.3), 227 ([M^+ – C_6H_5], 5.4). Calcd analysis for $C_{19}H_{16}N_2O_2$: C, 74.98; H, 5.30; N, 9.20. Found, %: C, 72.63; H, 5.10; N, 9.41.

4.1.1.9. 2-Nitro-11-phenyl-7,8,9,10-tetrahydro-6H-cyclohepta[b]quinoline ***14***. Reaction time 3 min, it was triturated at rt with EtOH, white solid, mp (179–181)°C, yield 44%. ^{1}H NMR ($DCCl_3$): δ 1.05–1.08 (2H, m, CH_2), 1.52–1.56 (2H, m, CH_2), 1.58–1.67 (2H, m, CH_2), 2.87–2.97 (2H, m, CH_2), 3.01–3.08 (2H, m, CH_2), 7.33–7.54 (5H, m, Ph–H), 8.13 (1H, d, J = 9.0 Hz, Het–H), 8.25 (1H, d, J = 2.1 Hz, Het–H), 8.42 (1H, dd, J = 2.1 and 9.0 Hz, Het–H). ^{13}C NMR ($DCCl_3$): δ 21.3, 22.1, 24.7, 28.8, 36.3, 120.9, 121.4, 125.2, 127.5, 128.4, 129.3, 130.1, 130.6, 136.7, 143.2, 148.4, 165.7. MS (*m*/*z*): 322 (M^+, 70.0), 272 ([M^+ – NO_2], 37.0), 245 ([M^+ – C_6H_5], 19.0), 198 ([M^+ – C_5H_{10}], 100.0). Calcd analysis for $C_{20}H_{18}N_2O_2$: C, 75.45; H, 5.70; N, 8.80. Found, %: C, 75.63; H, 5.90; N, 8.73.

4.1.1.10. 3,3-Dimethyl-7-nitro-9-phenyl-3,4-dihydroacridin-1(2H)-one ***15***. Reaction time 4 min, it was crystallized from acetone, yellow solid, mp (167–169)°C, yield 76%. ^{1}H NMR (DMSO-d_6): δ 1.12 (2H, s, CH_2), 2.51 (6H, s, CH_3), 3.35 (2H, s, CH_2), 7.28–7.29 (2H, m, Ph–H), 7.55–7.56 (3H, m, Ph–H), 8.21 (1H, d, J = 2.5 Hz, Het–H), 8.25 (1H, d, J = 9.2 Hz, Het–H), 8.54 (1H, dd, J = 2.5 and 9.2 Hz, Het–H). ^{13}C NMR (DMSO-d_6): δ 28.3, 32.3, 48.0, 53.7, 124.5, 124.6, 125.3, 126.4, 128.5, 128.7, 128.8, 130.9, 136.5, 145.5, 150.6, 151.9, 165.9, 197.4. Calcd analysis for $C_{21}H_{18}N_2O_3$: C, 72.82; H, 5.24; N, 8.09. Found, %: C, 72.90; H, 5.30; N, 8.12.

4.1.1.11. 8-Nitro-10-phenyl-11H-indeno[1,2-b]quinoline ***16***. Reaction time 2.50 min, it was triturated at rt with EtOH, yellow solid, mp > 300 °C, yield 70% ^{1}H NMR ($CDCl_3$): δ 3.96 (2H, s, CH_2), 7.52 (2H, d, J = 6.5 Hz, Ph–H), 7.57–7.68 (6H, m, Ph–H), 8.37 (1H, d, J = 9.0 Hz, Het–H), 8.39 (1H, d, J = 2.7 Hz, Ph–H), 8.49–8.51 (1H, dd, J = 2.5 and 9.3 Hz, Het–H), 8.69 (1H, d, J = 2.5 Hz, Ph–H). ^{13}C NMR ($CDCl_3$): δ 34.1, 122.4, 122.8, 122.9, 125.4, 125.5, 127.9, 129.1, 129.2, 130.7, 131.3, 134.8, 134.9, 144.9, 145.4, 145.8. Calcd analysis for $C_{22}H_{14}N_2O_2$: C, 78.09; H, 4.17; N, 8.28. Found, %: C, 78.13; H, 4.22; N, 8.31.

4.1.1.12. 8-Nitro-10-phenyl-11H-indeno[1,2-b]quinolin-11-one ***17***. Reaction time 2.5 min, it was crystallized from acetone-water, yellow solid, mp (297–299)°C, yield 65%. ^{1}H NMR (DMSO-d_6): δ 7.37–7.42 (4H, m, Ph–H), 7.44–7.57 (5H, m, Ph–H), 8.51 (1H, d, J = 9.2 Hz, Het–H), 8.58 (1H, d, J = 9.2 Hz, Het–H), 8.62 (1H, s, Het–H). ^{13}C NMR (DMSO-d_6): δ 118.1, 121.3, 121.4, 123.6, 124.2, 125.7, 127.4, 128.5, 129.7, 130.4, 130.7, 131.3, 133.7, 136.3, 137.1, 142.4, 145.5, 148.3, 150.7, 193.8. Calcd analysis for $C_{22}H_{12}N_2O_3$: C, 74.99; H, 3.43; N, 7.95. Found, %: C, 75.12; H, 3.50; N, 8.00.

Table 3
MBC results for compounds **9**, **13** and **14**.

Compound	CBM (μg/mL)	Colony count (CFU)
9	50	0
13	–[a]	TNTC[b]
14	–	TNTC
Rifampin	1.56	39

[a] MBC above the established rejection value of ≥40 colonies.
[b] TNTC = too numerous to count.

4.2. Antituberculosis tests

4.2.1. In vitro activity against M. tuberculosis (Mtb)

All manipulations of Mtb $H_{37}Rv$ were conducted in accordance with the Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition in Biosafety Level 3 containment laboratories [14].

Table 4
Macrophage and MTT results.

Compd.	Macrophage log reduction (low conc.)	Macrophage log reduction (mid conc.)	Macrophage log reduction (high conc.)	MTT % viability (low conc.)	MTT % viability (mid conc.)	MTT % viability (high conc.)
9	1.00	1.82	3.12	86	79	<10
13	1.80	1.25	1.72	67	63	25
14	1.24	1.19	1.41	82	79	<10
Rifampin (pos control)	0.46	1.83	1.85	82	86	78

The assay uses Mtb $H_{37}Rv$ in a 384-well plate format with in-plate DMSO carrier controls, 3.198 μM amikacin, 0.17 μM amikacin controls and 320 compounds. The Mtb $H_{37}Rv$ + DMSO carrier control provides a 100% growth control for each plate. The 3.198 μM amikacin completely inhibits growth of the bacteria and is used in place of uninoculated media as the background control; whereas, the 0.17 μM amikacin control approximates the MIC of amikacin ranging from 30 to 80% inhibition indicative of positive growth inhibition and proper assay performance. The media used for both compound preparation and Mtb $H_{37}Rv$ plating was assessed for contamination by plating two 384-well plates with media alone. The plates were checked for contamination by visual inspection and end-point detection. The compounds were evaluated in 10-point stacked plate dose response method. Compounds were serially diluted 1:2 from 100 μM thru 0.195 μM. For end-point detection the Promega reagent BacTiter-Glo™ Microbial Cell Viability (BTG) was used. The BTG reagent lyses the bacteria for the ATP quantification using luciferase production as an end-point detection point. After seven days of incubation, the assay plates were removed from the incubator and allowed to equilibrate to room temperature. Twenty-five microliters of BTG reagent, one third of the final volume of the well, was added using a Matrix WellMate. The BTG plates were briefly incubated for 20 min at room temperature, sealed with Perkin Elmer clear TopSeal A and read from the top using luminescence on a Perkin Elmer Envision.

4.2.2. Minimal inhibitory concentration (MIC)

The broth microdilution assay format following guidelines established by the Clinical and Laboratory Standards Institute (CLSI) is routinely utilized for MIC testing. Briefly, testing was conducted using 96-well, U-bottom microplates with an assay volume of 0.2 mL/well. First, the test media, Middlebrook 7H9 broth supplemented with OADC Enrichment (BD BioSciences; Sparks, MD), was added (0.1 mL/well) to each well. The test compounds, solubilized in appropriate solvent and subsequently diluted in test media, were subsequently added (0.1 mL/well) to appropriate wells at twice the intended starting concentration and serially diluted two-fold across the plate. The plates were then inoculated (0.1 mL/well) with a targeted concentration of 1.0 × 106 CFU/mL *M. tuberculosis* and incubated at 37 °C for 7 days in approximately 90% humidity. Following incubation, the plates were read visually and individual wells scored for turbidity, partial clearing or complete clearing. Testing was conducted in duplicate and the following controls were included in each test plate: i) medium only (sterility control); ii) organism in medium (negative control); and iii) rifampin or isoniazid (positive control). The MIC is reported as the lowest concentration (μg/mL) of drug that visually inhibits growth of the organism.

4.2.3. Minimal bactericidal concentration (MBC)

The MBC is determined subsequent to MIC testing by subculturing diluted aliquots from wells that fail to exhibit macroscopic growth. The sample aliquots were inoculated onto Middlebrook 7H10 agar plates and subsequently incubated for 16–21 days at 37 °C. Once growth was readily apparent, the bacterial colonies were enumerated. The MBC is defined as the lowest concentration (μg/mL) of compound exhibiting 99.9% kill over the same time period used to determine the MIC (18–24 h). MBC values greater than 16 times the MIC typically indicate antimicrobial tolerance.

4.2.4. Low-oxygen recovery assay (LORA)

Briefly, microplates were prepared in the same manner as the MIC testing format. Instead of incubating aerobically, the plates are placed under anaerobic conditions using a MACS MIC automated jar gassing system and incubated for 7 days at 37 °C. The plates were subsequently transferred to an ambient gaseous condition (5% CO_2) for 7 days after which the plates are read visually and individual wells scored for turbidity, partial clearing or complete clearing. Testing was conducted in duplicate and the following controls were included in each test plate: i) medium only (sterility control); ii) organism in medium (negative control); and iii) rifampin or isoniazid (positive control). Results are reported as the lowest concentration (μg/mL) of drug that visually inhibits growth of the organism.

4.2.5. Intracellular drug activity

Briefly, the murine J774 cell line was propagated in RPMI 1640 supplemented with L-glutamine and fetal bovine serum (FBS). Cells were maintained in tissue culture flasks at 37 °C in the presence of 5% CO_2. For infection studies, J774 cells were transferred to 12-well tissue culture chambers in 1 mL volumes at a density of 2.0 × 105 in the presence of 10% FBS. After overnight incubation, the medium was replaced with fresh medium containing 1% FBS to stop macrophage division while maintaining cell viability. Twenty-four hours later, the macrophage monolayer was enumerated with an ocular micrometer for total number of cells per well to determine the infection ratio. The medium was removed and replaced with 1 mL of fresh medium with 1% FBS containing Mtb at a multiplicity of infection (MOI) of 5 Mycobacteria/macrophage. The cells are infected for 4 h after which time non phagocytosed Mycobacteria were washed from the monolayers and fresh medium added. Drugs were then added, using 3 concentrations, and infection allowed proceeding for 7 days. At 0 and 7 days, the macrophages were lysed with sodium dodecyl sulfate, treated with DNAase, diluted and plated onto 7H10 agar to determine the cell number or colony forming units (CFU). Each drug concentration was tested in duplicate and rifampin was used as the positive control drug. A drug cytotoxicity control plate assay (MTT proliferation) was also conducted in parallel using uninfected macrophages to confirm that concentrations utilized for testing were not toxic to the macrophages.

4.2.6. Bacteria

MIC screening was conducted for Mtb $H_{37}Rv$ (SRI 1345), isoniazid (INH)-resistant Mtb (SRI 1369), rifampin (RMP)-resistant Mtb (SRI 1367), and ofloxacin (OFX)-resistant Mtb (SRI 4000). MBC, LORA and intracellular drug screening assays were conducted using only Mtb $H_{37}Rv$ (SRI 1345).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.12.013.

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