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Synthesis and antimycobacterial evaluation of newer 1-cyclopropyl-1,4-dihydro-6-fluoro-7-(substituted secondary amino)-8-methoxy-5-(sub)-4-oxoquinoline-3-carboxylic acids

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Abstract—Thirty-four newer 1-cyclopropyl-1,4-dihydro-6-fluoro-7-(substituted secondary amino)-8-methoxy-5-(sub)-4-oxoquinoline-3-carboxylic acids were synthesized from 1,2,3,4-tetrafluoro benzene and evaluated for in vitro and in vivo antimycobacterial activities against *Mycobacterium tuberculosis* H37Rv (MTB), multi-drug resistant *M. tuberculosis* (MDR-TB) and *Mycobacterium smegmatis* (MC^2) and also tested for the ability to inhibit the supercoiling activity of DNA gyrase. Among the synthesized compounds, 7-(1-(4-methoxybenzyl)-3,4,5,6,7,8-hexahydroisoquinolin-2(1*H*)-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-5-nitro-4-oxoquinoline-3-carboxylic acid (**13n**) was found to be the most active compound in vitro with MIC of 0.16 and 0.33 μM against MTB and MDR-TB, respectively. In the in vivo animal model **13n** decreased the bacterial load in lung and spleen tissues with 2.54 and 2.92 – log10 protections, respectively, at the dose of 50 mg/kg body weight. Compound **13n** also inhibited the supercoiling activity of mycobacterial DNA gyrase with IC_{50} of 30.0 μg/ml.

1. Introduction

Tuberculosis (TB) continues to be a major health concern. The World Health Organisation (WHO) declared TB a global emergency in 1993.[1] The emergence of AIDS and decline of socioeconomic standards have contributed to the disease's resurgence in industrialized countries. In most developing countries, although the disease has always been endemic, its severity has increased because of the global HIV pandemic and extensive social restructuring due to rapid industrialization and conflicts. There is now recognition that new drugs to treat TB are urgently required, specifically for use in shorter treatment regimens than are possible with the current agents and which can be employed to treat multi-drug resistant and latent disease. During the past decade, several of the fluoroquinolone antibacterial drugs have been examined as potential chemotherapeutics for *Mycobacterium tuberculosis* infection because of their favorable pharmacokinetic profiles such as easily absorbed after oral administration and readily penetrated into mammalian cells.[2] Fluoroquinolones exhibit potent in vitro and in vivo antimycobacterial activity.[3] Quinolones inhibit both bacterial type II topoisomerase, DNA gyrase and topoisomerase IV,[4] which are essential enzymes catalyzing DNA supercoiling and decatenation reactions, respectively. There is also a considerable effort to discover and develop newer fluoroquinolones, and some of them might have value in the treatment of TB.[5] Several of the quinolone antibacterial drugs have been examined as inhibitors of MTB as well as other mycobacterial infections.[6] As a result, gatifloxacin and moxifloxacin (8-methoxy quinolones) are drugs in pipeline and to be approved by FDA for the treatment of TB by the year 2010. Herewith we report the synthesis of newer 8-methoxy quinolone analogues containing unreported bulky secondary amino function at 7th position and nitro group at 5th position of 8-methoxy quinolone and the study of the influence of lipophilic character and nitro group on activity against MTB.

Keywords: Antimycobacterial activity; Antitubercular activity; 8-Methoxyquinolone carboxylic acids.
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2. Results and discussion

2.1. Synthesis

Thirty-four new 8-methoxyquinolone carboxylic acids with variation at C5 and C7 positions were synthesized from 1,2,3,4-tetrafluoro benzene (**1**) according to the literature method[7–9] with modification in some steps. Briefly, compound **1** was converted to 1-bromo-2,3,4,5-tetrafluorobenzene (**2**) using bromine and aluminium bromide in the presence of oleum, which was further methoxylated using sodium methoxide in methanol to yield 1-bromo-3-methoxy-2,4,5-trifluoro benzene (**3**). Compound **3** on treatment with cuprous cyanide and *N*-methyl-2-pyrrollidone gave the corresponding cyano derivative 3-methoxy-2,4,5-trifluorobenzonitrile (**4**). Compound **4** was reduced to give the corresponding amide (**5**) which was further treated with sulfuric acid to form 3-methoxy-2,4,5-trifluoro benzoic acid (**6**). Compound **6** on reaction with 1,1′-carbonyldiimidazole in tetrahydrofuran afforded the corresponding imidazolide, which, in situ, was treated with neutral magnesium salt of ethyl potassium malonate in the presence of tri-ethyl amine to yield ethyl 3-(2,4,5-trifluoro-3-methoxyphenyl)-3-oxopropanoate (**7**). Ethyl 3-(cyclopropylamino)-2-(2,4,5-trifluoro-3-methoxybenzoyl)acrylate (**9**) was prepared by a two-step one-pot reaction. First treatment of the keto ester **7** with triethyl orthoformate in acetic anhydride gave the one-carbon homologue enol ether intermediate ethyl 3-ethoxy-2-(2,4,5-trifluoro-3-methoxybenzoyl)acrylate (**8**) as an oil, which on reaction with cyclopropylamine at 0 °C affords 67% of **9** as an oily residue. Compound **9** on cyclization with the base potassium carbonate in dimethylsulfoxide yielded ethyl 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylate (**10**), when compared to earlier report,[7] this step proceeds smoothly without using violently reactive sodium hydride. Ethyl ester was finally hydrolysed in acidic condition to yield 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic acid (**11a**), which on nitration at C5 position with sulfuric acid and potassium nitrate yielded 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-5-nitro-4-oxoquinoline-3-carboxylic acid (**11b**). The titled compounds **12** and **13a–p** were prepared by treating **11a** and **11b** with appropriate secondary amines in the presence of potassium carbonate under microwave irradiation in DMSO (Scheme 1). When compared to conventional method[7] of 12 h process, microwave-assisted synthesis was performed with short reaction times (3–6 min), with ease and was environment friendly. Compounds **13c** and **13n** were further reduced by catalytic reduction of the nitro group to yield **14c** and **14n**. The purity of the synthesized compounds was monitored by thin-layer chromatography (TLC) and elemental analyses and the structures were identified by spectral data.

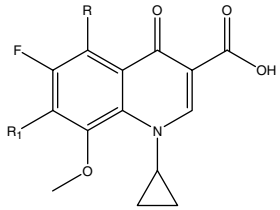
2.2. Antimycobacterial activity

The compounds were screened for their in vitro antimycobacterial activity against MTB, MDR-TB and *Mycobacterium smegmatis* ATCC 14468 (MC^2) by agar dilution method for the determination of MIC in duplicate.[10] The MDR-TB clinical isolate was resistant to isoniazid, rifampicin, ethambutol and ofloxacin. The minimum inhibitory concentration (MIC) is defined as the minimum concentration of the compound required to give complete inhibition of bacterial growth and MICs of the synthesized compounds along with the standard drugs for comparison are presented in Table 1.

In the first phase of screening against MTB, all the compounds showed excellent in vitro activity against MTB with MIC less than 15 μM. Five compounds (**13c**, **13g**, **12l**, **13l** and **13n**) inhibited MTB with MIC of less than 1 μM and were more potent than standard gatifloxacin (MIC: 1.04 μM). When compared to isoniazid (MIC: 0.36 μM), one compound (**13n**) was found to be more active against MTB and one compound (**13c**) was found to be equally active to isoniazid. Compound 7-(1-(4-methoxybenzyl)-3,4,5,6,7,8-hexahydroisoquinolin-2(1*H*)-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-5-nitro-4-oxoquinoline-3-carboxylic acid (**13n**) was found to be the most active compound in vitro with MIC of 0.16 μM against MTB and was 2.25 and 6.5 times more potent than isoniazid and gatifloxacin, respectively. Subsequently some of the compounds were evaluated against MDR-TB, and among the 22 compounds screened, all the compounds inhibited MDR-TB with MIC ranging from 0.33 to 3.99 μM and were found to be more active than isoniazid (MIC: 45.57 μM) and gatifloxacin (MIC: 8.34 μM). Five compounds (**13c**, **12e**, **13l**, **13n** and **13p**) inhibited MDR-TB with MIC of less than 1 μM. Compound **13n** was found to be the most active in vitro with MIC of 0.33 μM against MDR-TB and was 25 and 138 times more potent than gatifloxacin and isoniazid, respectively. The compounds were also evaluated against MC^2 in which all the compounds inhibited MC^2 with MIC

Scheme 1. Synthetic protocol of the compounds.

Table 1. Physical constants, in vitro antimycobacterial activities and cytotoxicity



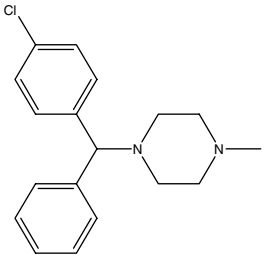
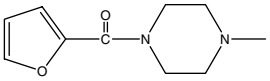
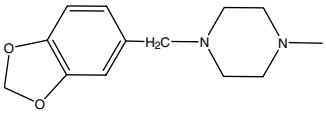
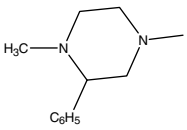
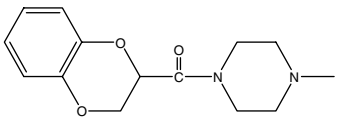
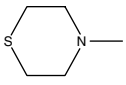
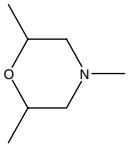
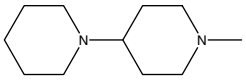
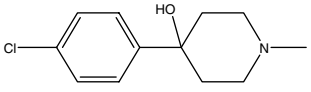
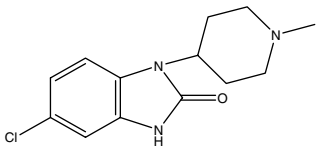
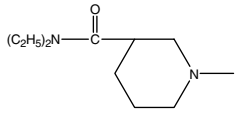
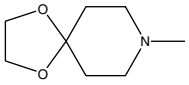
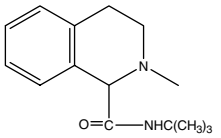
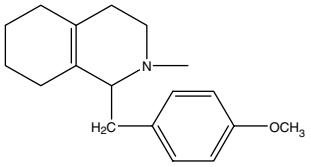
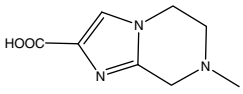
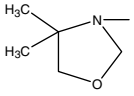
Compound	R	R_1	IC_{50} (μM)	MIC (μM)		
				MTB	MDRTB	MC^2
12a	H		NT	11.12	NT	44.48
13a	NO_2		102.96	1.28	1.28	10.29
12b	H		NT	13.72	NT	13.72
13b	NO_2		NT	6.25	NT	12.49
12c	H		126.14	1.57	1.57	3.15
13c	NO_2		115.63	0.35	0.35	1.44
14c	NH_2		NT	3.06	NT	6.13
12d	H		NT	13.84	NT	27.69
13d	NO_2		125.88	3.14	3.14	6.30
12e	H		119.39	1.49	0.74	1.49
13e	NO_2		109.94	1.37	2.74	2.74
12f	H		165.16	2.06	2.06	4.12
13f	NO_2		147.61	1.84	1.84	3.68
12g	H		160.09	3.99	3.99	16.01
13g	NO_2		143.55	0.89	1.79	1.79
12h	H		NT	7.06	NT	14.09
13h	NO_2		NT	6.41	NT	12.79
12i	H		NT	6.43	NT	12.84
13i	NO_2		NT	5.88	NT	11.75

Table 1 (*continued*)

Compound	R	R_1	IC_{50} (μM)	MIC (μM)		
				MTB	MDRTB	MC^2
12j	H		NT	5.94	NT	5.94
13j	NO_2		NT	5.47	NT	10.93
12k	H		136.01	1.69	3.39	0.85
13k	NO_2		123.88	1.55	1.55	12.39
12l	H		149.37	0.93	1.86	1.86
13l	NO_2		134.87	0.84	0.41	6.75
12m	H		NT	12.31	NT	49.26
13m	NO_2		113.11	1.41	2.82	2.82
12n	H		117.35	1.46	1.46	2.93
13n	NO_2		108.21	0.16	0.33	0.68
14n	NH_2		114.13	2.85	2.85	5.72
12o	H		141.27	3.53	1.76	7.08
13o	NO_2		128.23	3.120	1.60	6.42
12p	H		166.06	4.14	2.07	16.61
13p	NO_2		148.32	1.85	0.93	3.70
Gati	—	—	>155.3	1.04	8.34	2.08
INH	—	—	>455.8	0.36	45.57	45.57

NT indicates not tested.

ranging from 0.68 to 49.26 μM; 33 compounds were found to be more active than isoniazid (MIC: 45.57 μM) and six compounds were more active than gatifloxacin (MIC: 2.08 μM).

With respect to structure–MTB activity relationship, the results demonstrated that the antimycobacterial activity was enhanced to varying degrees (upto 9-fold) by the introduction of nitro group at C5 position. Reduction of nitro group at C5 position to amino group (**14c** and **14n**) reduces the activity indicating that favourable substitution at C5 was $NO_2 > H > NH_2$. At C7 position we have studied with various substituted piperazines (**12** and **13a–e**), (thio) morpholines (**12** and **13f–g**), substituted piperidines (**12** and **13h–k**), fused piperazines and piperidines (**12** and **13l–p**). A comparison of the substitution pattern at C7 demonstrated that the order of activity was fused piperazines and piperidines ⩾ (thio) morpholines > substituted piperazines > substituted piperidines. By introducing bulky lipophilic secondary amines at C7, enhanced the antimycobacterial activity was enchanced which might be due to more penetration of these compounds into mycobacterial cells.

2.3. In vitro cytotoxicity

Some compounds were further examined for toxicity (IC_{50}) in a mammalian Vero cell line upto 62.5 μg/ml concentrations.[11] After 72 h of exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product and the results are reported in Table 1. Twenty-one compounds when tested showed IC_{50}

values ranging from 102.96 to 166.06 μM. A comparison of the substitution pattern at C5 demonstrated that nitro group was more cytotoxic than the unsubstituted derivatives. These results are important as the C5 nitro substituted compounds with their increased cytoliability are much less attractive in the development of a quinolone for the treatment of TB. This is primarily due to the fact that the eradication of TB requires a lengthy course of treatment, and the need for an agent with a high margin of safety becomes a primary concern. The IC_{50} value of compound **13n** was found to be 108.21 μM and it showed selectivity index (IC_{50}/MIC) of 676.31.

2.4. In vivo antimycobacterial activity

Subsequently, compounds **12l** and **13n** were tested for in vivo efficacy against MTB at a dose of 50 mg/kg (Table 2) in CD-1 mice.[12] The mice were infected intravenously with *M. tuberculosis* ATCC 35801. Drug treatment by intra-peritoneal route began after 10 days of inoculation of the animal with microorganism and continued for 10 days. After 35 days post-infection the spleens and right lungs were aseptically removed, and the number of viable organisms was determined and compared with the counts from negative (vehicle treated) controls (mean culture forming units (CFU) in lung: 7.99 ± 0.16 and in spleen: 9.02 ± 0.21). Compound **12l** decreased the bacterial load in lung and spleen tissues with 1.87 and 2.61 − log10 protections, respectively, and was considered to be promising in reducing bacterial count in lung and spleen tissues. Compound **13n** decreased the bacterial load in lung and spleen tissues with 2.54 and 2.92 − log10 protections, respectively, and was considered to be promising in reducing bacterial count in lung and spleen tissues. When compared to gatifloxacin at the same dose level **12l** decreased the bacterial load with 0.51 − log10 protections and **13n** with 0.61 and 0.82 − log10 protections in lung and spleen tissues, respectively.

2.5. DNA gyrase inhibition

DNA gyrase is a target for different classes of natural and synthetic compounds. The compounds are broadly classified into (1) quinolones and fluoroquinolones, which target GyrA subunit of gyrase, (2) coumarins and (3) cyclothialidines, both of which inhibit the ATPase activity associated with GyrB subunit of DNA gyrase. The 1-cyclopropyl-1,4-dihydro-6-fluoro-7-(substituted secondary amino)-8-methoxy-5-(sub)-4-oxoquinoline-3-carboxylic acid derivatives synthesized and studied in this report were tested for their ability to inhibit supercoiling activity of DNA gyrase. Earlier studies have revealed that DNA gyrase from MTB and that from MC^2 share many of the properties.[13] The supercoiling assay results with various compounds using MC^2 DNA gyrase are presented in Fig. 1a–d. The IC_{50} values are presented in Table 3. Of all the newly synthesized compounds, compound **13n** inhibited supercoiling reaction with an IC_{50}value of 30 μg/ml. The compound **13n** also showed highest in vivo efficacy against MTB, MDR-TB and MC^2 amongst all the compounds tested in this report (Table 1). The other compounds that showed significant supercoiling inhibition include compounds **12l** and **13f,** with IC_{50} values of 40 and 50 μg/ml, respectively. Rest of the compounds tested did not show comparable inhibition of the enzyme (IC_{50} values > 50μg/ml).

Table 2. In vivo activity data of **12l**, **13n**, gatifloxacin and isoniazid against *M. tuberculosis* ATCC 35801 in mice

Compound	Lungs (log CFU ± SEM)	Spleen (log CFU ± SEM)
Control	7.99 ± 0.16	9.02 ± 0.21
Gatifloxacin (50 mg/kg)	6.02 ± 0.23	6.92 ± 0.07
Isoniazid (25 mg/kg)	5.86 ± 0.23	4.71 ± 0.10
12l	6.12 ± 0.11	6.41 ± 0.12
13n	5.41 ± 0.21	6.10 ± 0.18

2.6. Phototoxic evaluation

Quinolones in general have favorable safety profiles; phototoxicity has become a significant factor in the clinical use of some. Indeed, the first quinolone, nalidixic acid, caused light-induced dermal effects. This type of response has now been demonstrated for almost all fluoroquinolones, although the relative phototoxic potential varies greatly among compounds. Phototoxicity is considered to be an acute, light-induced irritation response characterized by dermal inflammation, with erythema and oedema as primary clinical endpoints. Phototoxicity with the quinolones is generally thought to result from the absorption of light by the parent compound or a metabolite in tissue. This photosensitized chromophore may then transfer its absorbed photoenergy to oxygen molecules, creating an environment for the production of reactive oxygen species such as singlet oxygen. These reactive species are then thought to attack cellular lipid membranes, initiating the inflammatory process.

Five (**13c**, **12e**, **13g**, **12k** and **13n**) compounds were evaluated for potential phototoxicity in a standardized in vivo test system that has been used previously to assess quinolone antibiotics.[14] The test compounds (140 mg/kg) and the positive control lomefloxacin hydrochloride (140 mg/kg) were evaluated for phototoxicity and both ears of each mouse were evaluated for changes indicative of a positive response: erythema, oedema or a measurable increase in ear thickness. Change from baseline was calculated separately for each animal and time point and analyzed for statistical significance and are presented in Table 4. The drug and time factors were analyzed by separate univariate methods. Orthogonal contrasts were used to test for both linear and quadratic trends over time in each group by Student's *t*-tests to test whether the change from baseline ear thickness was significantly different from zero. The results indicated that lomefloxacin showed significant increase in ear thickness from 4–96 to 24–96 h when compared within time points and with the control, respectively. The test compounds were found to show a significant difference in ear thickness at various time-points when compared with the pre-drug reading (0 h) but were less toxic when compared with the

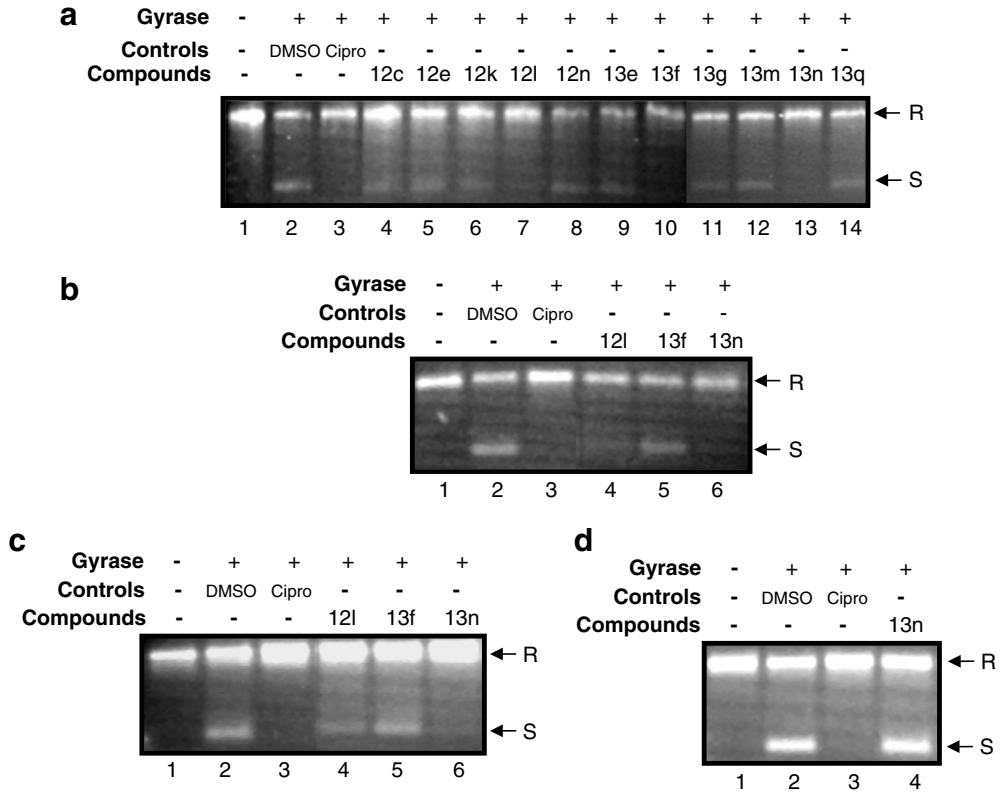


Figure 1. DNA gyrase supercoiling assay. The assays were performed as described in the text. DNA gyrase was pre-incubated with the indicated concentrations of the compounds and then rest of the components of the reaction including relaxed circular DNA were added. (a) Lane 1, relaxed circular DNA; lane 2, supercoiling reaction in presence of solvent control, 5% DMSO; lane 3, ciprofloxacin at 10 μg/ml concentration, used as a positive control of gyrase inhibition; lanes 4–14, reactions in the presence of 50 μg/ml of compounds **12c**, **12e**, **12k**, **12l**, **12n**, **13e**, **13f**, **13g**, **13m**, **13n** and **13q**, respectively. (b) Lane 1, relaxed circular DNA; lane 2, supercoiling reaction in the presence of 5% DMSO; lane 3, ciprofloxacin at 10 μg/ml concentration; lanes 4–6, reactions in the presence of 40 μg/ml of compounds **12l**, **13f** and **13n**, respectively. (c) Lane 1, relaxed circular DNA; lane 2, supercoiling reaction in presence of 5% DMSO; lane 3, ciprofloxacin at 10 μg/ml concentration; lanes 4–6, reactions in the presence of 30 μg/ml of compounds **12l**, **13f** and **13n**, respectively. (d) Lane 1, relaxed circular DNA; lane 2, supercoiling reaction in the presence of 5% DMSO; lane 3, ciprofloxacin at 10 μg/ml concentration; lanes 4, reaction in the presence of 20 μg/ml of compound **13n**. R and S indicate relaxed and supercoiled DNA, respectively.

Table 3. IC_{50} values for DNA gyrase inhibition

Compounds	IC_{50} (μg/ml)
12c	>50
12e	30
12k	>50
12l	>50
12n	40
13e	>50
13f	>50
13g	50
13m	>50
13n	30
13q	>50

negative (vehicle-treated) and positive controls (lomefloxacin). Compounds **13c** and **13g** showed a slight increase in ear thickness from 48–96 to 24–96 h, respectively, but were less toxic than the positive control (lomefloxacin). No erythema occurred in mice dosed with 140 mg/kg of **12e** and **13n** throughout the 96 h study, while compounds **13g** and **12k** showed a significant erythema after irradiation until 4 h only and compound **13c** developed erythema from 48 h.

3. Materials and methods

Melting points were taken on an electrothermal melting point apparatus (Buchi BM530) in open capillary tubes and are uncorrected. Infrared spectra (KBr disc) were run on Jasco IR Report 100 spectrometer. ^{1}H NMR spectra were scanned on a JEOL Fx 300 MHz NMR spectrometer using DMSO-d_6 as solvent. Chemical shifts are expressed in δ (ppm) relative to tetramethylsilane. ^{13}C NMR spectra were recorded on Bruker AC 200/DPX 400 MHz. Elemental analyses (C, H and N) were performed on Perkin-Elmer model 240 C analyzer and the data were within ±0.4% of the theoretical values.

3.1. Synthesis of 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-5-nitro-4-oxoquinoline-3-carboxylic acid (11b)

A solution of **10** (3 g, 1.00 equiv) in concentrated sulfuric acid (30 ml) was treated portionwise with potassium nitrite (1.5 equiv) over 30 min. The reaction mixture was stirred at room temperature for 6 h. Then poured into ice-cold water and the solid obtained was filtered and washed with water and dissolved in dichloromethane.

Table 4. Phototoxic evaluation of newer 8-methoxy quinolones

Group	Ear thickness (mm)[a]						Erythema[b]					
	Time (approximately) after start of irradiation (h)[c]											
	0	4	24	48	72	96	0	4	24	48	72	96
Control[d]	0.37 ± 0.03	0.36 ± 0.02	0.38 ± 0.03	0.37 ± 0.03	0.37 ± 0.03	0.38 ± 0.03	0	0	0	0	0	0
13c	0.26 ± 0.01	0.27 ± 0.02	0.28 ± 0.01	0.30 ± 0.01	0.33 ± 0.01	0.31 ± 0.02	0	0	0	2	3	4
12e	0.33 ± 0.01	0.31 ± 0.01	0.32 ± 0.01	0.33 ± 0.01	0.33 ± 0.01	0.32 ± 0.02	0	0	0	0	0	0
13g	0.28 ± 0.02	0.29 ± 0.01	0.35 ± 0.02	0.36 ± 0.01	0.34 ± 0.01	0.33 ± 0.01	0	2	0	0	0	0
12k	0.39 ± 0.04	0.38 ± 0.04	0.36 ± 0.05	0.37 ± 0.06	0.39 ± 0.07	0.39 ± 0.07	0	2	0	0	0	0
13n	0.32 ± 0.01	0.33 ± 0.01	0.32 ± 0.02	0.33 ± 0.01	0.33 ± 0.01	0.32 ± 0.02	0	0	0	0	0	0
Lomefloxacin	0.31 ± 0.01	0.40 ± 0.02	0.48 ± 0.02	0.53 ± 0.02	0.64 ± 0.04	0.60 ± 0.06	6	6	6	6	6	6

[a] Mean ear thickness ± SEM; left and right ears were averaged.
[b] Number of mice with erythema.
[c] Time zero = pre-dose (mice exposed to UV light immediately after dosing); 4 h = end of irradiation period.
[d] Control = 0.5% aqueous solution of sodium carboxymethylcellulose (4 Ns/m^2) dosed at 10 ml/kg.

The DCM layer was washed successively with water and 5% sodium bicarbonate, dried over sodium sulfate, distilled recrystallised from methanol (Yield: 88%, mp >250 °C).

3.2. Synthesis of 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic acid (11a)

The product **10** (3 g) was suspended in 6 N HCl (50 ml) and refluxed for 3 h and then cooled to 0 °C. The precipitate obtained was filtered, washed with water and dried (Yield: 90%, mp >250 °C).

3.3. Synthesis of 1-cyclopropyl-1,4-dihydro-6-fluoro-7-(substituted secondary amino)-8-methoxy-5-(sub)-4-oxoquinoline-3-carboxylic acid (12 and 13a–p)

To a solution of **11a**,**b** (0.295 g, 0.34 g, 1.0 equiv) in dimethylsulfoxide (10 ml) and substituted secondary amines (1.1 equiv), potassium carbonate (0.165 g, 1.2 equiv) was added and placed in microwave and allowed to react at 320 V for a period of 2 min. After completion of the reaction, the mixture was poured into ice-cold water and kept overnight, filtered and washed with water to yield **12** and **13a**–**p**.

3.3.1. 7-(4-((4-Chlorophenyl)(phenyl)methyl)piperazin-1-yl)-1-cyclopropyl-1,4-dihydro-6-fluoro-8-methoxy-4-oxoquinoline-3-carboxylic acid (12a). Yield: 69%; mp 115–117°C; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28–0.48 (m, 4H, cyclopropyl), 1.35 (m, 1H, cyclopropyl), 2.59 (t, 4H, 3,5-CH_2 of piperazine), 3.32 (t, 4H, 2,6-CH_2 of piperazine), 3.77 (s, 3H, OCH_3), 5.2 (s, 1H, CH of diphenylmethyl), 7.0–7.18 (m, 9H, Ar-H), 7.9 (s, 1H, C_5-H), 8.79 (s, 1H, C_2-H), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 5.6, 36.0, 49.6, 50.6, 56.1, 73.6, 108.6, 109.5, 118.8, 126.3, 128.3, 129.4, 131.9, 132.8, 142.8, 146.5, 145.3, 148.7, 166.3, 177.5. Anal. ($C_{31}H_{29}ClFN_3O_4$) C, H, N. Calculated C, 66.25; H, 5.20; N, 7.48. Found: C, 66.22; H, 5.19; N, 7.48.

3.3.2. 7-(4-((4-Chlorophenyl)(phenyl)methyl)piperazin-1-yl)-1-cyclopropyl-1,4-dihydro-6-fluoro-8-methoxy-5-nitro-4-oxoquinoline-3-carboxylic acid (13a). Yield: 62%; mp 168–170°C; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28–0.48 (m, 4H, cyclopropyl), 1.35 (m, 1H, cyclopropyl), 2.59 (t, 4H, 3,5-CH_2 of piperazine), 3.32 (t, 4H, 2,6-CH_2 of piperazine), 3.77 (s, 3H, OCH_3), 5.2 (s, 1H, CH of diphenylmethyl), 7.0–7.18 (m, 9H, Ar-H), 8.79 (s, 1H, C_2-H), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 5.6, 36.0, 49.6, 50.6, 56.1, 73.6, 107.6, 109.5, 118.8, 126.3, 128.3, 129.4, 130.8, 131.9, 132.8, 142.8, 146.5, 148.7, 166.3, 177.5. Anal. ($C_{31}H_{28}ClFN_4O_6$) C, H, N. Calculated C, 61.34; H, 4.65; N, 9.23. Found: C, 61.34; H, 4.66; N, 9.22.

3.3.3. 1-Cyclopropyl-1,4-dihydro-6-fluoro-7-(4-(2-furoyl)-piperazin-1-yl)-8-methoxy-4-oxoquinoline-3-carboxylic acid (12b). Yield: 65%; mp 215–217°C; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28–0.48 (m, 4H, cyclopropyl), 1.35 (m, 1H, cyclopropyl), 3.16 (t, 4H, 3,5-CH_2 of piperazine), 3.26 (t, 4H, 2,6-CH_2 of piperazine), 3.52 (s, 3H, OCH_3), 6.5–7.28 (m, 3H, Ar-H), 7.91 (s, 1H, C_5-H), 8.81 (s, 1H, C_2-H), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 5.6, 36.2, 47.5, 49.5, 56.2, 107.7, 109.3, 111.4, 113.5, 130.6, 131.8, 133.0, 147.1, 148.1, 155.2, 166.3, 177.5. Anal. ($C_{23}H_{22}FN_3O_6$) C, H, N. Calculated C, 60.66; H, 4.87; N, 9.23. Found: C, 60.64; H, 4.88; N, 9.21.

3.3.4. 1-Cyclopropyl-1,4-dihydro-6-fluoro-7-(4-(2-furoyl)-piperazin-1-yl)-8-methoxy-5-nitro-4-oxoquinoline-3-carboxylic acid (13b). Yield: 66%; mp 118–120 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28–0.48 (m, 4H, cyclopropyl), 1.35 (m, 1H, cyclopropyl), 3.16 (t, 4H, 3,5-CH_2 of piperazine), 3.26 (t, 4H, 2,6-CH_2 of piperazine), 3.52 (s, 3H, OCH_3), 6.5–7.28 (m, 3H, Ar-H), 8.81 (s, 1H, C_2-H), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 5.6, 36.2, 47.5, 49.5, 56.2, 107.7, 109.3, 111.4, 113.5, 130.6, 131.8, 133.0, 145.0, 147.1, 148.1, 155.2, 166.3, 177.5. Anal. ($C_{23}H_{21}FN_4O_8$) C, H, N. Calculated C, 55.20; H, 4.23; N, 11.20. Found: C, 55.1; H, 4.23; N, 11.21.

3.3.5. 7-(4-((Benzo[*d*][1,3]dioxol-5-yl)methyl)piperazin-1-yl)-1-cyclopropyl-1,4-dihydro-6-fluoro-8-methoxy-4-oxoquinoline-3-carboxylic acid (12c). Yield: 68%; mp 95–97 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28–0.48 (m, 4H, cyclopropyl), 1.35 (m, 1H, cyclopropyl), 2.82 (t, 4H, 3,5-CH_2 of piperazine), 3.1 (t, 4H, 2,6-CH_2 of

piperazine), 3.55 (s, 3H, OCH_3), 3.6 (s, 2H, CH_2 of piperanoyl), 5.86 (s, 2H, –OCH_2O–), 6.42–6.62 (m, 3H, Ar-H), 7.8 (s, 1H, C_5-H), 8.8 (s, 1H, C_2-H), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 5.6, 36.1, 50.3, 52.2, 56.1, 60.4, 108.2, 109.5, 101.3, 113.9, 115.1, 119.4, 122.2, 128.8, 129.9, 132.5, 145.8, 147.3, 148.5, 148.1, 166.3, 177.5. Anal. ($C_{26}H_{26}FN_3O_6$) C, H, N. Calculated C, 63.02; H, 5.29; N, 8.48. Found: C, 63.00; H, 5.29; N, 8.46.

3.3.6. 7-(4-((Benzo[d][1,3]dioxol-5-yl)methyl)piperazin-1-yl)-1-cyclopropyl-1,4-dihydro-6-fluoro-8-methoxy-5-nitro-4-oxoquinoline-3-carboxylic acid (13c). Yield: 80%; mp 188–190 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28–0.48 (m, 4H, cyclopropyl), 1.35 (m, 1H, cyclopropyl), 2.82 (t, 4H, 3,5-CH_2 of piperazine), 3.1 (t, 4H, 2,6-CH_2 of piperazine), 3.55 (s, 3H, OCH_3), 3.6 (s, 2H, CH_2 of piperanoyl), 5.86 (s, 2H, –OCH_2O–), 6.42–6.62 (m, 3H, Ar-H), 8.8 (s, 1H, C_2-H), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 5.6, 36.1, 50.3, 52.2, 56.1, 60.4, 108.2, 109.5, 101.3, 113.9, 115.1, 119.4, 122.2, 128.8, 129.9, 130.7, 131.8, 132.5, 147.3, 148.5, 148.1, 166.3, 177.5. Anal. ($C_{26}H_{25}FN_4O_8$) C, H, N. C, H, N. Calculated C, 57.78; H, 4.66; N, 10.37. Found: C, 57.81; H, 4.64; N, 10.37.

3.3.7. 1-Cyclopropyl-1,4-dihydro-6-fluoro-8-methoxy-7-(4-methyl-3-phenylpiperazin-1-yl)-4-oxoquinoline-3-carboxylic acid (12d). Yield: 66%; mp 115–117 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28–0.52 (m, 4H, cyclopropyl), 1.38 (m, 1H, cyclopropyl), 2.2 (s, 3H, CH_3), 2.6 (t, 2H, 5-CH_2 of piperazine), 3.15 (t, 2H, 6-CH_2 of piperazine), 3.4 (d, 2H, 2-CH_2 of piperazine), 3.53 (s, 3H, OCH_3), 4.12 (t, 1H, 3-CH of piperazine), 7.0–7.2 (m, 5H, Ar-H), 7.81 (s, 1H, C_5-H), 8.5 (s, 1H, C_2-H), 14.4 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 5.6, 36.0, 40.5, 52.2, 56.2, 59.0, 63.4, 108.4, 109.4, 119.8, 127.3, 128.5, 131.5, 135.4, 137.4, 145.3, 148.2, 152.7, 166.3, 177.5. Anal. ($C_{25}H_{26}FN_3O_4$) C, H, N. Calculated C, 66.51; H, 5.08; N, 9.31. Found: C, 66.52; H, 5.08; N, 9.31.

3.3.8. 1-Cyclopropyl-1,4-dihydro-6-fluoro-8-methoxy-7-(4-methyl-3-phenylpiperazin-1-yl)-5-nitro-4-oxoquinoline-3-carboxylic acid (13d). Yield: 80%; mp 154–156 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28–0.52 (m, 4H, cyclopropyl), 1.38 (m, 1H, cyclopropyl), 2.2 (s, 3H, CH_3), 2.6 (t, 2H, 5-CH_2 of piperazine), 3.15 (t, 2H, 6-CH_2 of piperazine), 3.4 (d, 2H, 2-CH_2 of piperazine), 3.53 (s, 3H, OCH_3), 4.12 (t, 1H, 3-CH of piperazine), 7.0–7.2 (m, 5H, Ar-H), 8.5 (s, 1H, C_2-H), 14.4 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 5.6, 36.0, 40.5, 52.2, 56.2, 59.0, 63.4, 107.8, 109.4, 127.3, 128.5, 130.8, 131.5, 135.4, 137.4, 148.2, 152.7, 166.3, 177.5. Anal. ($C_{25}H_{25}FN_4O_6$) C, H, N. Calculated C, 60.48; H, 5.08; N, 11.28. Found: C, 60.46; H, 5.05; N, 11.30.

3.3.9. 1-Cyclopropyl-1,4-dihydro-7-(4-(2,3-dihydrobenzo[*b*][1,4]dioxin-2-oyl))(piperazin-1-yl)-6-fluoro-8-methoxy-4-oxoquinoline-3-carboxylic acid (12e). Yield: 64%; mp 171–172 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28–0.48 (m, 4H, cyclopropyl), 1.35 (m, 1H, cyclopropyl), 3.26 (t, 4H, 2,6-CH_2 of piperazine), 3.4 (t, 4H, 3,5-CH_2 of piperazine), 3.51 (s, 3H, OCH_3), 4.6 (d, 2H, 3-CH_2 of dihydrobenzodioxinyl), 5.14 (t, 1H, 2-CH of dihydrobenzodioxinyl), 6.5–6.71 (m, 4H, Ar-H), 7.75 (s, 1H, C_5-H), 8.79 (s, 1H, C_2-H), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 5.6, 36.1, 47.4, 48.5, 66.2, 85.9, 108.9, 109.3, 115.0, 119.2, 121.0, 129.8, 132.4, 145.6, 146.7, 148.1, 166.3, 168.7, 177.5. Anal. ($C_{27}H_{26}FN_3O_7$) C, H, N. Calculated C, 61.95; H, 5.01; N, 8.03. Found: C, 61.95; H, 5.00; N, 8.02.

3.3.10. 1-Cyclopropyl-1,4-dihydro-7-(4-(2,3-dihydrobenzo[*b*][1,4]dioxin-2-oyl))(piperazin-1-yl)-6-fluoro-8-methoxy-5-nitro-4-oxoquinoline-3-carboxylic acid (13e). Yield: 83%; mp 172–174 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28–0.48 (m, 4H, cyclopropyl), 1.35 (m, 1H, cyclopropyl), 3.26 (t, 4H, 2,6-CH_2 of piperazine), 3.4 (t, 4H, 3,5-CH_2 of piperazine), 3.51 (s, 3H, OCH_3), 4.6 (d, 2H, 3-CH_2 of dihydrobenzodioxinyl), 5.14 (t, 1H, 2-CH of dihydrobenzodioxinyl), 6.5–6.71 (m, 4H, Ar-H), 8.79 (s, 1H, C_2-H), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 5.6, 36.1, 47.4, 48.5, 66.2, 85.9, 107.9, 109.3, 115.0, 119.2, 121.0, 129.8, 130.5, 131.7, 132.4, 146.7, 148.1, 152.5, 166.3, 168.7, 177.5. Anal. ($C_{27}H_{25}FN_4O_9$) C, H, N. Calculated C, 57.04; H, 4.43; N, 9.86. Found: C, 57.07; H, 4.46; N, 9.86.

3.3.11. 1-Cyclopropyl-1,4-dihydro-6-fluoro-8-methoxy-7-thiomorpholinoquinoline-4-oxoquinoline-3-carboxylic acid (12f) (CAS No. 114213-99-9). Yield: 70%; mp 168–170 °C.

3.3.12. 1-Cyclopropyl-1,4-dihydro-6-fluoro-8-methoxy-5-nitro-7-thiomorpholinoquinoline-4-oxoquinoline-3-carboxylic acid (13f). Yield: 81%; mp 163–165 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28–0.54 (m, 4H, cyclopropyl), 1.36 (m, 1H, cyclopropyl), 2.64 (t, 4H, 3,5-CH_2 of thiomorpholine), 3.38 (t, 4H, 2,6-CH_2 of thiomorpholine), 3.51 (s, 3H, OCH_3), 8.9 (s, 1H, C_2-H), 14.4 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 5.6, 28.2, 36.3, 50.2, 56.2, 107.8, 109.5, 130.7, 131.7, 133.6, 148.2, 152.7, 166.3, 177.5. Anal. ($C_{18}H_{18}FN_3O_6S$) C, H, N. Calculated C, 51.06; H, 4.28; N, 9.92. Found: C, 51.02; H, 4.25; N, 9.91.

3.3.13. 1-Cyclopropyl-1,4-dihydro-7-(2,6-dimethylmorpholino)-6-fluoro-8-methoxy-4-oxoquinoline-3-carboxylic acid (12g). Yield: 65%; mp 229–231 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28–0.48 (m, 4H, cyclopropyl), 1.35 (m, 1H, cyclopropyl), 1.2 (d, 6H, 2,6-CH_3 of morpholino), 3.0 (d, 4H, 2,6-CH_2 of morpholine), 3.56 (s, 3H, OCH_3), 3.9 (m, 2H, 3,5-CH of morpholine), 7.1 (s, 1H, C_5-H), 7.9 (s, 1H, C_2-H), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 5.6, 36.3, 56.3, 69.5, 65.4, 108.9, 109.3, 119.5, 129.9, 132.6, 145.9, 148.2, 166.3, 177.5. Anal. ($C_{20}H_{23}FN_2O_5$) C, H, N. Calculated C, 61.53; H, 5.94; N, 7.18. Found: C, 61.53; H, 5.96; N, 7.19.

3.3.14. 1-Cyclopropyl-1,4-dihydro-7-(2,6-dimethylmorpholino)-6-fluoro-8-methoxy-5-nitro-4-oxoquinoline-3-carboxylic acid (13g). Yield: 84%; mp 124–126 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28–0.48 (m, 4H, cyclopropyl), 1.35 (m, 1H, cyclopropyl), 1.2 (d, 6H, 2,6-CH_3 of mor-

pholino), 3.0 (d, 4H, 2,6-CH_2 of morpholine), 3.56 (s, 3H, OCH_3), 3.9 (m, 2H, 3,5-CH of morpholine), 7.9 (s, 1H, C_2-H), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 5.6, 36.3, 56.3, 69.5, 65.4, 107.9, 109.3, 119.5, 129.9, 130.9, 131.5, 133.4, 148.2, 152.3, 166.3, 177.5. Anal. ($C_{20}H_{22}FN_3O_7$) C, H, N. Calculated C, 55.17; H, 5.09; N, 9.65. Found: C, 55.17; H, 5.09; N, 9.65.

3.3.15. 1-Cyclopropyl-1,4-dihydro-6-fluoro-8-methoxy-7-(4-(piperidin-1-yl)piperidin-1-yl)-4-oxoquinoline-3-carboxylic acid (12h). Yield: 63%; mp 163–165 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28–0.48 (m, 4H, cyclopropyl), 1.35 (m, 1H, cyclopropyl), 1.5–1.6 (m, 10H, 5-CH_2), 2.2 (t, 4H, 2-CH_2), 2.7 (m, 1H, CH), 2.8 (t, 4H, 2-CH_2), 3.57 (s, 3H, OCH_3), 7.32 (s, 1H, C_5-H), 8.2 (s, 1H, C_2-H), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 5.6, 36.2, 25.7, 26.7, 28.5, 47.1, 52.8, 56.2, 58.5, 109.3, 107.8, 108.9, 129.8, 132.5, 145.6, 146.6, 148.1, 166.3, 177.5. Anal. ($C_{24}H_{30}FN_3O_4$) C, H, N. Calculated C, 64.99; H, 6.82; N, 9.47. Found: C, 65.01; H, 6.80; N, 9.47.

3.3.16. 1-Cyclopropyl-1,4-dihydro-6-fluoro-8-methoxy-5-nitro-7-(4-(piperidin-1-yl)piperidin-1-yl)-4-oxoquinoline-3-carboxylic acid (13h). Yield: 69%; mp 167–169 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28–0.48 (m, 4H, cyclopropyl), 1.35 (m, 1H, cyclopropyl), 1.5–1.6 (m, 10H, 5-CH_2), 2.2 (t, 4H, 2-CH_2), 2.7 (m, 1H, CH), 2.8 (t, 4H, 2-CH_2), 3.57 (s, 3H, OCH_3), 8.2 (s, 1H, C_2-H), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 5.6, 36.2, 25.7, 26.7, 28.5, 47.1, 52.8, 56.2, 58.5, 109.3, 107.8, 130.7, 131.8, 133.5, 148.1, 152.7, 166.3, 177.5. Anal. ($C_{24}H_{29}FN_4O_6$) C, H, N. Calculated C, 59.01; H, 5.98; N, 11.47. Found: C, 59.01; H, 5.97; N, 11.49.

3.3.17. 7-(4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl)-1-cyclopropyl-1,4-dihydro-6-fluoro-8-methoxy-4-oxoquinoline-3-carboxylic acid (12i). Yield: 68%; mp 112–114 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28–0.46 (m, 4H, cyclopropyl), 1.33 (m, 1H, cyclopropyl), 2.0 (t, 4H, 3,5-CH_2 of piperidine), 2.7 (t, 4H, 2,6-CH_2 of piperidine), 3.51 (s, 3H, OCH_3), 7.1–7.18 (m, 4H, Ar-H), 7.82 (s, 1H, C_5-H), 8.88 (s, 1H, C_2-H), 10.0 (br s, 1H, OH), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 5.6, 36.0, 38.2, 42.6, 56.1, 74.5, 108.9, 109.3, 118.8, 129.6, 131.5, 138.1, 145.7, 148.2, 166.3, 177.5. Anal. ($C_{25}H_{24}ClFN_2O_5$) C, H, N. Calculated C, 61.67; H, 4.97; N, 5.75. Found: C, 61.69; H, 4.99; N, 5.74.

3.3.18. 7-(4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl)-1-cyclopropyl-1,4-dihydro-6-fluoro-8-methoxy-5-nitro-4-oxoquinoline-3-carboxylic acid (13i). Yield: 76%; mp 136–138 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28–0.46 (m, 4H, cyclopropyl), 1.33 (m, 1H, cyclopropyl), 2.0 (t, 4H, 3,5-CH_2 of piperidine), 2.7 (t, 4H, 2,6-CH_2 of piperidine), 3.51 (s, 3H, OCH_3), 7.1–7.18 (m, 4H, Ar-H), 8.88 (s, 1H, C_2-H), 10.0 (br s, 1H, OH), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 5.6, 36.0, 38.2, 42.6, 56.1, 74.5, 107.9, 109.3, 118.8, 129.6, 130.6, 131.5, 138.1, 148.2, 152.4, 166.3, 177.5. Anal. ($C_{25}H_{23}ClFN_3O_7$) C, H, N. Calculated C, 56.45; H, 4.36; N, 7.90. Found: C, 56.47; H, 4.33; N, 7.91.

3.3.19. 7-(4-(6-Chloro-1,2-dihydro-2-oxobenzo[*d*]imidazol-3-yl)piperidin-1-yl)-1-cyclopropyl-1,4-dihydro-6-fluoro-8-methoxy-4-oxoquinoline-3-carboxylic acid (12j). Yield: 70%; mp 259–261 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28–0.48 (m, 4H, cyclopropyl), 1.35 (m, 1H, cyclopropyl), 1.6–2.4 (m, 8H, 4-CH_2 of piperidine), 3.52 (s, 3H, OCH_3), 4.1 (br m, 1H, CH of piperidine), 6.8–7.7 (m, 3H, Ar-H), 7.88 (s, 1H, C_5-H), 8.3 (s, 1H, C_2-H), 10.8 (s, 1H, NH), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 5.7, 36.2, 26.9, 46.3, 50.3, 108.8, 109.3, 122.2, 123.4, 124.7, 129.8, 130.1, 131.3, 132.3, 145.6, 148.1, 151.8, 166.3, 177.5. Anal. ($C_{26}H_{24}ClFN_4O_5$) C, H, N. Calculated C, 59.26; H, 4.59; N, 10.63. Found: C, 59.26; H, 4.61; N, 10.61.

3.3.20. 7-(4-(6-Chloro-1,2-dihydro-2-oxobenzo[*d*]imidazol-3-yl)piperidin-1-yl)-1-cyclopropyl-1,4-dihydro-6-fluoro-8-methoxy-5-nitro-4-oxoquinoline-3-carboxylic acid (13j). Yield: 78%; mp 178–180 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28–0.48 (m, 4H, cyclopropyl), 1.35 (m, 1H, cyclopropyl), 1.6–2.4 (m, 8H, 4-CH_2 of piperidine), 3.52 (s, 3H, OCH_3), 4.1 (br m, 1H, CH of piperidine), 6.8–7.7 (m, 3H, Ar-H), 8.3 (s, 1H, C_2-H), 10.8 (s, 1H, NH), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 5.7, 36.2, 26.9, 46.3, 50.3, 107.8, 109.3, 122.2, 123.4, 124.7, 130.1, 131.3, 133.3, 148.1, 151.8, 166.3, 177.5. Anal. ($C_{26}H_{23}ClFN_5O_7$) C, H, N. Calculated C, 54.60; H, 4.05; N, 12.24. Found: C, 54.61; H, 4.06; N, 12.23.

3.3.21. 1-Cyclopropyl-7-(3-(diethylcarbamoyl)piperidin-1-yl)-1,4-dihydro-6-fluoro-8-methoxy-4-oxoquinoline-3-carboxylic acid (12k). Yield: 70%; mp 85–87 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28–0.48 (m, 4H, cyclopropyl), 1.35 (m, 1H, cyclopropyl), 1.2 (t, 6H, 2-CH_3 of ethyl), 1.78–2.7 (m, 9H, H of piperidine), 3.24 (q, 4H, 2-CH_2 of ethyl), 3.53 (s, 3H, OCH_3), 7.8 (s, 1H, C_5-H), 8.94 (s, 1H, C_2-H), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 5.6, 36.3, 12.9, 22.3, 28.2, 41.3, 42.6, 51.0, 54.4, 108.9, 109.3, 118.8, 129.8, 132.6, 145.4, 146.2, 148.1, 166.3, 177.5. Anal. ($C_{24}H_{30}FN_3O_5$) C, H, N. Calculated C, 62.73; H, 6.58; N, 9.14. Found: C, 62.72; H, 6.60; N, 9.14.

3.3.22. 1-Cyclopropyl-7-(3-(diethylcarbamoyl)piperidin-1-yl)-1,4-dihydro-6-fluoro-8-methoxy-5-nitro-4-oxoquinoline-3-carboxylic acid (13k). Yield: 75%; mp 128–130 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28–0.48 (m, 4H, cyclopropyl), 1.35 (m, 1H, cyclopropyl), 1.2 (t, 6H, 2-CH_3 of ethyl), 1.78–2.7 (m, 9H, H of piperidine), 3.24 (q, 4H, 2-CH_2 of ethyl), 3.53 (s, 3H, OCH_3), 8.94 (s, 1H, C_2-H), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 5.6, 36.3, 12.9, 22.3, 28.2, 41.3, 42.6, 51.0, 54.4, 107.9, 109.3, 118.8, 129.8, 130.6, 131.7, 133.6, 148.1, 152.5, 166.3, 177.5. Anal. ($C_{24}H_{29}FN_4O_7$) C, H, N. Calculated C, 57.14; H, 5.79; N, 11.11. Found: C, 57.15; H, 5.81; N, 11.11.

3.3.23. 1-Cyclopropyl-1,4-dihydro-7-(1,4-dioxa-8-azaspiro[4.5]dec-8-yl)-6-fluoro-8-methoxy-4-oxoquinoline-3-carboxylic acid (12l) (CAS No 848070-83-7). Yield: 73%; mp 254–256 °C.

3.3.24. 1-Cyclopropyl-1,4-dihydro-7-(1,4-dioxa-8-azaspiro[4.5]dec-8-yl)-6-fluoro-8-methoxy-5-nitro-4-oxoquinoline-3-carboxylic acid (13l). Yield: 69%; mp 152–154 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28–0.52 (m, 4H, cyclopropyl), 1.38 (m, 1H, cyclopropyl), 1.78–2.4 (m, 8H, 4-CH_2 of azaspirodecane), 3.51 (s, 3H, OCH_3), 3.96 (m, 4H, 2-CH_2 of azaspirodecane), 8.9 (s, 1H, C_2-H), 14.4 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 5.6, 34.0, 36.0, 39.8, 56.2, 64.3, 107.8, 109.6, 130.6, 131.7, 133.5, 148.1, 152.7, 166.3, 177.5. Anal. ($C_{21}H_{22}FN_3O_8$) C, H, N. Calculated C, 54.43; H, 4.79; N, 9.07. Found: C, 54.43; H, 4.78; N, 9.05.

3.3.25. 7-(1-(*tert*-Butylcarbamoyl)-3,4-dihydroisoquinolin-2(1*H*)-yl)-1-cyclopropyl-1,4-dihydro-6-fluoro-8-methoxy-4-oxoquinoline-3-carboxylic acid (12m). Yield: 63%; mp 70–72 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28–0.48 (m, 4H, cyclopropyl), 1.35 (m, 1H, cyclopropyl), 1.3 (s, 9H, 3-CH_3), 2.66–2.9 (m, 4H, 2-CH_2 of isoquinoline), 3.53 (s, 3H, OCH_3), 4.85 (s, 1H, CH of isoquinoline), 6.7–7.1 (m, 4H, Ar-H), 7.8 (s, 1H, C_5-H), 8.8 (s, 1H, C_2-H), 10.2 (s, 1H, NH), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 5.6, 36.3, 25.9, 47.4, 48.3, 56.1, 65.5, 108.8, 109.3, 118.8, 127.0, 127.9, 128.6, 129.5, 132.1, 135.4, 138.1, 145.7, 146.2, 148.1, 166.3, 168.7, 177.5. Anal. ($C_{28}H_{30}FN_3O_5$) C, H, N. Calculated C, 66.26; H, 5.96; N, 8.28. Found: C, 66.26; H, 5.95; N, 8.28.

3.3.26. 7-(1-(*tert*-Butylcarbamoyl)-3,4-dihydroisoquinolin-2(1*H*)-yl)-1-cyclopropyl-1,4-dihydro-6-fluoro-8-methoxy-5-nitro-4-oxoquinoline-3-carboxylic acid (13m). Yield: 82%; mp 172–174 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28–0.48 (m, 4H, cyclopropyl), 1.35 (m, 1H, cyclopropyl), 1.3 (s, 9H, 3-CH_3), 2.66–2.9 (m, 4H, 2-CH_2 of isoquinoline), 3.53 (s, 3H, OCH_3), 4.85 (s, 1H, CH of isoquinoline), 6.7–7.1 (m, 4H, Ar-H), 8.8 (s, 1H, C_2-H), 10.2 (s, 1H, NH), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 5.6, 36.3, 25.9, 47.4, 48.3, 56.1, 65.5, 108.8, 109.3, 118.8, 127.0, 127.9, 128.6, 130.6, 131.7, 133.5, 135.4, 138.1, 148.1, 152.3, 166.3, 168.7, 177.5. Anal. ($C_{28}H_{29}FN_4O_7$) C, H, N. Calculated C, 60.86; H, 5.29; N, 10.14. Found C, 60.86; H, 5.30; N, 10.17.

3.3.27. 1-Cyclopropyl-7-(8-(4-methoxybenzyl)-3,4,5,6,7,8-hexahydroisoquinolin-2(1*H*)-yl)-1,4-dihydro-6-fluoro-8-methoxy-4-oxoquinoline-3-carboxylic acid (12n). Yield: 62%; mp 176–178 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28–0.54 (m, 4H, cyclopropyl), 1.36 (m, 1H, cyclopropyl), 1.6–1.95 (m, 8H, 4-CH_2 of isoquinolinyl), 2.2–3.4 (m, 7H, 2-CH_2 and 1-CH of isoquinolinyl, and CH_2), 3.52 (s, 3H, OCH_3), 3.73 (s, 3H, –OCH_3), 6.8–7.1 (m, 4H, Ar-H), 7.88 (s, 1H, C_5-H), 8.56 (s, 1H, C_2-H), 14.4 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 5.6, 24.3, 25.5, 28.6, 30.4, 36.0, 37.9, 42.9, 56.4, 108.7, 109.3, 114.1, 119.2, 123.8, 129.1, 135.4, 145.6, 146.5, 148.2, 158.4, 166.3, 177.5. Anal. ($C_{31}H_{33}FN_2O_5$) C, H, N. Calculated C, 69.91; H, 6.25; N, 5.26. Found C, 69.90; H, 6.26; N, 5.25.

3.3.28. 1-Cyclopropyl-7-(8-(4-methoxybenzyl)-3,4,5,6,7,8-hexahydroisoquinolin-2(1*H*)-yl)-1,4-dihydro-6-fluoro-8-methoxy-5-nitro-4-oxoquinoline-3-carboxylic acid (13n). Yield: 60%; mp 108–110 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28–0.54 (m, 4H, cyclopropyl), 1.36 (m, 1H, cyclopropyl), 1.6–1.95 (m, 8H, 4-CH_2 of isoquinolinyl), 2.2-3.4 (m, 7H, 2-CH_2 and 1-CH of isoquinolinyl, and CH_2), 3.52 (s, 3H, OCH_3), 3.73 (s, 3H, –OCH_3), 6.8–7.1 (m, 4H, Ar-H), 8.56 (s, 1H, C_2-H), 14.4 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 5.6, 24.3, 25.5, 28.6, 30.4, 36.0, 37.9, 42.9, 56.4, 107.7, 109.3, 114.1, 123.8, 129.1, 130.5, 131.9, 135.4, 148.2, 152.7, 158.4, 166.3, 177.5. Anal. ($C_{31}H_{32}FN_3O_7$) C, H, N. Calculated C, 64.46; H, 5.58; N, 7.27. Found: C, 64.44; H, 5.55; N, 7.27.

3.3.29. 7-(2-Carboxy-5,6-dihydroimidazo[1,2-a]pyrazin-7(8H)-yl)-1-cyclopropyl-1,4-dihydro-6-fluoro-8-methoxy-4-oxoquinoline-3-carboxylic acid (12o). Yield: 79%; mp 169–171 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28–0.48 (m, 4H, cyclopropyl), 1.35 (m, 1H, cyclopropyl), 3.1–3.8 (m, 6H, 3-CH_2), 3.55 (s, 3H, OCH_3), 7.6 (s, 1H, CH), 7.63 (s, 1H, C_5-H), 8.78 (s, 1H, C_2-H), 12.12 (s, 1H, 2-COOH), 14.6 (s, 1H, 3-COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 5.7, 36.3, 37.4, 50.5, 55.9, 57.2, 109.3, 118.8, 130.6, 132.2, 139.4, 145.6, 148.2, 158.4, 166.3, 167.9, 177.5. Anal. ($C_{21}H_{19}FN_4O_6$) C, H, N. Calculated C, 57.01; H, 4.33; N, 12.66. Found: C, 57.01; H, 4.33; N, 12.66.

3.3.30. 7-(2-Carboxy-5,6-dihydroimidazo[1,2-*a*]pyrazin-7(8*H*)-yl)-1-cyclopropyl-1,4-dihydro-6-fluoro-8-methoxy-5-nitro-4-oxoquinoline-3-carboxylic acid (13o). Yield: 84%; mp 208–210 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28–0.48 (m, 4H, cyclopropyl), 1.35 (m, 1H, cyclopropyl), 3.1–3.8 (m, 6H, 3-CH_2), 3.55 (s, 3H, OCH_3), 7.6 (s, 1H, CH), 8.78 (s, 1H, C_2-H), 12.12 (s, 1H, 2-COOH), 14.6 (s, 1H, 3-COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 5.7, 36.3, 37.4, 50.5, 55.9, 57.2, 107.7, 109.3, 130.6, 131.7, 132.2, 133.6, 139.4, 148.2, 152.5, 158.4, 166.3, 167.9, 177.5. Anal. ($C_{21}H_{18}FN_5O_8$) C, H, N. Calculated C, 51.75; H, 3.72; N, 14.37. Found: C, 51.75; H, 3.73; N, 14.35.

3.3.31. 1-Cyclopropyl-1,4-dihydro-7-(4,4-dimethyloxazolidin-3-yl)-6-fluoro-8-methoxy-4-oxoquinoline-3-carboxylic acid (12p). Yield: 62%; mp 180–182 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28–0.48 (m, 4H, cyclopropyl), 1.35 (m, 1H, cyclopropyl), 1.16 (s, 6H, 2-CH_3), 3.41 (s, 2H, 5-CH_2 of oxazolidinyl), 3.57 (s, 3H, OCH_3), 4.6 (s, 2H, 2CH of oxazolidinyl), 7.69 (s, 1H, C_5-H), 8.85 (s, 1H, C_2-H), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 5.6, 36.5, 55.9, 65.5, 83.6, 84.4, 108.8, 109.1, 119.3, 129.7, 131.4, 132.4, 135.4, 145.6, 146.5, 149.4, 166.3, 177.5. Anal. ($C_{19}H_{21}FN_2O_5$) C, H, N. Calculated C, 60.63; H, 5.62; N, 7.44. Found: C, 60.60; H, 5.65; N, 7.47.

3.3.32. 1-Cyclopropyl-1,4-dihydro-7-(4,4-dimethyloxazolidin-3-yl)-6-fluoro-8-methoxy-5-nitro-4-oxoquinoline-3-carboxylic acid (13p). Yield: 77%; mp 160–162 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28–0.48 (m, 4H, cyclopropyl), 1.35 (m, 1H, cyclopropyl), 1.16 (s, 6H, 2-CH_3), 3.41 (s, 2H, 5-CH_2 of oxazolidinyl), 3.57 (s, 3H, OCH_3), 4.6 (s, 2H, 2-CH of oxazolidinyl), 8.85 (s, 1H, C_2-H), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 5.6, 36.5, 55.9, 65.5, 83.6, 84.4, 107.8, 109.1, 131.4, 135.4, 149.4, 152.9, 166.3, 177.5. Anal.

($C_{19}H_{20}FN_3O_7$) C, H, N. Calculated C, 54.16; H, 4.78; N, 9.97. Found: C, 54.14; H, 4.77; N, 9.99.

3.4. Synthesis of 5-amino-1-cyclopropyl-1,4-dihydro-6-fluoro-7-(substituted secondary amines)-8-methoxy-4-oxoquinoline-3-carboxylic acid (14c,n)

Compounds **13c** and **13n** were further reduced by catalytic reduction of the nitro group to yield **14c** and **14n**.

3.4.1. 5-Amino-1-cyclopropyl-1,4-dihydro-6-fluoro-7-(4-((benzo[*d*][1,3]dioxol-5-yl)methyl)piperazin-1-yl)-8-methoxy-4-oxoquinoline-3-carboxylic acid (14c). Yield: 76%; mp 125–127 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28–0.48 (m, 4H, cyclopropyl), 1.35 (m, 1H, cyclopropyl), 2.82 (t, 4H, 3,5-CH_2 of piperazine), 3.1 (t, 4H, 2,6-CH_2 of piperazine), 3.55 (s, 3H, OCH_3), 3.6 (s, 2H, CH_2 of piperanoyl), 5.86 (s, 2H, –OCH_2O–), 6.42–6.62 (m, 3H, Ar-H), 11.8 (br s, 2H, NH_2), 8.8 (s, 1H, C_2-H), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 177.5, 166.3, 148.1, 148.5, 147.3, 145.8, 132.5, 129.9, 128.8, 122.2, 119.4, 115.1, 113.9, 101.3, 109.5, 108.2, 60.4, 56.1, 52.2, 50.3, 36.1, 5.6. Anal. ($C_{26}H_{27}FN_4O_6$) C, H, N. Calculated C, 61.17; H, 5.33; N, 10.97. Found: C, 61.16; H, 5.35; N, 10.95.

3.4.2. 5-Amino-1-cyclopropyl-7-(8-(4-methoxybenzyl)-3,4,5,6,7,8-hexahydroisoquinolin-2(1*H*)-yl)-1,4-dihydro-6-fluoro-8-methoxy-4-oxoquinoline-3-carboxylic acid (14n). Yield: 67%; mp 152–153 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28–0.54 (m, 4H, cyclopropyl), 1.36 (m, 1H, cyclopropyl), 1.6–1.95 (m, 8H, 4-CH_2 of isoquinolinyl), 2.2–3.4 (m, 7H, 2-CH_2 and 1-CH of isoquinolinyl, and CH_2), 3.52 (s, 3H, OCH_3), 3.73 (s, 3H, –OCH_3), 6.8–7.1 (m, 4H, Ar-H), 8.56 (s, 1H, C_2-H), 12.1 (br s, 2H, NH_2), 14.4 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 5.6, 24.3, 25.5, 28.6, 30.4, 36.0, 37.9, 42.9, 56.4, 99.3, 109.3, 114.1, 123.8, 128.9, 130.5, 131.9, 135.4, 136.8, 148.2, 158.4, 166.3, 177.5. Anal. ($C_{31}H_{34}FN_3O_5$) C, H, N. Calculated C, 67.99; H, 6.26; N, 7.67. Found: C, 68.00; H, 6.26; N, 7.67.

3.5. In vitro antimycobacterial activity

All compounds were screened for their in vitro antimycobacterial activity against MTB, MDR-TB and MC^2 in Middlebrook 7H11agar medium supplemented with OADC by agar dilution method similar to that recommended by the National Committee for Clinical Laboratory Standards for the determination of MIC in duplicate. The MDR-TB clinical isolate was obtained from Tuberculosis Research Center, Chennai, India, and was resistant to isoniazid, rifampicin, ethambutol and ofloxacin. The minimum inhibitory concentration (MIC) is defined as the minimum concentration of compound required to give complete inhibition of bacterial growth.

3.6. Cytotoxicity

Some compounds were further examined for toxicity (IC_{50}) in a mammalian Vero cell line at concentrations of 62.5 μg/ml. After 72 h of 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.

3.7. In vivo antimycobacterial activity

One compound was tested for efficacy against MTB at a dose of 25 mg/kg in 6-week-old female CD-1 mice six per group. In this model, the mice were infected intravenously through caudal vein with approximately 10^7 viable *M. tuberculosis* ATCC 35801. Drug treatment by intraperitoneal route began after 10 days of inoculation of the animal with microorganism and continued for 10 days. After 35 days post-infection the spleens and right lungs were aseptically removed and ground in a tissue homogenizer, the number of viable organisms was determined by serial 10-fold dilutions and subsequent inoculation onto 7H10 agar plates. Cultures were incubated at 37 °C in ambient air for 4 weeks prior to counting. Bacterial counts were measured and compared with the counts from negative controls (vehicle treated) in lung and in spleen.

3.8. DNA gyrase supercoiling assay

The enzyme was isolated from MC^2 cells and purified as described.[10] The compounds tested were dissolved in DMSO and pre-incubated with the enzyme. The supercoiling assays were carried out as described previously.[10] Briefly, supercoiling assays were carried out by incubating 400 ng of relaxed circular pUC18 in supercoiling buffer (35 mM Tris–HCl, pH 7.5, 5 mM $MgCl_2$, 25 mM potassium glutamate, 2 mM spermidine, 2 mM ATP, 50 μg/ml bovine serum albumin and 90 μg/ml yeast t-RNA in 5% (v/v) glycerol) for 30 min. Ciprofloxacin at final concentration of 10 μg/ml was used as a positive control and another control reaction having 5% DMSO in absence of the compounds was also performed. The reaction samples were heat-inactivated at 65 °C for 15 min and applied onto 1% agarose gel for electrophoresis in Tris–acetate–EDTA buffer for 12 h. The gels were stained with ethidium bromide to visualize the DNA topoisomers.

3.9. Phototoxicity evaluation

Female swiss albino mice, approximately 2 months old and weighing 20–25 g, were used in this study. Before oral dosing, they were fasted overnight for at least 18 h. Food was returned at the end of the 4 h photo-irradiation period. Eighteen mice were randomly distributed into three dosing groups. First group received a single dose of screened compound at 140 mg/kg by oral gavage. A second group received a single dose of 140 mg of lomefloxacin HCl/kg. This lomefloxacin dose is one that, in preliminary experiments in this test system, produced a consistent erythema and ear thickening response. The final group served as a vehicle control and received 10 ml/kg of the methylcellulose vehicle only. Test animals were exposed to UV light in a manner adapted from that described previously. Animals were irradiated for 4 h, equal to a total UV light irradiation of approximately 18 J/cm^2. Before dosing, at the end

of the irradiation period and at approximately 24, 48, 72 and 96 h after dosing, both ears of each mouse were evaluated for changes indicative of a positive response: erythema, oedema or a measurable increase in ear thickness.

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