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Novel ofloxacin derivatives: Synthesis, antimycobacterial and toxicological evaluation

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Abstract—Thirty novel 9-fluoro-2,3-dihydro-8,10-(mono/di-sub)-3-methyl-8-nitro-7-oxo-7*H*-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxylic acids were synthesized from 2,3,4,5-tetrafluoro benzoic acid and evaluated for in vitro and in vivo antimycobacterial activities against *Mycobacterium tuberculosis* H37Rv (MTB), multi-drug resistant *Mycobacterium tuberculosis* (MDR-TB), and *Mycobacterium smegmatis* (MC^2) and also tested for the ability to inhibit the supercoiling activity of DNA gyrase from mycobacteria. Among the synthesized compounds, 10-[2-carboxy-5,6-dihydroimidazo[1,2-*a*]pyrazin-7(8H)-yl]-9-fluoro-2,3-dihydro-3-methyl-8-nitro-7-oxo-7*H*-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxylic acid was found to be the most active compound in vitro with MIC99 of 0.19 μM and 0.09 μM against MTB and MTR-TB, respectively. In the in vivo animal model also the same compound decreased the bacterial load in lung and spleen tissues with 1.91 and 2.91 − log 10 protections, respectively, at the dose of 50 mg/kg body weight. Compound 10-[(4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)]-9-fluoro-2,3-dihydro-3-methyl-8-nitro-7-oxo-7*H*-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxylic acid was found to be the most active in the inhibition of the supercoiling activity of DNA gyrase with an IC_{50} of 10.0 μg/mL. The results demonstrate the potential and importance of developing new oxazino quinolone derivatives against mycobacterial infections.

Fluoroquinolones exhibit potent in vitro and in vivo antimycobacterial activity.[1] There is a significant effort to include fluoroquinolones as new front-line agents (ofloxacin and moxifloxacin) and second-line agents (ciprofloxacin) to combat tuberculosis (TB).[2] Experts from the World Health Organization favor a third-line regimen containing four drugs (an aminoglycoside, ethionamide, pyrazinamide, and ofloxacin) during the initial phase and two drugs (ethionamide and ofloxacin) during the continuation phase.[3] Quinolones inhibit bacterial type II topoisomerase, DNA gyrase, and topoisomerase IV,[4] which are essential enzymes that maintain the supercoiling of the DNA by carrying out two opposing reactions. The incidence of mycobacterial resistance to fluoroquinolones is relatively low at the present time, and there are no reports of cross-resistance or antagonism with other classes of antimycobacterial agents.[5] There is also a considerable effort to discover and develop newer fluoroquinolones, and some of them might have value in the treatment of TB.[6,7] As a part of the study attempting to further optimize the quinolones against *Mycobacterium tuberculosis* (MTB)[8,9] and multi-drug resistant *M. tuberculosis* (MDR-TB) as MTB is getting resistance to many quinolones,[10–12] herewith we report the synthesis, antimycobacterial and toxicological evaluation of novel 9-fluoro-2,3-dihydro-8,10-(mono/di-sub)-3-methyl-8-nitro-7-oxo-7*H*-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxylic acids.

Thirty new oxazinoquinolone carboxylic acids with variation at C_9 and C_{10} positions were synthesized from 2,3,4,5-tetrafluoro benzoic acid (**1**) according to the literature method[13] with modification in some steps (Scheme 1). Briefly, compound **1** 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 82% of ethyl 3-(2,3,4,5-tetrafluorophenyl)-3-oxopropanoate (**2**). Ethyl 2,3,4,5-tetrafluoro-α-[[(2-hydroxy-1-methyl-

Keywords: Oxazino[2,3,4-*ij*]quinoline-6-carboxylic acids; Antimycobacterial activity; Tuberculosis.
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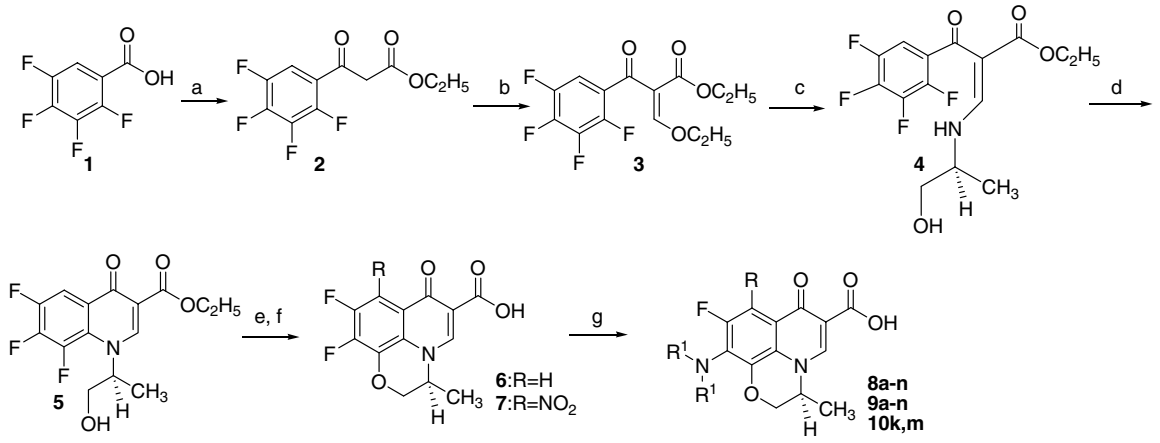


Scheme 1. Synthetic protocol of the compounds. Reagents and conditions: (a) CDI, $EtOCOCH_2CO_2K$, $MgCl_2$, Et_3N; (b) $(EtO)_3CH$, AC_2O; (c) (+)-2-amino-1-propanol, EtOH/Et_2O (50:50); (d) DMSO, K_2CO_3; (e) THF, 10% KOH; (f) H_2SO_4, KNO_3; (g) various secondary amines, DMSO, MWI; (h) H_2, Ran.Ni, DMF.

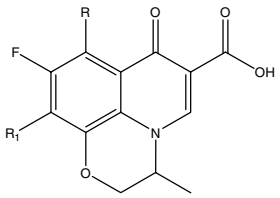
ethyl)amino]methylene]-β-oxo-benzenepropanoic acid (**4**) was prepared by a two-step one-pot reaction. First treatment of the keto ester **2** with tri-ethyl orthoformate in acetic anhydride gave the one-carbon homologue enol ether intermediate ethyl α-(ethoxymethylene)-2,3,4,5-tetrafluoro-β-oxo-benzenepropanoic acid (**3**) as an oil, which on reaction with (*S*)-(+)-2-amino-1-propanol at 0°C affords 67% of **4** as an oily residue. Compound **4** on cyclization with the base potassium carbonate in dimethylsulfoxide yielded 62% of ethyl 6,7,8-trifluoro-1,4-dihydro-1-(2-hydroxy-1-methylethyl)-4-oxo-3-quinolinecarboxylic acid (**5**), when compared to earlier report,[9] this step proceeds smoothly without using violently reactive sodium hydride. Further cyclization and hydrolysis of compound **5** was done by heating with 10% aqueous potassium hydroxide in tetrahydrofuran, affording 9,10-difluoro-2,3-dihydro-3-methyl-7-oxo-7*H*-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxylic acid (**6**) with 70% yield, which on nitration at C_8 position with sulfuric acid and potassium nitrate yielded 88% of 9,10-difluoro-2,3-dihydro-3-methyl-8-nitro-7-oxo-7*H*-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxylic acid (**7**). Compounds **6** and **7** on further reaction with various secondary amines under microwave irradiation in dimethylsulfoxide afforded titled compounds **8, 9a**–**n**. When compared to conventional method[13] of 12 h process, microwave assisted synthesis was performed with short reaction times (3–6 min), with ease, and was environment friendly. Compounds **9k** and **9m** were further reduced by catalytic reduction of the nitro group to yield **10k** and **10m**. The purity of the synthesized compounds was monitored by thin layer chromatography (TLC) and elemental analyses, and the structures were identified by spectral data.[14]

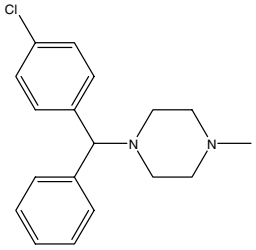
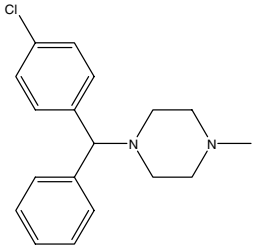
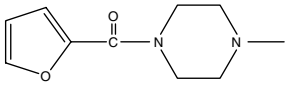
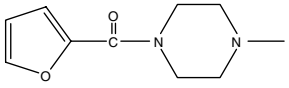
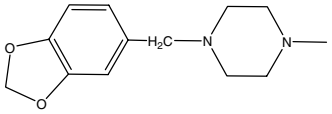
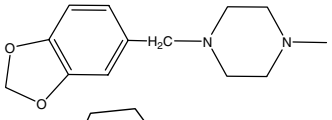
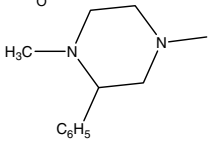
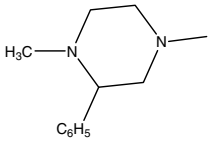
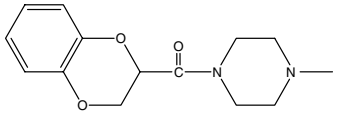
The compounds were screened for their in vitro antimycobacterial activity against MTB, MDR-TB, and *Mycobacterium smegmatis* ATCC 14468 (MC^2) by agar dilution method for the determination of MIC99 in duplicate.[15] The MDR-TB clinical isolate was resistant to isoniazid, rifampicin, ethambutol, and ofloxacin. The minimum inhibitory concentration (MIC99) is defined as the minimum concentration of compound required to give complete inhibition of bacterial growth and MICs of the synthesized compounds along with the standard drugs for comparison are reported in Table 1.

In the first phase of screening against MTB, all the compounds showed excellent in vitro activity against MTB with MIC99 less than 15 μM. Thirteen compounds (**9b**, **9g**, **9h**, **9i**, **8j**, **9j**, **8k**, **9k**, **9l**, **8m**, **9m**, **8n**, and **9n**) inhibited MTB with MIC99 of less than 2 μM and were more potent than standard ofloxacin (MIC99: 2.16 μM). When compared to isoniazid (MIC99: 0.36 μM), three compounds (**9j**, **9k**, and **9m**) were found to be more active against MTB. Compound 10-[2-carboxy-5,6-dihydroimidazo[1,2-*a*]pyrazin-7(8*H*)-yl]-9-fluoro-2,3-dihydro-3-methyl-8-nitro-7-oxo-7*H*-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxylic acid (**9m**) was found to be the most active compound in vitro with MIC99 of 0.19 μM against MTB, and was 2 and 11 times more potent than isoniazid and ofloxacin, respectively. Subsequently some of the compounds were evaluated against MDR-TB, and among the twenty compounds screened, all the compounds inhibited MDR-TB with MIC99 ranging from 0.09 to 8.30 μM and were found to be more active than isoniazid (MIC99: 45.57 μM) and ofloxacin (MIC99: 34.59 μM). Six compounds (**8j**, **9j**, **8k**, **9k**, **8m** and **9m**) inhibited MDR-TB with MIC99 of less than 1 μM. Compounds **8k**, **9k** and **9m** were found to be the most active compound in vitro with MIC99 of 0.09 μM against MDR-TB and were 384 and 506 times more potent than ofloxacin and isoniazid, respectively. The compounds were also evaluated against MC^2 in which all the compounds inhibited MC^2 with MIC99 ranging from 0.66 to 114.29 μM; twenty-three compounds were found to be more active than isoniazid (MIC99: 45.57 μM) and six compounds were more active than ofloxacin (MIC99: 2.16 μM).

With respect to structure–MTB activity relationship, the results demonstrated that the antimycobacterial activity was enhanced 1.1- to 8.8-fold by the introduction of nitro group at C_8 position. Reduction of nitro group at C_8 position to amino group (**10k** and **10m**) reduces the

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



Compound	R	R_1	Yield (%)	mp (°C)	IC_{50} (μM)	MIC99 (μM)		
						MTB	MDR-TB	MC^2
8a	H		68	168	NT	5.71	NT	45.62
9a	NO_2		62	>250	105.39	2.63	2.63	0.66
8b	H		73	118	NT	7.09	NT	3.53
9b	NO_2		66	216	64.25	0.80	1.60	3.21
8c	H		73	188	NT	6.50	NT	25.96
9c	NO_2		80	215	118.72	2.96	5.95	1.48
8d	H		69	154	NT	14.29	NT	114.29
9d	NO_2		80	211	129.54	3.23	3.23	6.49
8e	H		70	172	NT	12.27	NT	49.07

(*continued on next page*)

Table 1 (*continued*)

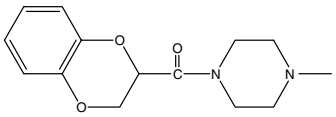
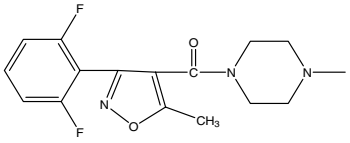
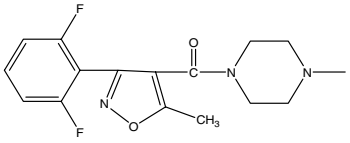
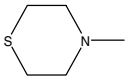
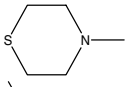
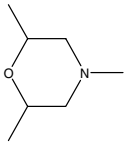
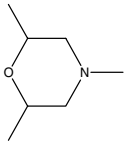
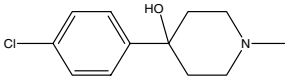
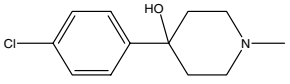
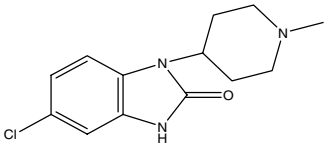
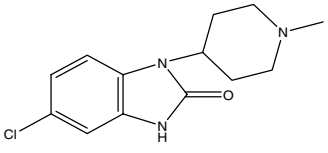
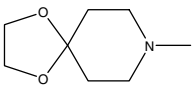
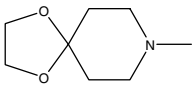
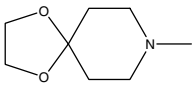
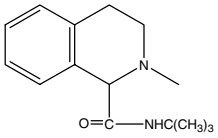
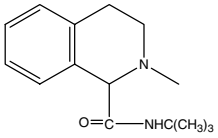
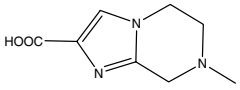
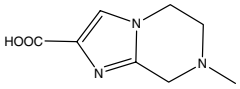
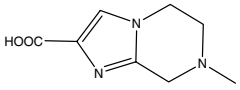
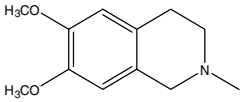
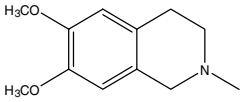
Compound	R	R_1	Yield (%)	mp (°C)	IC_{50} (μM)	MIC99 (μM)		
						MTB	MDR-TB	MC^2
9e	NO_2		83	224	112.72	2.81	1.41	11.27
8f	H		70	193	NT	10.99	NT	43.98
9f	NO_2		84	208	101.87	5.10	NT	1.27
8g	H		77	163	>171.52	2.14	8.59	4.28
9g	NO_2		81	>250	152.67	1.91	1.91	1.91
8h	H		73	124	>166.06	4.14	8.3	4.14
9h	NO_2		84	>250	148.32	1.85	3.70	1.85
8i	H		70	136	NT	6.62	NT	26.43
9i	NO_2		76	220	60.35	1.51	3.01	6.04
8j	H		67	178	>121.85	1.52	0.76	48.74
9j	NO_2		78	205	112.03	0.34	0.34	1.39
8k	H		64	152	154.55	0.47	0.09	3.86

Table 1 (*continued*)

Compound	R	R_1	Yield (%)	mp (°C)	IC_{50} (μM)	MIC99 (μM)		
						MTB	MDR-TB	MC^2
9k	NO_2		69	225	139.08	0.21	0.09	3.47
10k	NH_2		61	189	>149.02	3.72	3.72	11.16
8l	H		75	172	126.64	3.16	3.16	50.66
9l	NO_2		82	>250	116.06	1.45	1.45	2.89
8m	H		78	208	>145.90	0.91	0.44	29.18
9m	NO_2		84	>250	132.03	0.19	0.09	6.61
10m	NH_2		59	219	>140.96	0.88	1.76	5.28
8n	H		72	168	>137.53	1.72	3.43	13.75
9n	NO_2		75	191	62.5	1.56	3.12	12.51
Oflox	—	—	—	—	>155.3	2.16	34.59	2.16
INH	—	—	—	—	>455.8	0.36	45.57	45.57

NT indicates not tested.

activity indicating that favorable substitution at C_8 was $NO_2 > H > NH_2$. At C_9 position we have studied with various substituted piperazines (**8** and **9a–f**) (thio) morpholines (**8** and **9g–h**), substituted piperidines (**8** and **9i–j**), fused piperazines, and piperidines (**8** and **9k–n**). A comparison of the substitution pattern at C_9 demonstrated that the order of activity was fused piperazines and piperidines > substituted piperidines ⩾ (thio) morpholines > substituted piperazines.

Some compounds were further examined for toxicity (IC_{50}) in a mammalian Vero cell line at 62.5 μg/mL concentrations.[16] 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 62.35 to 171.52 μM. A comparison of the substitution pattern at C_8 demonstrated that nitro group was more cytotoxic than the unsubstituted derivatives. These results are important as the C_8 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 **9m** was found to be 132.03 μM and showed selectivity index (IC_{50}/MIC) of more than 1467.

Subsequently, compound **9m** was tested for in vivo efficacy against MTB at a dose of 50 mg/kg (Table 2) in CD-1 mice.[8] The mice were infected intravenously with *M. tuberculosis* ATCC 35801. Drug treatment by intraperitoneal route began after 10 days of inoculation of

Table 2. In vivo activity data of **9m**, ofloxacin, 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
Ofloxacin (50 mg/kg)	6.61 ± 0.15	7.61 ± 0.16
Isoniazid (25 mg/kg)	5.86 ± 0.23	4.71 ± 0.10
9m (50 mg/kg)	6.08 ± 0.10	6.11 ± 0.23

the animal with microorganism and continued for 10 days. After 35 days postinfection 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 **9m** decreased the bacterial load in lung and spleen tissues with 1.91 and 2.91 − log10 protections, respectively, and was considered to be promising in reducing bacterial count in lung and spleen tissues. When compared to ofloxacin at the same dose level **9m** decreased the bacterial load with 0.53 and 1.5 − log10 protections in lung and spleen tissues, respectively.

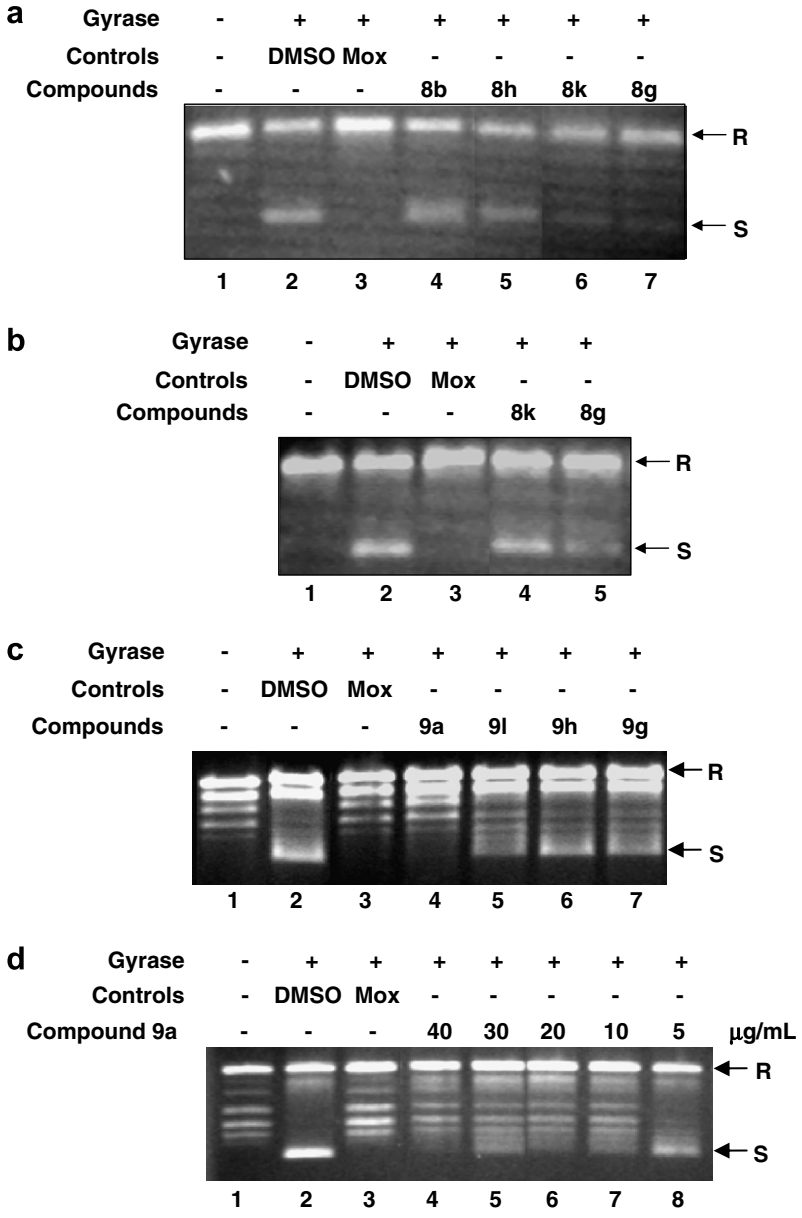


Figure 1. DNA gyrase inhibition. (a) Lane 1: relaxed circular DNA, lane 2: supercoiling reaction in the presence of 5% DMSO used as a solvent control, lane 3: moxifloxacin at 5 μg/mL concentration is used as the positive control. Lanes 4–7: reactions in the presence of 50 μg/mL of compounds **8b**, **8h**, **8k**, and **8g**, respectively. (b) Lane 1: relaxed circular DNA, lane 2: supercoiling reaction in the presence of 5% DMSO, lane 3: moxifloxacin (5 μg/mL concentration). Lanes 4 and 5: reactions in the presence of 40 μg/mL of compounds **8k** and **8g**, respectively. (c) Lane 1: relaxed circular DNA, lane 2: supercoiling reaction in the presence of 5% DMSO, lane 3: moxifloxacin (5 μg/mL). Lanes 4–7: reactions in the presence of 50 μg/mL of compounds **9a**, **9l**, **9h**, and **9g**, respectively. (d) Lane 1: relaxed circular DNA, lane 2: supercoiling reaction in the presence of 5% DMSO, lane 3: moxifloxacin at 5 μg/mL concentration. Lanes 4–8: reactions in the presence of 40, 30, 20, 10, and 5 μg/mL of compound **9a**, respectively. R, relaxed circular DNA substrate; S, supercoiled DNA product.

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

Compound	IC_{50} (μg/ml)
8b	>50
8h	>50
8k	50
8g	40
9a	10
9l	50
9h	>50
9g	50

The quinolones and fluoroquinolones inhibit two enzymes viz., DNA gyrase and topoisomerase IV. The reactions catalyzed by these ATP-dependent enzymes involve a transient double-stranded break in DNA and subsequent strand passage and religation reactions to facilitate topological changes in DNA. Amongst the two enzymes, DNA gyrase is unique in catalyzing the negative supercoiling of DNA and has essential roles during replication and transcription. Topoisomerase IV is not found in many eubacteria including mycobacteria. Hence DNA gyrase is the sole target for quinolones and fluoroquinolones in mycobacteria. Quinolones bind to the gyrase–DNA complex primarily through their interaction with amino acid residues located toward the amino terminus of GyrA subunit. The ability of the newly synthesized quinolone derivatives to inhibit DNA supercoiling by MC^2 gyrase was studied (Fig. 1). The IC_{50} values summarized in Table 3 were arrived at by taking various quantities of the compounds against the fixed amount of the enzyme in standard assay conditions.[17] Amongst all the compounds tested for the gyrase inhibition, compound **9a** showed lowest IC_{50} value of 10 μg/mL. Compound **8g** showed an IC_{50} value of 40 μg/mL and compounds **8k**, **9l**, and **9g** showed IC_{50} values of 50 μg/mL, respectively. Rest of the compounds tested for gyrase inhibition, that is, **8b**, **8h**, and **9h** do not inhibit the enzyme effectively as their IC_{50} values are higher (>50 μg/mL).

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 edema 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. Four (**9a**, **8b**, **9g**, and **8k**) compounds were evaluated for potential phototoxicity in a standardized in vivo test system that has been used previously to assess quinolone antibiotics.[18] The test compounds (140 mg/kg) and the positive controls lomefloxacin and ofloxacin hydrochloride (140 mg/kg) were evaluated for phototoxicity and both ears of each mouse were evaluated for changes indicative of a positive response: erythema, edema 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 is 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 to 96 h and 24 to 96 h when compared within time points and with the control, respectively, but ofloxacin did not show significant increase in ear thickness when compared within time and with the control, respectively. The test compounds were found to show a significant difference in ear thickness at various timepoints when compared with the pre-drug reading (0 h) but were less or non-toxic when compared with the negative (vehicle-treated) and positive controls (lomefloxacin and ofloxacin). No erythema occurred in mice dosed with 140 mg/kg of **9a** and **9g** throughout the 96-h study, while compound **8b** showed a significant erythema after irradiation untill 24 h only. Significant erythema was observed in the ears of mice dosed with compound **8k** and this response was maximal after

Table 4. Phototoxic evaluation

Group	Time (approximately) after start of irradiaton[c] (h)											
	Ear thickness[a] (mm)						Erythema[b]					
	0	4	24	48	72	96	0	4	24	48	72	96
Control[d]	0.29 ± 0.02	0.29 ± 0.01	0.32 ± 0.02	0.31 ± 0.01	0.34 ± 0.03	0.34 ± 0.03	0	0	0	0	0	0
9a	0.28 ± 0.01	0.28 ± 0.01	0.28 ± 0.02	0.29 ± 0.01	0.29 ± 0.01	0.31 ± 0.01	0	0	0	0	0	0
8b	0.29 ± 0.02	0.30 ± 0.02	0.29 ± 0.01	0.29 ± 0.02	0.29 ± 0.02	0.30 ± 0.02	0	4	0	0	0	0
9g	0.25 ± 0.01	0.28 ± 0.02	0.29 ± 0.01	0.29 ± 0.01	0.28 ± 0.02	0.28 ± 0.01	0	0	0	0	0	0
8k	0.34 ± 0.01	0.37 ± 0.02	0.32 ± 0.01	0.31 ± 0.01	0.32 ± 0.01	0.35 ± 0.01	0	6	6	2	1	0
Ofloxacin	0.29 ± 0.01	0.32 ± 0.01	0.29 ± 0.01	0.30 ± 0.01	0.35 ± 0.03	0.35 ± 0.04	0	6	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	0	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/m2) dosed at 10 mL/kg.

24 h but gradually subsided over 72 and 96 h of irradiation. Significant erythema was seen in animals treated with lomefloxacin throughout the 96-h study but ofloxacin showed significant erythema only at 4 h which subsided in next 24 h.

Screening of the antimycobacterial activity of these novel series identified newer potent antitubercular oxazino[2,3,4-*ij*]quinoline-6-carboxylic acids endowed with high activity toward MDR-TB, with MIC99 ranging from 0.09 to 8.30 μM. In conclusion, it has been shown that the potency, selectivity, and low cytotoxicity of these compounds make them valid leads for synthesizing new compounds that possess better activity. Further structure–activity and mechanistic studies should prove fruitful.

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References and notes

1. Shandil, R. K.; Jayaram, R.; Kaur, P.; Gaonkar, S.; Suresh, B. L.; Mahesh, B. N.; Jayashree, R.; Nandi, V.; Bharath, S.; Balasubramanian, V. *Antimicrob. Agents Chemother.* **2007**, *51*, 576.
2. Nuermberger, E. L.; Yoshimatsu, T.; Tyagi, S.; O'Brien, R. J.; Vernon, A. R.; Chaisson, R. E.; Bishai, W. R.; Grosset, J. H. *Am. J. Respir. Crit. Care Med.* **2004**, *169*, 421.
3. Veziris, N.; Truffot-Pernot, C.; Aubry, A.; Jarlier, V.; Lounis, N. *Antimicrob. Agents Chemother.* **2003**, *47*, 3117.
4. Maxwell, A. *Trends Microbiol.* **1997**, *5*, 102.
5. Bozeman, L.; Burman, W.; Metchock, B.; Welch, L.; Weiner, M. *Clin. Infect. Dis.* **2002**, *40*, 386.
6. Anquetin, G.; Greiner, J.; Mahmoudi, N.; Santillana-Hayat, M.; Gozalbes, R.; Farhati, K.; Derouin, F.; Aubry, A.; Cambau, E.; Vierling, P. *Eur. J. Med. Chem.* **2006**, *41*, 1478.
7. Janin, Y. L. *Bioorg. Med. Chem.* **2007**, *15*, 2479.
8. Sriram, D.; Yogeeswari, P.; Basha, S. J.; Radha, D. R.; Nagaraja, V. *Bioorg. Med. Chem.* **2005**, *13*, 5774.
9. Sriram, D.; Aubry, A.; Yogeeswari, P.; Fisher, L. M. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2982.
10. Ginsburg, A. S.; Grosset, J. H.; Bishai, W. R. *Lancet Infect. Dis.* **2003**, *3*, 432.
11. Aubry, A.; Veziris, N.; Cambau, E.; Truffot-Pernot, C.; Jarlier, V.; Fisher, L. M. *Antimicrob. Agents Chemother.* **2006**, *50*, 104.
12. Cheng, A. F.; Yew, W. W.; Chan, E. W.; Chin, M. L.; Hui, M. M.; Chan, R. C. *Antimicrob. Agents Chemother.* **2004**, *48*, 596.
13. Mitscher, L. A.; Sharma, P. N.; Chu, D. T. W.; Shen, L. L.; Pernets, A. G. *J. Med. Chem.* **1987**, *30*, 2283.
14. Representative analytical data for compound 10-[2-carboxy-5,6-dihydroimidazo[1,2-*a*]pyrazin-7(8*H*)-yl]-9-fluoro-2,3-dihydro-3-methyl-8-nitro-7-oxo-7*H*-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxylic acid (**9m**): Yield: %; mp °C; IR (KBr) cm^{-1}: 3400, 1750, 1660, 1590; ^1H NMR (DMSO-d_6) δ ppm: 1.60 (d, 3H, $CHCH_3$), 3.1–3.8 (m, 6H, 3-CH_2), 4.35 (br s, 2 H, OCH_2), 4.5 (m, 1H, $CHCH_3$), 7.6 (s, 1H, CH), 8.00 (s, 1H, C_5–H), 12.12 (s, 1H, 2-COOH), 14.4 (s, 1H, 6-COOH); Anal. ($C_{20}H_{16}FN_5O_8$): calcd C, 50.75; H, 3.41; N, 14.79; found C, 50.62; H, 3.47; N, 14.78.
15. National Committee for Clinical Laboratory Standards. Antimycobacterial susceptibility testing for *Mycobacterium tuberculosis*. Proposed standard M24-T. National Committee for Clinical Laboratory Standards, Villanova, PA, 1995.
16. Gundersen, L. L.; Meyer, N. J.; Spilsberg, B. *J. Med. Chem.* **2002**, *45*, 1383.
17. Manjunatha, U. H.; Dalal, M.; Chatterji, M.; Radha, D. R.; Visweswariah, S. S.; Nagaraja, V. *Nucleic Acids Res.* **2002**, *30*, 2144.
18. Mayne, T. N.; Johnson, N. J.; Kluwe, W. M.; Lencoski, D. L.; Polzer, R. J. *J. Antimicrob. Chemother.* **1997**, *39*, 67–73.