Antimycobacterial Activities of Novel 1-(Cyclopropyl/*tert*-butyl/4-fluorophenyl)-1,4-dihydro-6-nitro-4-oxo-7-(substituted secondary amino)-1,8-naphthyridine-3-carboxylic Acid

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Fifty-one 1-(cyclopropyl/*tert*-butyl/4-fluorophenyl)-1,4-dihydro-6-nitro-4-oxo-7-(substituted secondary amino)-1,8-naphthyridine-3-carboxylic acids were synthesized and evaluated for antimycobacterial *in vitro* and *in vivo* 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 *M. smegmatis*. Among the synthesized compounds, 1-*tert*-butyl-1,4-dihydro-7-(4,4-dimethyloxazolidin-3-yl)-6-nitro-4-oxo-1,8-naphthyridine-3-carboxylic acid (**10q**) was found to be the most active compound *in vitro* with an MIC of 0.1 μM against MTB and MDR-TB and was 3 and 455 times more potent than isoniazid against MTB and MDR-TB, respectively. In the *in vivo* animal model **10q** decreased the bacterial load in lung and spleen tissues with 2.39 and 3.89-log10protections respectively at the dose of 50 mg/kg body weight.

Tuberculosis (TB), which is caused by *Mycobacterium tuberculosis* (MTB[a]), is one of the most prevalent diseases and is responsible for the deaths of about one billion people during the last two centuries.[1] MTB is a particularly successful pathogen that latently infects about 2 billion people, about one-third of world population. Each year, there are about 8 million new TB cases and 2 million deaths worldwide. The increasing emergence of drug-resistant TB, especially multi-drug-resistant TB (MDR-TB, resistant to at least two frontline drugs such as isoniazid and rifampin), is particularly alarming. MDR-TB has already caused several fatal outbreaks[2] and poses a significant threat to the treatment and control of the disease in some parts of the world, where the incidence of MDR-TB can be as high as 14%. The standard TB therapy is ineffective in controlling MDR-TB in high MDR-TB incidence areas. Fifty million people have already been infected with drug-resistant TB.[1] There is much concern that the TB situation may become even worse with the spread of HIV worldwide, a virus that weakens the host immune system and allows latent TB to reactivate and makes the person more susceptible to reinfection with either drug-susceptible or drug-resistant strains. In the last 50 years, only a few drugs have been approved by the Food and Drug Administration (FDA) to treat TB, reflecting the inherent difficulties in discovery and clinical testing of new agents and the lack of pharmaceutical industry research in the area.[3] Hence, faster acting and effective new drugs to better combat TB, including multi-drug-resistant tuberculosis, are needed.

Several of the quinolone antibacterial have been examined as inhibitors of MTB as well as other mycobacterial infections.[4] As a result, gatifloxacin and moxifloxacin (Figure 1) are drugs in pipeline and to be approved by FDA for the treatment of TB by the year 2010. Quinolones inhibit bacterial type II topoisomerases, DNA gyrase, and topoisomerase IV,[5] which are essential enzymes that maintain the supercoils in DNA. 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.[6] A huge number of fluoroquinolones differing in the nature of the R7 and R1 substituents were elaborated to enable the establishment of structure/antituberculosis relationships[7−11] and only a few R6- or/and R8-substituted quinolones are known, probably due to the difficult access to precursors of these derivatives. Taking into account that fluoroquinolones exhibit fairly good antimycobacterial activity and having in mind the not fully explored antitubercular potential of nitroquinolone pharmacophore, in the present work, effort has been taken to study the effect of nitro substitution at sixth position of naphthyridinone. The substitutions at the N1 position include cyclopropyl/*tert*-butyl/4-fluorophenyl, in combination with various unreported bulky secondary amino functions to study the influence of lipophilic character at 7 position of naphthyridone on activity against MTB. We report herein the synthesis of newer 1-(cyclopropyl/*tert*-butyl/4-fluorophenyl)-

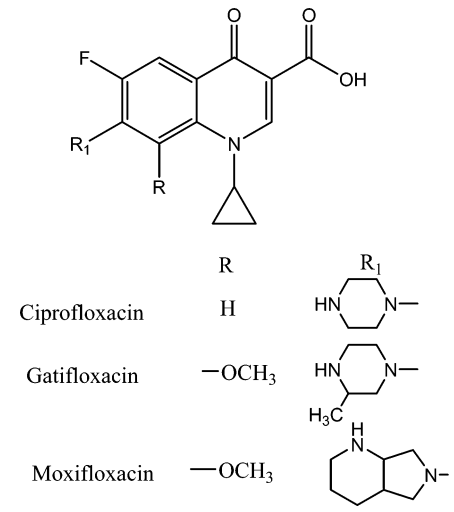

Figure 1. Standard fluoroquinolones.

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[a] Abbreviations: MTB: *Mycobacterium tuberculosis*; MDR-TB: MC^2: *M. smegmatis*; MIC: minimum inhibitory concentration.

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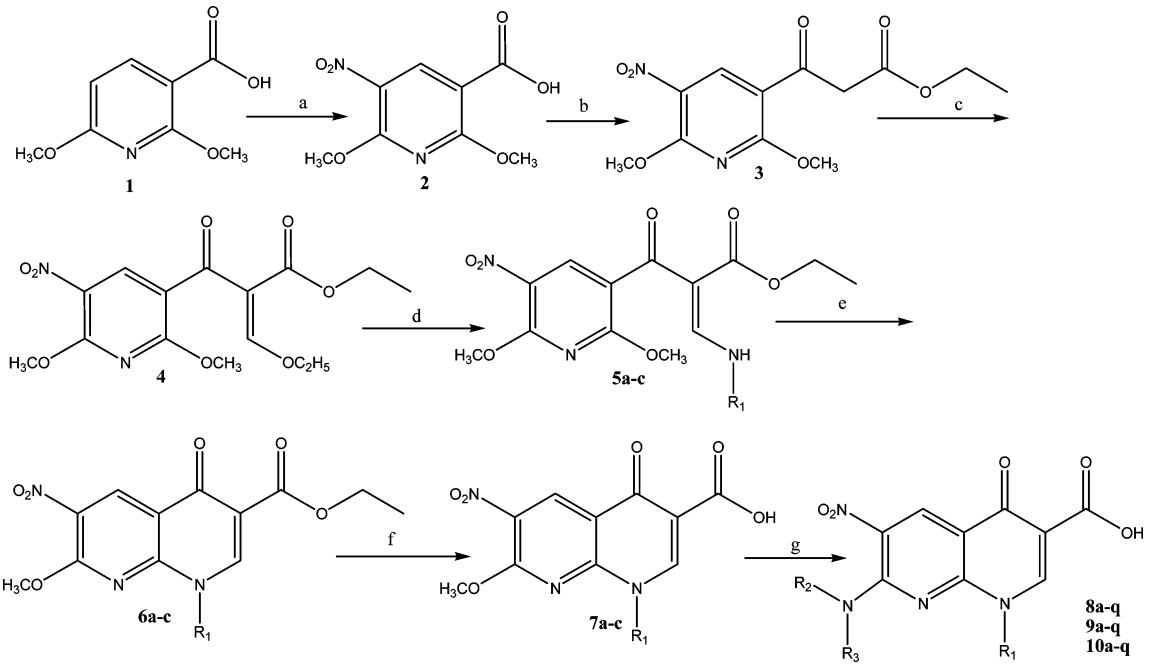
Scheme 1[a]



[a] (a) HNO_3, $(CH_3CO)O$; (b) CDI, $C_2H_5OOCCH_2COOK$, $MgCl_2$, $(C_2H_5)_3N$; (c) $(C_2H_5O)_3CH$, $(CH_3CO)_2O$; (d) R_1NH_2; (e) K_2CO_3; (f) HCl; (g) secondary amines, K_2CO_3, MWI.

1,4-dihydro-6-nitro-4-oxo-7-(substituted secondary amino)-1,8-naphthyridine-3-carboxylic acids and their antimycobacterial activities together with toxicological results.

Chemistry

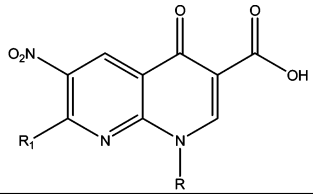
The synthesis of the titled compounds **8**−**10a**−**q** was accomplished as outlined in Scheme 1. The 2,6-dimethoxynicotinic acid (**1**) was converted to 2,6-dimethoxy-5-nitronicotinic acid (2) by treatment with nitric acid in presence of acetic anhydride. Compound **2** on reaction with 1,1′-carbonyldiimidazole in tetrahydrofuran afforded the corresponding imidazolide, which, *in situ* was treated with the neutral magnesium salt of ethyl potassium malonate in the presence of triethylamine to yield ethyl 3-(2,6-dimethoxy-5-nitropyridin-3-yl)-3-oxopropanoate (**3**). Ethyl 1-(cyclopropyl/*tert*-butyl/4-fluorophenyl)-7-methoxy-6-nitro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylates (**6a**−**c**) were prepared by a three-step one-pot reaction. First, treatment of the keto ester **3** with triethyl orthoformate in acetic anhydride gave the one-carbon homologue enol ether intermediate ethyl 2-[(2,6-dimethoxy-5-nitropyridin-3-yl)carbonyl]-3-ethoxyacrylate (**4**), which upon evaporation to dryness was allowed to react with a slight excess of appropriate amines under nitrogen atmosphere in a mixture of ether and ethanol at 0 °C. This gave the enamino ester ethyl 3-(substituted aminomethylene)-2-[(2,6-dimethoxy-5-nitropyridin-3-yl)carbonyl]acrylate (**5**), and base-catalyzed cyclization of **5a**−**c** with potassium carbonate in DMSO yielded naphthyridone **6a**−**c**. Ethyl esters were finally hydrolyzed in acidic condition to yield 1-(cyclopropyl/*tert*-butyl/4-fluorophenyl)-1,4-dihydro-7-methoxy-6-nitro-4-oxo-1,8-naphthyridine-3-carboxylic acid (**7a**−**c**). The titled compounds **8**−**10a**−**q** were prepared by treating **7a**−**c** with appropriate secondary amines in presence of potassium carbonate under microwave irradiation in DMSO. When compared to conventional method[12] of a 2−3 h process, microwave-assisted synthesis was performed with short reaction times (2−3 min) with ease and was environmentally friendly. The purity of the synthesized compounds was monitored by thin layer chromatography (TLC) and elemental analyses, and the structures were identified by spectral data.

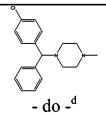
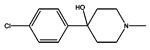
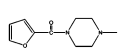
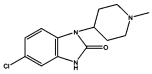
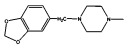
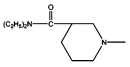
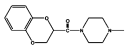
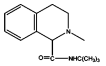
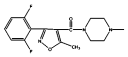
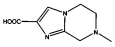
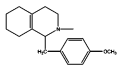
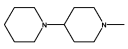
Biological Results and Discussion

Antimycobacterial Activity. The compounds were screened for their *in vitro* antimycobacterial activity against MTB, MDR-TB, and *M. smegmatis* ATCC 14468 (MC^2) by agar dilution method[13] for the determination of MIC in duplicate. 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 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 an MIC of less than 12 μM. Eleven compounds (**9d**, **10d**, **9g**, **10g**, **9h**, **10m**, **9o**, **10o, 8q**, **9q**, and **10q**) inhibited MTB with an MIC of less than 1 μM and were more potent than the standard fluoroquinolone gatifloxacin (MIC: 1.04 μM). When compared to isoniazid (MIC: 0.36 μM), two compounds (**9q** and **10q**) were found to be more active against MTB. 1-*tert*-Butyl-1,4-dihydro-7-(4,4-dimethyloxazolidin-3-yl)-6-nitro-4-oxo-1,8-naphthyridine-3-carboxylic acid (**10q**) was found to be the most active compound *in vitro* with an MIC of 0.1 μM against MTB and was 3.6 and 10.4 times more potent than isoniazid and gatifloxacin, respectively. Subsequently, some of the compounds were evaluated against MDR-TB, and among the thirty three compounds screened, all of them inhibited MDR-TB with an MIC ranging from 0.08 to 6.19 μM and were found to be more active than isoniazid (MIC: 45.57 μM) and gatifloxacin (MIC: 8.34 μM). Fifteen compounds (**9a**, **9d**, **10d**, **9g**, **10g**, **9h**, **9j**, **8k**, **9k**, **10m**, **9o**, **10o**, **8p**, **9q**, and **10q**) inhibited MDR-TB with an MIC of less than 1 μM. 7-(2-Carboxy-5,6-dihydroimidazo-[1,2-a]pyrazin-7(8H)-yl)-1-(4-fluorophenyl)-1,4-dihydro-6-nitro-4-oxo-1,8-naphthyridine-3-carboxylic acid (**9o**) was found to be the most active compound *in vitro* with an MIC of 0.08 μM against MDR-TB and was 104 and 570 times more potent than

Table 1. Physical Constants, *in Vitro* Antimycobacterial Activities, and Cytotoxicity



No	R	R_1	IC_{50} (μM)	MIC (μM) MTB	MIC (μM) MDR TB	MIC (μM) MC^2	No	R	R_1	IC_{50} (μM)	MIC (μM) MTB	MIC (μM) MDR TB	MIC (μM) MC^2
8a	CP[a]		111.6	5.59	NT	22.32	**8j**	CP		64.4	6.46	NT	25.78
9a	4FP[b]	- do -[d]	101.8	1.27	0.31	5.09	**9j**	4FP	- do -	59.6	2.89	0.72	11.59
10a	tBu[c]	- do -	>108.5	1.35	NT	173.59	**10j**	tB	- do -	124.8	3.11	1.56	24.95
8b	CP		68.9	6.90	3.44	27.57	**8k**	CP		55.5	2.97	0.74	23.81
9b	4FP	- do -	123.2	3.07	1.54	24.63	**9k**	4FP	- do -	53.9	2.69	0.62	10.79
10b	tBu	- do -	133.1	3.32	3.32	6.67	**10k**	tB	- do -	57.7	2.88	2.88	11.53
8c	CP		126.7	3.16	1.58	25.33	**8l**	CP		>136.7	3.41	NT	109.29
9c	4FP	- do -	57.1	11.42	NT	45.66	**9l**	4FP	- do -	>122.2	3.05	1.52	12.22
10c	tBu	- do -	>122.7	1.53	3.06	6.14	**10l**	tB	- do -	>131.9	3.29	NT	105.59
8d	CP		139.1	6.96	NT	27.81	**8m**	CP		150.1	7.52	NT	30.02
9d	4FP	- do -	124.1	0.77	0.18	3.09	**9m**	4FP	- do -	132.9	6.65	NT	26.57
10d	tBu	- do -	>134.3	0.84	0.84	13.43	**10m**	tBu	- do -	144.5	0.90	0.44	3.61
8e	CP		59.9	11.98	1.49	6.00	**8n**	CP		123.6	3.09	6.19	24.73
9e	4FP	- do -	>54.3	10.86	NT	21.72	**9n**	4FP	- do -	111.7	2.79	1.39	11.17
10e	tBu	- do -	116.3	1.45	2.90	5.82	**10n**	tBu	- do -	119.8	2.99	1.49	6.00
8f	CP		107.7	21.53	NT	86.13	**8o**	CP		141.9	3.54	1.77	28.39
9f	4FP	- do -	98.5	9.85	NT	39.39	**9o**	4FP	- do -	>126.4	0.38	0.08	1.58
10f	tBu	- do -	104.8	2.62	1.31	10.48	**10o**	tBu	- do -	>136.9	0.42	0.42	6.86
8g	CP		166.1	8.32	NT	33.21	**8p**	CP		117.8	5.89	0.74	1.47
9g	4FP	- do -	72.6	0.44	0.91	1.81	**9p**	4FP	- do -	<53.5	10.69	NT	85.53
10g	tBu	- do -	79.6	0.99	0.48	1.99	**10p**	tBu	- do -	>114.3	5.73	NT	11.43
8h	CP		>160.9	4.02	4.02	32.19	**8q**	CP		167.1	0.51	NT	4.17
9h	4FP	- do -	141.3	0.43	0.43	1.79	**9q**	4FP	- do -	>145.9	0.21	<0.10	1.82
10h	tBu	- do -	159.3	7.74	NT	15.45	**10q**	tBu	- do -	>160.1	0.10	0.10	3.99
8i	CP		>141.6	7.09	NT	28.31	**Gati**	-	-	>155.3	1.04	8.34	2.08
9i	4FP	- do -	>126.1	3.15	1.57	25.23	**INH**	-	-	>455.8	0.36	45.57	45.57
10i	tBu	- do -	>136.6	1.70	1.70	6.84							

[a] Cyclopropyl. [b] 4-Fluorophenyl. [c] *tert*-Butyl. [d] Same as above.

gatifloxacin and isoniazid respectively. The compounds were also evaluated against MC^2 in which all the compounds inhibited MC^2 with an MIC ranging from 1.47 to 173.59 μM, and 45 compounds were found to be more active than isoniazid (MIC: 45.57 μM).

With respect to the structure−MTB activity relationship, the results demonstrated that the antimycobacterial activity imparted by the in N1 substituent was in the order of *tert*-butyl > 4-flurophenyl > cyclopropyl. This result was in contrast to the other antibacterial fluoroquinolones, where the cyclopropyl group was the favorable substituent. This result correlates well with the previous report on the importance of *tert*-butyl group as the antimycobacterial pharmacophore.[14] The enhanced activity by the *tert*-butyl group could be supported by its better electron-donating property and its higher hydrophobicity than by the cyclopropyl group, which provides support for the cooperative-binding model for the inhibition of DNA gyrase proposed by Shen, in which two quinolone molecules self-associate by π−π ring stacking and tail-to-tail hydrophobic interaction between the N1 substituents.[15] At the C7 position we have studied various substituted piperazines (**8**−**10a**−**f**), (thio)morpholines (**8**−**10g**,**h**), substituted piperidines (**8**−**10i**−**n**), fused piperazines and piperidines (**8**−**10o**,**p**), and oxazolidine (**8**−**10q**). A comparison of the substitution pattern at C_7 demonstrated that the order of activity was oxazolidine > fused piperazines and piperidines > (thio)morpholines > substituted piperazines ≥ substituted piperidines. The results demonstrated that the contribution of the C7 position to antimycobacterial activity was dependent on the substituent at N1 and was in the order of oxazolidine > fused piperazines and piperidines > (thio)-morpholines > substituted piperidines > substituted piperazines > (thio)morpholines when N1 was cyclopropyl; oxazolidine >

Table 2. *In Vivo* Activity Data of **10q**, 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
10q (50 mg/kg)	5.60 ± 0.08	5.13 ± 0.16

(thio)morpholines > substituted piperazines > substituted piperidines > fused piperazines and piperidines when N1 was 4-fluorophenyl; oxazolidine > substituted piperazines substituted piperidines > (thio)morpholines > > fused piperazines and piperidines when N_1 was *tert*-butyl. In MDR-TB, the results demonstrated that the antimycobacterial activity imparted by the in N1 substituents was in the order 4-flurophenyl > *tert*-butyl > cyclopropyl. Introduction of bulky lipophilic secondary amines at C7 enhanced the antimycobacterial activity, which might be due to more penetration of these compounds into mycobacterial cells.

All the compounds were further examined for toxicity (IC_{50}) in a mammalian Vero cell line up to 62.5 μg/mL concentrations by a serial dilution method. 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 nonradioactive cell proliferation assay,[16] and the results are reported in Table 1. Fifteen compounds when tested showed IC_{50} values ranging from 55.5 to 160.1 μM. A comparison of the substitution pattern at C_7 demonstrated that piperidine-substituted analogues were more cytotoxic than the substituted piperazines. These results are important, as the piperdine-substituted compounds with their increased cytoliability are much less attractive in the development of a quinolone for the treatment of TB. This is primarily because 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. Compound **10q** was found to be nontoxic up to 62.5 μg/mL (160.1 μM) and showed a selectivity index (IC_{50}/MIC) of more than 1600.

Prior to animal screening, the maximum tolerated dose (MTD) was performed for compound **51** using C57BL/6 female mice by administration of a one-time dose/animal of an escalating dose of drug (100, 300, and 500 mg/kg). The nine mice (three mice/dose) in each study were observed for a total of 1 week. Surviving mice were sacrificed and the organs examined for signs of overt toxicity. Compound **10q** showed no effect or adverse reactions/toxicity at the maximum dose tested. Subsequently, compound **10q** was tested for efficacy against MTB at a dose of 50 mg/kg (Table 2) in CD-1 mice. In this model,[11] the mice were infected intravenously with *M. tuberculosis* ATCC 35801. Drug treatment by the intraperitoneal route began after 10 days of inoculation of the animal with the microorganism and continued for 10 days. After 35 days postinfection, the spleens and right lungs were aseptically removed, and the number of viable organisms were 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 **10q** decreased the bacterial load in lung and spleen tissues with 2.39 and 3.89-log10protections, 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, **10q** decreased the bacterial load with 0.42 and 1.79-log10protections in lung and spleen tissues, respectively. Compound **10q** was found to be less active than isoniazid in the *in vivo* study. This lower *in vivo* activity might be due to the low bioavailability of the compound.

DNA Gyrase Inhibition. The naphthyridone derivatives synthesized and studied in this report were tested for their ability to inhibit supercoiling activity of DNA gyrase. The bacterial targets for quinolones and fluroquinolones are the type II DNA topoisomerases, DNA gyrase and topoisomerase IV. These ATP-dependent enzymes act by a transient double-stranded DNA break, followed by strand passage and religation reactions to facilitate DNA transactions processes.[17] DNA gyrase is unique in catalyzing the negative supercoiling of DNA and is essential for DNA replication, transcription, and recombination. In all species of mycobacteria including MTB, DNA gyrase is the sole type II topoisomerase carrying out the reactions of both type II topoisomerases. Further, our earlier studies have revealed that DNA gyrase from *M. tuberculosis* and *M. smegmatis* are highly similar in protein level, antigenic properties, and catalytic activities.[18] The supercoiling assay results with various compounds using *M. smegmatis* DNA gyrase is presented in Figure 2. The IC_{50} values presented in Table 3 show that the compounds **8p**, **9d**, **9q**, **10g**, and **10m** inhibit DNA gyrase at 50 μg/mL or lower concentrations (56.54−116.72 μM). The other compounds, viz. **9g**, **9h**, **9o**, and **10q**, have MIC values greater than 50 μg/mL (202.27−256.15 μM) and yet show comparable antimicrobial activity to those which inhibit DNA gyrase at lower concentrations (Table 1).

All the molecules tested do not show inhibition of supercoiling activity at very low concentrations. The IC_{50} values were higher compared to that of moxifloxacin and ciprofloxacin, two well characterized fluoroquinolones, used as positive controls. However, in cell-based assays, most of the compounds exhibited higher potency. Thus, there is an inverse correlation between IC_{50} values and enzyme inhibition data. Generally the inhibitory molecules that show very good inhibition in enzyme assays have a lower degree of efficacy in cell-based *in vitro* assays because of poor permeability, intracellular instability.[19] The difference in the extent of inhibition found in enzyme assays versus *in vivo* susceptibility tests may be due to the following reasons: (1) The molecules may act as prodrugs and upon entering the cell undergo enzymatic modifications to become a much more potent drug.[20] (2) Other proteins and/or ionic environment *in vivo* may influence binding characteristics to better accommodate the compounds to the binding site in the enzyme. Since selected compounds showed only weak inhibition activity against the target enzyme, it is supposed that the mode of action of these compounds is different from inhibition of DNA gyrase.

Phototoxic Evaluation. Quinolones in general have favorable safety profiles; phototoxicity has become a significant factor in the clinical use of some[21]quinolones. Indeed, the first quinolone, nalidixic acid, caused light-induced dermal effects. This type of response has now been demonstrated for almost all fluoroquinolones,[22] 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.[23] This photosensitized chromophore may then transfer its absorbed photo energy 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. Six (**9g**, **10g**, **9h**, **9o**, **8p**, and **10q**) compounds were evaluated for potential phototoxicity in a standardized *in vivo* test system that has been used

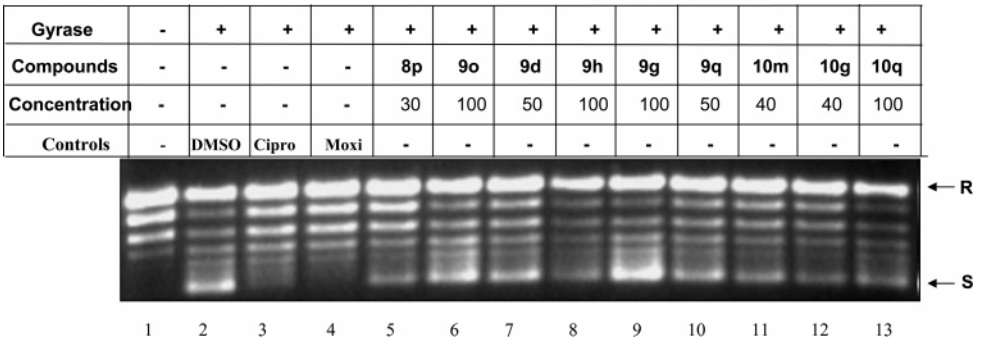


Figure 2. DNA gyrase supercoiling assay. The assays were performed as described in Experimental Section. DNA gyrase was incubated with indicated concentrations of the compounds before the addition of rest of the components. Lane 1: relaxed DNA, lane 2: supercoiling reaction in presence of 5% DMSO; ciprofloxacin (lane 3) and moxifloxacin at concentration of 5 μg/mL (lane 4) were used as the positive controls for inhibition of enzyme. Lane 5: 30 μg/mL **8p**, lane 6: 100 μg/mL **9o**, lane 7: 50 μg/mL **9d**, lane 8: 100 μg/mL **9h**, lane 9: 100 μg/mL **9g**, lane 10: 50 μg/mL **9q**, lane 11: 40 μg/mL **10m**, lane 12: 40 μg/mL **10g**, lane 13: 100 μg/mL **10q**. R and S indicate relaxed and supercoiled pUC18 DNA, respectively.

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

compound	IC_{50} (μM)
8p	56.54
9d	99.31
9g	>232.34
9h	226.04
9o	>202.27
9q	116.72
10g	101.93
10m	92.50
10q	256.15
ciprofloxacin	15.09

previously to assess quinolone antibiotics.[24] 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, 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 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 from 24 to 96 h when compared with 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 predrug reading (0 h) but were less or not toxic when compared with the negative (vehicle-treated) and positive controls (lomefloxacin). No erythema occurred in mice dosed with 140 mg/kg of **9g**, **10g**, **9h**, and **8p** throughout the 96 h study, while compound **9o** showed a significant erythema after irradiation till 24 h only and compound **10q** developed erythema from 48 h.

Experimental Section

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 240C analyzer, and the data were within $\pm$0.4% of the theoretical values.

2,6-Dimethoxy-5-nitropyridine-3-carboxylic Acid (2). This compound was prepared according to the literature method[12] with slight modification. Briefly, to a mixture of concentrated nitric acid (4 mL) (**1**) and acetic anhydride (12 mL) was added dropwise 2,6-dimethoxynicotinicacid (16.3 mM) at 0−5 °C, and the mixture was stirred for 3 h. The temperature was brought up to room temperature, and the mixture was stirred for an additional 4 h. The resulting precipitate was poured into ice-cold water and filtered. The precipitate was dissolved in ether and extracted with saturated sodium bicarbonate solution, and the aqueous layer upon acidification with 2 N HCl yielded **2** with 80% yield; mp 236−238 °C (lit. ref 230 °C).

Ethyl 3-(2,6-Dimethoxy-5-nitropyridin-3-yl)-3-oxopropanoate (3). This compound was prepared from **2** according to the literature method[12] with 80% yield.

Ethyl 1-(Cyclopropyl/*tert*-butyl/4-fluorophenyl)-7-methoxy-6-nitro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylate (6a−c). A mixture of **3** (1.00 equiv), triethyl orthoformate (1.5 equiv), and acetic anhydride (2.5 equiv) were refluxed at 140 °C for 1 h. The ethyl acetate produced as a byproduct was distilled simultaneously under atmospheric pressure. After completion of reaction, the reaction mixture was concentrated under reduced pressure to yield ethyl 2-[(2,6-dimethoxy-5-nitropyridin-3-yl)carbonyl]-3-ethoxyacrylate (**4**). The above residue was dissolved in a mixture of ether (20 mL) and ethanol (20 mL). The corresponding primary amine (1.1 equiv) was added at 0 °C and stirred for 30 min under nitrogen atmosphere, followed by distillation to yield ethyl 3-(substituted aminomethylene)-2-[(2,6-dimethoxy-5-nitropyridin-3-yl)-carbonyl]acrylate (**5a−c**). The above crude solid (1.0 equiv) was dissolved in DMSO (30 mL), and anhydrous potassium carbonate (1.6 equiv) was added and refluxed at 60 °C for 3 h. After completion of reaction, the mixture was diluted with ice cold water and neutralized with 20% HCl to a pH of 5−6. The precipitate thus formed was filtered and washed with water followed by 2-propanol to yield ethyl 1-(cyclopropyl/*tert*-butyl/4-fluorophenyl)-7-methoxy-6-nitro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylates **6a−c**.

Ethyl 1-cyclopropyl-7-methoxy-6-nitro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylate (6a). Yield: 92%; mp: 210−212 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28−0.53 (m, 4H, cyclopropyl), 1.32 (t, 3H, CH_3 of OCH_2CH_3), 1.35 (m, 1H, cyclopropyl), 3.74 (s, 3H, OCH_3), 4.22 (m, 2H, OCH_2), 9.26 (s, 1H, C_5-H), 9.56 (s, 1H, C_2-H); ^{13}C NMR (DMSO-d_6) δ ppm: 177.5, 165.3, 164.7, 159.9, 149.9, 135.2, 126.1, 112.2, 109.4, 61.4, 54.9, 36.0, 14.4, 5.6; Anal. ($C_{15}H_{15}N_3O_6$) C, H, N.

Ethyl 1-(4-fluorophenyl)-7-methoxy-6-nitro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylate (6b). Yield: 92%; mp: >250 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 1.32 (t, 3H, CH_3 of OCH_2CH_3), 1.76 (s, 9H, *t*-butyl), 3.74 (s, 3H, OCH_3), 4.22 (m, 2H, OCH_2), 9.26 (s, 1H, C_5-H), 9.56 (s, 1H, C_2-H); ^{13}C NMR (DMSO-d_6) δ ppm: 177.5, 167.4, 165.3, 164.7, 152.9, 146.6, 139.8, 135.2, 126.1,

Table 4. Phototoxic Evaluation of the Titled Compounds

	time (approximately) after start of irradiaton (h)[a]											
	ear thickness (mm)[b]						erythema[c]					
group	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
9g	0.25 ± 0.01	0.27 ± 0.01	0.26 ± 0.01	0.26 ± 0.01	0.29 ± 0.01	0.29 ± 0.02	0	0	0	0	0	0
10g	0.27 ± 0.01	0.28 ± 0.02	0.28 ± 0.02	0.29 ± 0.02	0.30 ± 0.02	0.32 ± 0.02	0	0	0	0	0	0
9h	0.26 ± 0.01	0.25 ± 0.01	0.28 ± 0.01	0.27 ± 0.01	0.3 ± 0.01	0.3 ± 0.01	0	0	0	0	0	0
9o	0.27 ± 0.01	0.32 ± 0.01	0.33 ± 0.03	0.32 ± 0.02	0.33 ± 0.01	0.33 ± 0.02	0	4	4	0	0	0
8p	0.33 ± 0.01	0.34 ± 0.02	0.34 ± 0.01	0.38 ± 0.02	0.36 ± 0.01	0.33 ± 0.02	0	0	0	1[e]	0	0
10q	0.24 ± 0.01	0.28 ± 0.02	0.26 ± 0.01	0.29 ± 0.02	0.30 ± 0.02	0.30 ± 0.02	0	0	0	4	4	4
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] Time zero = predose (mice exposed to UVA light immediately after dosing); 4 h = end of irradiation period. [b] Mean ear thickness ± SEM; left and right ears were averaged. [c] Number of mice with erythema. [d] Control = 0.5% aqueous solution of sodium carboxymethylcellulose (4000 cps) dosed at 10 mL/kg. [e] Very slight erythema, not considered to be drug-related.

116.3, 117.9, 110.2, 109.4, 61.4, 54.9, 14.4; Anal. ($C_{16}H_{19}N_3O_6$) C, H, N.

Ethyl 1-*tert*-butyl-7-methoxy-6-nitro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylate (6c). Yield: 92%; mp: 204−206 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 1.32 (t, 3H, CH_3 of OCH_2CH_3), 3.74 (s, 3H, OCH_3), 4.22 (m, 2H, OCH_2), 6.45−6.74 (m, 4H, Ar-H), 9.26 (s, 1H, C_5-H), 9.56 (s, 1H, C_2-H); ^{13}C NMR (DMSO-d_6) δ ppm: 177.5, 165.3, 164.7, 159.9, 149.9, 135.2, 126.1, 112.2, 109.4, 65.0, 61.4, 54.9, 28.5, 14.4; Anal. ($C_{18}H_{14}N_3O_6$) C, H, N.

1-(Cyclopropyl/*tert*-butyl/4-fluorophenyl)-1,4-dihydro-7-methoxy-6-nitro-4-oxo-1,8-naphthyridine-3-carboxylic Acid (7a-c). Compound **6a−c** (1.0 equiv) was suspended in 6 N HCl (5 mL), refluxed for 6 h, and then cooled to 0 °C. The precipitate obtained was filtered and washed with water followed by 20% ethyl acetate yielded **7a−c**.

1-Cyclopropyl-1,4-dihydro-7-methoxy-6-nitro-4-oxo-1,8-naphthyridine-3-carboxylic Acid (7a). Yield: 92%; mp: 232−234 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28−0.46 (m, 4H, cyclopropyl), 1.38 (m, 1H, cyclopropyl), 4.28 (s, 3H, methoxy), 9.26 (s, 1H, C_5-H), 9.56 (s, 1H, C_2-H), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 177.5, 166.3, 164.1, 159.9, 152.7, 135.2, 126.0, 111.3, 109.4, 54.9, 36.0, 5.6; Anal. ($C_{13}H_{11}N_3O_6$) C, H, N.

1−4-Fluorophenyl-1,4-dihydro-7-methoxy-6-nitro-4-oxo-1,8-naphthyridine-3-carboxylic Acid (7b). Yield: 92%; mp: 239−241 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 1.6 (S, 9H, *tert*-butyl), 4.32 (s, 3H, methoxy), 9.26 (s, 1H, C_5-H), 9.54 (s, 1H, C_2-H), 14.68 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 177.5, 167.4, 166.3, 164.1, 152.9, 149.4, 139.8, 135.2, 126.0, 116.3, 117.9, 109.4, 54.9; Anal. ($C_{14}H_{15}N_3O_6$) C, H, N.

1-*tert*-Butyl-1,4-dihydro-7-methoxy-6-nitro-4-oxo-1,8-naphthyridine-3-carboxylic Acid (7c). Yield: 92%; mp: 196−198 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 4.34 (s, 3H, methoxy), 6.48−6.68 (m, 4H, Ar-H), 9.24 (s, 1H, C_5-H), 9.56 (s, 1H, C_2-H), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 177.5, 166.3, 164.1, 159.9, 152.7, 135.2, 126.0, 111.3, 109.4, 65.0, 54.9, 28.5; Anal. ($C_{16}H_{10}FN_3O_6$) C, H, N.

1-(Cyclopropyl/*tert*-butyl/4-fluorophenyl)-1,4-dihydro-6-nitro-4-oxo-7-(substituted secondary amines)-1,8-naphthyridine-3-carboxylic Acid (8−10a−q). Compound **7a−c** (1.0 equiv) in dimethyl sulfoxide (2.5 mL) and appropriate secondary amines (1.1 equiv) were irradiated in a microwave oven at an intensity of 80% with 30 s/cycle. The number of cycles in turn depended on the completion of the reaction, which was checked by TLC. The reaction timing varied from 1.5 to 3 min. After completion of the reaction, the mixture was poured into ice cold water and washed with water and 2-propanol to give titled products.

7-(4-((4-Chlorophenyl)(phenyl)methyl)piperazin-1-yl)-1-cyclopropyl-1,4-dihydro-6-nitro-4-oxo-1,8-naphthyridine-3-carboxylic Acid 8a. Yield: 70%; mp: 146−148 °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), 3.12 (t, 4H, 2,6-CH_2 of piperazine), 4.2 (s, 1H, CH of diphenylmethyl), 7.0−7.18 (m, 9H, Ar-H), 9.26 (s, 1H, C_5-H), 9.56 (s, 1H, C_2-H), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 177.5, 166.3, 161.2, 160.7, 152.7, 142.8, 140.9, 135.4, 131.8, 129.4, 128.3, 126.3, 118.8, 111.5, 73.6, 49.3, 49.6, 36.0, 5.6; Anal. ($C_{29}H_{26}ClN_5O_5$) C, H, N.

1,4-Dihydro-1-(4-fluorophenyl)-7-(4-(2-furoyl)piperazin-1-yl)-6-nitro-4-oxo-1,8-naphthyridine-3-carboxylic Acid 9b. Yield: 76%; mp: 174−176 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 3.16 (t, 4H, 3,5-CH_2 of piperazine), 3.26 (t, 4H, 2,6-CH_2 of piperazine), 6.5−7.68 (m, 7H, Ar-H), 9.26 (s, 1H, C_5-H), 9.56 (s, 1H, C_2-H), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 176.5, 168.7, 167.3, 161.0, 155.2, 152.9, 149.4, 147.1, 145.0, 139.8, 135.0, 119.0, 117.9, 116.3, 113.5, 111.4, 109.3, 48.5, 47.5; Anal. ($C_{24}H_{18}FN_5O_7$) C, H, N.

1-*tert*-Butyl-7-(4-((benzo[*d*][1,3]dioxol-6-yl)methyl)piperazin-1-yl)-1,4-dihydro-6-nitro-4-oxo-1,8-naphthyridine-3-carboxylic Acid 10c. Yield: 75%; mp: 180−182 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 1.76 (s, 9H, *t*-butyl), 2.82 (t, 4H, 3,5-CH_2 of piperazine), 3.1 (t, 4H, 2,6-CH_2 of piperazine), 3.6 (s, 2H, CH_2 of piperanoyl), 5.86 (s, 2H, OCH_2O), 6.42−6.62 (m, 3H, Ar-H), 9.26 (s, 1H, C_5-H), 9.56 (s, 1H, C_2-H), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 177.5, 166.3, 161.2, 160.7, 152.7, 148.5, 147.3, 135.4, 128.9, 122.2, 118.8, 113.9, 111.3, 101.2, 65.0, 60.4, 52.2, 49.0, 28.5; Anal. ($C_{25}H_{27}N_5O_7$) C, H, N.

1-Cyclopropyl-1,4-dihydro-7-(4-methyl-3-phenylpiperazin-1-yl)-6-nitro-4-oxo-1,8-naphthyridine-3-carboxylic Acid 8d. Yield: 75%; mp: 245−247 °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), 4.12 (t, 1H, 3-CH of piperazine), 7.0−7.2 (m, 5H, Ar-H), 9.28 (s, 1H, C_5-H), 9.56 (s, 1H, C_2-H), 14.4 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 177.5, 166.3, 161.2, 160.7, 152.7, 137.4, 135.4, 129.6, 128.5, 127.3, 118.8, 111.7, 63.4, 59.0, 52.2, 49.0, 40.5, 36.0, 5.6; Anal. ($C_{23}H_{23}N_5O_5$) C, H, N.

7-(4-(2,3-Dihydrobenzo[*b*][1,4]dioxin-2-oyl)(piperazin-1-yl)-1,4-dihydro-1-(4-fluorophenyl)-6-nitro-4-oxo-1,8-naphthyridine-3-carboxylic Acid 9e. Yield: 85%; mp: 176−178 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 3.26 (t, 4H, 2,6-CH_2 of piperazine), 3.4 (t, 4H, 3,5-CH_2 of piperazine), 4.6 (d, 2H, 3-CH_2 of dihydrobenzodioxinyl), 5.14 (t, 1H, 2-CH of dihydrobenzodioxinyl), 6.5−6.98 (m, 8H, Ar-H), 9.26 (s, 1H, C_5-H), 9.56 (s, 1H, C_2-H), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 177.5, 168.7, 166.3, 160.7, 152.9, 149.4, 146.7, 139.8, 135.4, 121.0, 118.8, 117.9, 116.3, 115.0, 111.9, 109.3, 85.9, 66.2, 48.5, 47.4; Anal. ($C_{28}H_{22}FN_5O_8$) C, H, N.

1-*tert*-Butyl-7-(3-(2,6-difluorophenyl)-5-methylisoxazol-4-oyl)-(piperazin-1-yl)-1,4-dihydro-6-nitro-4-oxo-1,8-naphthyridine-3-carboxylic Acid 10f. Yield: 70%; mp: >250 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 1.74 (s, 9H, *t*-butyl), 2.3 (s, 3H, 5-CH_3 of isoxazolyl), 3.12 (t, 4H, 3,5-CH_2 of piperazine), 3.28 (t, 4H, 2,6-CH_2 of piperazine), 6.82−7.08 (m, 3H, Ar-H), 9.28 (s, 1H, C_5-H), 9.54 (s, 1H, C_2-H), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 177.5, 173.0, 168.9, 166.3, 161.2, 160.7, 159.8, 152.7, 135.4, 132.4, 118.8, 111.9, 111.3, 65.0, 48.5, 47.1, 28.5, 6.5; Anal. ($C_{28}H_{26}F_2N_6O_7$) C, H, N.

1-Cyclopropyl-1,4-dihydro-6-nitro-4-oxo-7-(4-thiomorpholino)-1,8-naphthyridine-3-carboxylic Acid 8g. Yield: 76%; mp: 200−

202 °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), 9.28 (s, 1H, C_5-H), 9.56 (s, 1H, C_2-H), 14.4 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 177.5, 166.3, 161.2, 160.7, 152.7, 135.4, 118.8, 111.9, 111.3, 50.2, 36.0, 28.2, 5.6; Anal. ($C_{16}H_{16}N_4O_5S$) C, H, N.

1,4-Dihydro-1-(4-fluorophenyl)-7-(2,6-dimethylmorpholino)-6-nitro-4-oxo-1 ,8-naphthyridine-3-carboxylic Acid 9h. Yield: 81%; mp: 105−107 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 1.2 (d, 6H, 2,6 CH_3 of morpholino), 3.0 (d, 4H, 2,6-CH_2 of morpholine), 3.9 (m, 2H, 3,5-CH of morpholine), 6.4−6.7 (m, 4H, Ar-H), 9.26 (s, 1H, C_5-H), 9.56 (s, 1H, C_2-H), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 177.5, 168.7, 166.3, 160.7, 152.9, 149.4, 139.8, 135.4, 118.8, 117.9, 116.3, 111.9, 109.3, 65.4, 69.5, 21.2; Anal. ($C_{21}H_{19}FN_4O_6$) C, H, N.

1-*tert*-Butyl-1,4-dihydro-6-nitro-4-oxo-7-(4-(piperidin-1-yl)piperidin-1-yl)-1,8-naphthyridine-3-carboxylic Acid 10i. Yield: 76%; mp: 154−156 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 1.5−1.6 (m, 10H, 5 CH_2), 1.78 (s, 9H, *t*-butyl), 2.2 (t, 4H, 2 CH_2), 2.7 (m, 1H, CH), 2.8 (t, 4H, 2 CH_2), 9.28 (s, 1H, C_5-H), 9.54 (s, 1H, C_2-H), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 177.5, 166.3, 161.2, 160.7, 152.7, 135.4, 118.8, 111.9, 111.3, 65.0, 58.5, 52.8, 47.1, 28.5, 27.9, 26.7, 25.7; Anal. ($C_{23}H_{31}N_5O_5$) C, H, N.

7-(4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl)-1-cyclopropyl-1,4-dihydro-6-nitro-4-oxo-1,8-naphthyridine-3-carboxylic Acid 8j. Yield: 79%; mp: 162−164 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28−0.46 (m, 4H, cyclopropyl), 1.32 (m, 1H, cyclopropyl), 2.0 (t, 4H, 3,5-CH_2 of piperidine), 2.7 (t, 4H, 2,6-CH_2 of piperidine), 7.1−7.18 (m, 4H, Ar-H), 9.26 (s, 1H, C_5-H), 9.56 (s, 1H, C_2-H), 10.0 (bs, 1H, OH), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 177.5, 166.3, 161.2, 160.7, 152.7, 138.1, 135.4, 131.5, 129.6, 118.8, 111.9, 111.3, 74.5, 42.6, 38.2, 36.0, 5.6; Anal. ($C_{23}H_{21}ClN_4O_6$) C, H, N.

7-(4-(6-Chloro-1,2-dihydro-2-oxobenzo[d]imidazol-3-yl)piperidin-1-yl)-1,4-dihydro-1-(4-fluorophenyl)-6-nitro-4-oxo-1,8-naphthyridone-3-carboxylic Acid 9k. Yield: 78%; mp: >250 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 1.6−2.4 (m, 8H, 4 CH_2 of piperidine), 4.1 (bm, 1H, CH of piperidine), 6.4−6.9 (m, 7H, Ar-H), 9.26 (s, 1H, C_5-H), 9.56 (s, 1H, C_2-H), 10.8 (s, 1H, NH), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 177.5, 168.7, 166.3, 160.7, 152.9, 151.8, 149.4, 139.8, 135.4, 131.3, 130.1, 124.7, 123.4, 122.2, 118.8, 117.9, 116.3, 111.9, 109.3, 50.3, 46.3, 26.9; Anal. ($C_{27}H_{20}ClFN_6O_6$) C, H, N.

1-*tert*-Butyl-7-(3-(diethylcarbamoyl)piperidin-1-yl)-1,4-dihydro-6-nitro-4-oxo-1,8-naphthyridine-3-carboxylic Acid 10l. Yield: 70%; mp: >250 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 1.2 (t, 6H, 2-CH_3 of ethyl), 1.7 (s, 9H, *t*-butyl), 1.78−2.7 (m, 9H, H of piperidine), 3.24 (q, 4H, 2-CH_2 of ethyl), 9.28 (s, 1H, C_5-H), 9.54 (s, 1H, C_2-H), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 177.5, 175.3, 166.3, 161.2, 160.7, 152.7, 135.4, 118.8, 111.9, 111.3, 65.0, 54.4, 51.0, 42.6, 41.3, 28.5, 28.2, 22.3, 12.9; Anal. ($C_{23}H_{31}N_5O_6$) C, H, N.

1-Cyclopropyl-1,4-dihydro-7-(1,4-dioxa-8-azaspiro[4.5]dec-8-yl)-6-nitro-4-oxo-1,8-naphthyridine-3-carboxylic Acid 8m. Yield: 78%; mp: 124−126 °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), 3.96 (m, 4H, 2-CH_2 of azaspirodecane), 9.28 (s, 1H, C_5-H), 9.56 (s, 1H, C_2-H), 14.4 (s, 1H, COOH);); ^{13}C NMR (DMSO-d_6) δ ppm: 177.5, 166.3, 161.2, 160.7, 152.7, 135.4, 118.8, 111.9, 111.3, 109.6, 64.3, 39.8, 36.0, 34.0, 5.6; Anal. ($C_{19}H_{20}N_4O_7$) C, H, N.

7-(1-(*tert*-Butylcarbamoyl)-3,4-dihydroisoquinolin-2(1*H*)-yl)-1,4-dihydro-1-(4-fluorophenyl)-6-nitro-4-oxo-1,8-naphthyridine-3-carboxylic Acid 9n. Yield: 82%; mp: 128−130 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 1.3 (s, 9H, 3 CH_3), 2.66−2.9 (m, 4H, 2 CH_2 of isoquinoline), 4.85 (s, 1H, CH of isoquinoline), 6.7−7.1 (m, 8H, Ar-H), 9.26 (s, 1H, C_5-H), 9.56 (s, 1H, C_2-H), 10.2 (s, 1H, NH), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 177.5, 168.9, 168.7, 166.3, 160.7, 152.9, 149.4, 139.8, 138.1, 135.4, 129.5, 128.6, 127.9, 127.0, 118.8, 117.9, 116.3, 111.9, 109.3, 65.5, 48.3, 47.4, 30.6, 25.9; Anal. ($C_{29}H_{26}FN_5O_6$) C, H, N.

1-*tert*-Butyl-7-(2-carboxy-5,6-dihydroimidazo[1,2-*a*]pyrazin-7(8*H*)-yl)-1,4-dihydro-6-nitro-4-oxo-1,8-naphthyridine-3-carboxylic Acid 10o. Yield: 72%; mp: 212−214 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 1.74 (s, 9H, *t*-butyl), 3.1−3.8 (m, 6H, 3-CH_2), 7.6 (s, 1H, CH), 9.28 (s, 1H, C_5-H), 9.54 (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: 177.5, 167.9, 166.3, 161.2, 160.7, 158.4, 152.7, 139.4, 135.4, 130.6, 118.8, 111.9, 111.3, 65.0, 57.2, 50.5, 37.4, 28.5; Anal. ($C_{20}H_{20}N_6O_7$) C, H, N.

1-Cyclopropyl-1,4-dihydro-7-(8-(4-methoxybenzyl)-3,4,5,6,7,8-hexahydroisoquinolin-2(1*H*)-yl)-6-nitro-4-oxo-1,8-naphthyridine-3-carboxylic Acid 8p. Yield: 77%; mp: 101−103 °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), 3.73 (s, 3H, OCH_3), 6.8−7.1 (m, 4H, Ar-H), 9.28 (s, 1H, C_5-H), 9.56 (s, 1H, C_2-H), 14.4 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 177.5, 166.3, 161.2, 160.7, 158.4, 152.7, 135.4, 131.9, 129.1, 123.8, 118.8, 114.1, 111.9, 111.3, 61.5, 56.4, 42.9, 37.9, 36.0, 30.4, 28.6, 25.5, 24.3, 5.6; Anal. ($C_{29}H_{30}N_4O_6$) C, H, N.

1,4-Dihydro-7-(4,4-dimethyloxazolidin-3-yl)-1-(4-fluorophenyl)-6-nitro-4-oxo-1,8-naphthyridine-3-carboxylic Acid 9q. Yield: 81%; mp: 192−194 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 1.16 (s, 6H, 2-CH_3), 3.41 (s, 2H, 5-CH_2 of oxazolidinyl), 4.6 (s, 2H, 2-CHof oxazolidinyl), 6.4−6.7 (m, 4H, Ar-H), 9.26 (s, 1H, C_5-H), 9.56 (s, 1H, C_2-H), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 177.5, 168.7, 166.3, 160.7, 152.9, 149.4, 139.8, 135.4, 118.8, 117.9, 116.3, 111.9, 84.4, 83.6, 65.5, 22.4; Anal. ($C_{20}H_{17}FN_4O_6$) C, H, N.

MIC Determination. 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.[13] 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.

Cytotoxicity.

All the compounds were further examined for toxicity (IC_{50}) in a mammalian Vero cell line up to concentrations of 62.5 μg/mL[16] by a serial dilution method. 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.

***In Vivo* Studies.**

One compound was tested for efficacy against MTB at a dose of 25 mg/kg in six-week-old female CD-1 mice six per group. In this model, the mice were infected intravenously through caudal vein 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 postinfection, the spleens and right lungs were aseptically removed and ground in a tissue homogenizer, and 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.

DNA Gyrase Supercoiling Assay.

DNA gyrase was purified from *M. smegmatis* cells as described previously.[25] The compounds tested were dissolved in DMSO and preincubated with gyrase. Supercoiling assays were carried out as described previously,[11] by incubating 400 ng of relaxed pUC18 at 37 °C 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 1 h. Moxifloxacin at 5 μg/mL and ciprofloxacin at 10 μg/mL final concentration were used as positive

controls, and a control reaction having 5% DMSO in absence of compounds was also performed. The reaction samples were heat inactivated and applied onto 1% agarose gel after electrophoresis in Tris-acetate-EDTA buffer for 12 h. The gels were stained with ethidium bromide and recorded by gel documentation.

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 photoirradiation period. Eighteen mice were randomly distributed into three dosing groups. The first groups 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 UVA light in a manner adapted from that described previously.[24] Animals were irradiated for 4 h, equal to a total UV light irradiation of approximately 18 J/cm2. 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, edema, or a measurable increase in ear thickness.

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Supporting Information Available: Spectral and elemental data and gel pictures of DNA gyrase inhibition studies of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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