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Original article

Synthesis and antimycobacterial activities of novel 6-nitroquinolone-3-carboxylic acids

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Abstract

Various 1-(substituted)-1,4-dihydro-6-nitro-4-oxo-7-(sub-secondary amino)-quinoline-3-carboxylic acids were synthesized from 2,4-dichlorobenzoic acid by six step synthesis. The compounds were 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 48 synthesized compounds, 7-(4-((benzo[*d*][1,3]dioxol-5-yl)methyl)piperazin-1-yl)-1-cyclopropyl-1,4-dihydro-6-nitro-4-oxoquinoline-3-carboxylic acid (**8c**) was found to be the most active compound *in vitro* with MIC of 0.08 and 0.16 μM against MTB and MDR-TB, respectively. In the *in vivo* animal model **8c** decreased the bacterial load in lung and spleen tissues with 2.78 and 4.15-log 10 protections, respectively, at the dose of 50 mg/kg body weight.

Keywords: Nitroquinolones; Quinolones; Antitubercular; Antimycobacterial; Tuberculosis

1. Introduction

Tuberculosis (TB) is one of the most prevalent infectious diseases known to man. About 32% of the world's population is infected with TB. Every year, approximately 8 million of these infected people develop active TB. It is the leading cause of mortality and morbidity among the infectious diseases with about 2.2 million deaths every year worldwide [1]. In the recent past, TB is receiving the increased attention that this global public health problem deserves. Governments, nongovernmental organizations, and philanthropic organizations have begun investing funds required to control and eventually eliminate this scourge. Until now, progress in TB drug development has been impeded by two major factors: (a) the belief that there was little need for new agents, and (b) the high cost of development coupled with the perception that the potential global market was insufficient to guarantee return on investment. 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. Recently World Health Organization (WHO) recommended third-line regimen for the treatment of TB which includes quinolone antibacterial ofloxacin. Quinolones act by interfering with the action of the bacterial DNA gyrase, resulting in double strand breaks in DNA which in turn leads to the degradation of chromosomal DNA. Breaks in the genome caused by quinolones also leads to termination of chromosomal replication and interference with cell division and gene expression [2]. Various nitroheterocycles like nitrofuranylamides [3,4], 6-nitro-2,3-dihydroimidazo[2,1-*b*]oxazoles [5], nitroimidazopyran [6], 5-nitro-2,3-dihydroimidazo-oxazole [7] and nitroimidazole [8] inhibited *Mycobacterium tuberculosis* H37Rv (MTB) *in vitro* and *in vivo*. Till date few 6-nitroquinolones were studied [9,10] and were mostly bearing identical substituents at positions 1 and 7 of

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the quinolone moiety. Taking into account that quinolones 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 explore the effect of nitro substitution at 6th position of quinolone in combination with N_1 cyclopropyl/*t*-butyl/4-fluorophenyl, with various bulky secondary amino function at 7th position. We report herein the synthesis of newer 1-(substituted)-1,4-dihydro-6-nitro-4-oxo-7-(sub-secondary amino)-quinoline-3-carboxylic acids and evaluation of their antimycobacterial activities together with toxicological results.

2. Synthesis

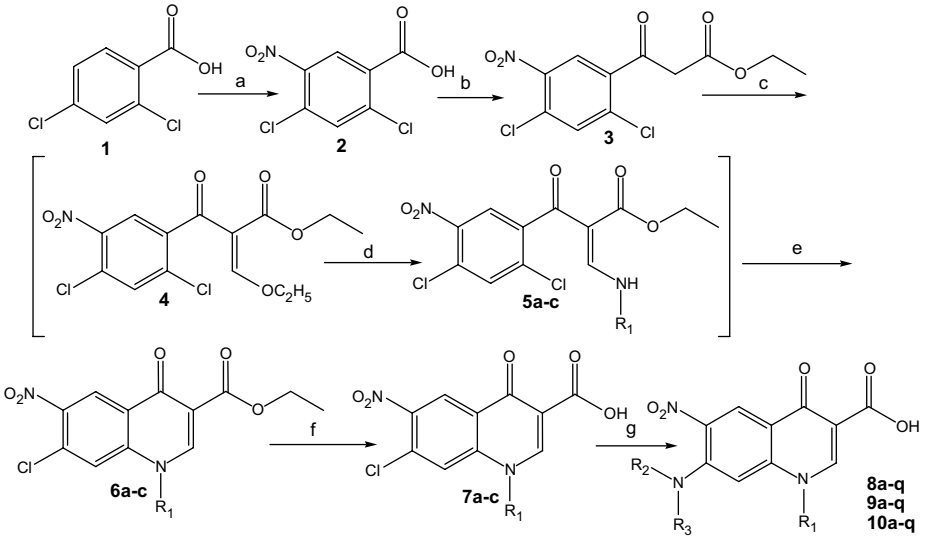
The synthesis of various 6-nitroquinolone (**8–10a–p**) is outlined in Scheme 1. 2,4-Dichlorobenzoic acid (**1**) was converted to 2,4-dichloro-5-nitrobenzoic acid (**2**) by treatment with nitric acid in the presence of sulphuric acid at 0–5 °C. Compound **2** on reaction with 1,1′-carbonyldiimidazole (CDI) 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-dichloro-5-nitrophenyl)-3-oxopropanoate (**3**). Ethyl 1-(cyclopropyl/*t*-butyl/4-fluorophenyl)-7-chloro-6-nitro-4-oxo-1,4-dihydro-quinoloine-3-carboxylates (**6a–c**) were prepared by a three-step one-pot reaction. First treatment of the keto ester **3** with tri-ethylorthoformate in acetic anhydride gave the one-carbon homologue enol ether intermediate ethyl 2-(2,4-dichloro-5-nitrobenzoyl)-3-ethoxyacrylate (**4**), which upon evaporation to dryness was allowed to react with slight excess of appropriate amines under nitrogen atmosphere in a mixture of ether and ethanol at 0 °C gave the enamino ester ethyl 2-(2,4-dichloro-5-nitrobenzoyl)-3-(cyclopropyl/4-fluorophenyl/*t*-butyl amino)acrylate (**5a–c**), and base catalyzed cyclization of **5a–c** with potassium carbonate in DMSO yielded quinolones **6a–c**. Ethyl esters were finally hydrolyzed in acidic condition to yield 1-(cyclopropyl/*t*-butyl/4-fluorophenyl)-1,4-dihydro-7-chloro-6-nitro-4-oxo-quinoline-3-carboxylic acid (**7a–c**). The titled compounds **8–10a–p** were prepared by treating **7a–c** with appropriate secondary amines under microwave irradiation in DMSO in closed system. When compared to conventional method [11] of 24 h process, microwave assisted synthesis was performed with short reaction times (2–3 min), with ease and was environment 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. In general, infrared spectra (IR) showed C=O stretching peak of carboxylic acid at 1720–1730 cm^{-1}; C=O stretching peak of pyridine carbonyl at 1612–1620 cm^{-1} and absorption of aryl nitro group at 1470–1370 cm^{-1}. In the nuclear magnetic resonance spectra (^{1}H NMR) the signals of the respective protons of the prepared derivatives were verified on the basis of their chemical shifts, multiplicities and coupling constants. The spectra showed a broad singlet at δ 14.6 ppm corresponding to COOH proton; singlet at δ 9.52 ppm corresponding to C_2 proton; singlet at δ 8.3 ppm corresponding to C_8 proton; singlet at δ 9.34 ppm corresponding to C_5 proton; N_1 cyclopropyl protons showed multiplets at δ 1.42 (1H) and 0.28–0.48 (4H) ppm; N_1 *t*-butyl protons showed singlet at δ 1.62 (9H) ppm; and N_1 4-fluorophenyl protons showed multiplet at δ 6.50–6.72 (4H) ppm. The elemental analysis results were within ±0.4% of the theoretical values.

3. Results and discussion

3.1. Antimycobacterial activity

The compounds were screened for their *in vitro* antimycobacterial activity against MTB, MDR-TB and *Mycobacterium*



Scheme 1. (a) HNO_3, H_2SO_4; (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.

smegmatis ATCC 14468 (MC^2) by agar dilution method 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 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 reported in Table 1.

In the first phase of screening against MTB, all the compounds showed good *in vitro* activity against MTB with MIC less than 15 μM. Five compounds (**8b–d**, **8i** and **8j**) inhibited MTB with MIC of less than 1 μM and were relatively more potent than standard fluoroquinolone gatifloxacin (MIC: 1.04 μM). When compared to isoniazid (MIC: 0.36 μM), two compounds (**8c** and **8d**) were found to be more active against MTB. Compound 7-(4-((benzo[*d*][1,3]dioxol-5-yl)methyl)piperazin-1-yl)-1-cyclopropyl-1,4-dihydro-6-nitro-4-oxoquinoline-3-carboxylic acid (**8c**) was found to be the most active compound *in vitro* with MIC of 0.08 μM against MTB and was 4.50 and 13 times more potent than isoniazid and gatifloxacin, respectively. Subsequently some of the compounds were evaluated against MDR-TB, and among the 18 compounds screened, all the compounds inhibited MDR-TB with MIC ranging from 0.16 to 6.01 μM and were found to be more active than isoniazid (MIC: 45.57 μM), and gatifloxacin (MIC: 8.34 μM). Eight compounds (**8b–d**, **9e**, **10f**, **8i**, **8j** and **8o**) inhibited MDR-TB with MIC of less than 1 μM. Compound **8c** was found to be the most active compound *in vitro* with MIC of 0.16 μM against MDR-TB and was 52 and 284 times more potent than gatifloxacin and isoniazid, respectively. The compounds were also evaluated against MC^2 strain in which all the compounds inhibited the bacteria with MIC ranging from 0.79 to 64.36 μM and 45 compounds were found to be more active than isoniazid (MIC: 45.57 μM).

With respect to structure–MTB activity relationship, the results demonstrated that the antimycobacterial activity imparted by the in N_1 substituent was in the order of cyclopropyl > 4-flurophenyl > *tert*-butyl. This result correlated with the other antibacterial fluoroquinolones, wherein cyclopropyl group was the favorable substituent. This result also correlated well with the previous report on the importance of cyclopropyl group as antimycobacterial pharmacophore [12]. The enhanced activity of the cyclopropyl group could be supported by a hypothesis based on the belief that the antibacterial activity of quinolones is related to the amount of un-ionized drug that is able to penetrate the cell membranes. This in turn is associated with a highly acidic carboxyl group and a less basic C_7 amino substituent [13]. The carboxyl group becomes more acidic if the N_1 substituent is electron donating in nature. The greater activity of cyclopropyl-substituted quinolones was therefore associated with the electron donating effect of this moiety. At C_7 position we have studied with various substituted piperazines (**8–10a–f**), (thio) morpholines (**8–10g** and **10h**), substituted piperidines (**8–10i–l**), fused piperazines and piperidines (**8–10m–p**). A comparison of the substitution pattern at C_7 demonstrated that the order of activity was substituted piperazines > substituted piperidines > fused piperazines and piperidines ≥ (thio) morpholines. The results demonstrated that the contribution of the C_7 position to antimycobacterial activity was dependent on the substituent at N_1 and was in the order of substituted piperazines > substituted piperidines > fused piperazines and piperidines > (thio) morpholines when N_1 was cyclopropyl; substituted piperazines ≥ substituted piperidines and (thio) morpholines > fused piperazines and piperidines when N_1 was 4-fluorophenyl; substituted piperazines ≥ substituted piperidines, fused piperazines and piperidines and (thio) morpholines when N_1 was *tert*-butyl.

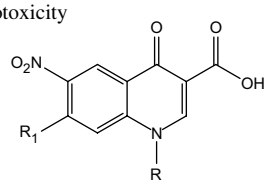
Some compounds were further examined for toxicity (CC_{50}) in a mammalian Vero cell line till 62.50 μg/ml concentrations. After 72 h of exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product and the results are reported in Table 1. Nine compounds when tested showed CC_{50} values ranging from 54.39 to 142.30 μM. A comparison of the substitution pattern at N_1 demonstrated that 4-fluorophenyl group was more cytotoxic than the cyclopropyl group. These results are important as the N_1 phenyl 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. Compound **8c** was found to be non-toxic up to 62.50 μg/ml (126.90 μM) and showed selectivity index (CC_{50}/MIC) of more than 1586.

Subsequently, compound **8c** was tested for *in vivo* efficacy against MTB at the dose of 50 mg/kg (Table 2) in CD-1 mice. The mice were infected intravenously with *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 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 **8c** decreased the bacterial load in lung and spleen tissues with 2.78 and 4.15-log 10 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 **8c** decreased the bacterial load with 0.81 and 2.05-log 10 protections in lung and spleen tissues, respectively. Compound **8c** was found to be less active than isoniazid in the *in vivo* study on dose basis.

3.2. Inhibition of DNA gyrase activity

The 6-nitroquinolone-3-carboxylic acid derivatives described above were evaluated for their ability to inhibit DNA supercoiling activity of DNA gyrase isolated from *M. smegmatis*. Quinolones and fluoroquinolones are synthetic compounds which inhibit bacterial type II DNA topoisomerases. These enzymes facilitate DNA replication, transcription and other DNA transaction processes. They do so by transiently creating a double strand break in the DNA followed by religation of the broken strands after the passage of intact duplex DNA through

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



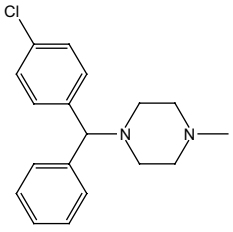
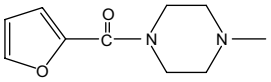
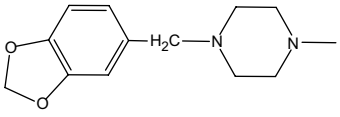
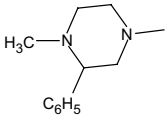
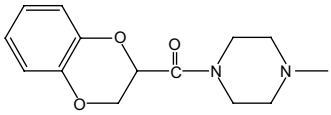
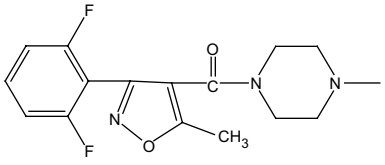
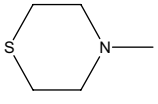
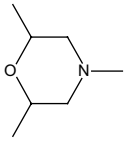
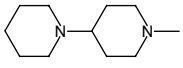
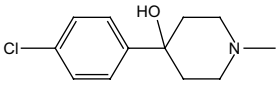
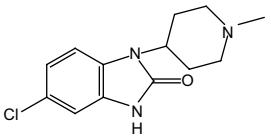
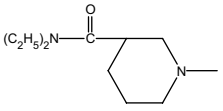
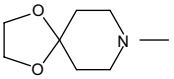
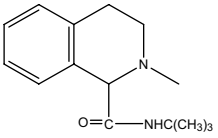
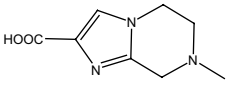
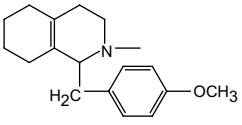
No.	R	R_1	Yield (%)	M.p. (°C)	CC_{50} (μM)	MIC (μM)		
						MTB	MDRTB	MC[2]
8a	Cyclopropyl		68	192	NT	5.59	NT	5.59
9a	4-F-phenyl		62	134	NT	10.19	NT	40.78
10a	*t*-Butyl		71	128	NT	5.44	NT	10.87
8b	Cyclopropyl		73	237	111.2	0.69	0.34	1.39
9b	4-F-Phenyl		66	127	NT	6.18	NT	24.68
10b	*t*-Butyl		68	222	NT	6.68	NT	13.34
8c	Cyclopropyl		73	148	126.9	0.08	0.16	0.79
9c	4-F-phenyl		80	108	NT	2.85	0.71	22.87
10c	*t*-Butyl		71	182	NT	3.07	1.53	24.58
8d	Cyclopropyl		69	198	136.36	0.20	0.20	0.87
9d	4-F-phenyl		80	126	NT	6.23	NT	24.88
10d	*t*-Butyl		67	147	NT	16.09	NT	64.36
8e	Cyclopropyl		70	134	NT	2.99	6.01	12.01
9e	4-F-phenyl		83	70	54.39	2.72	0.68	10.88
10e	*t*-Butyl		71	231	NT	5.83	NT	23.29
8f	Cyclopropyl		70	241	NT	2.69	2.69	10.79
9f	4-F-phenyl		84	223	NT	4.94	NT	19.73
10f	*t*-Butyl		81	>250	104.9	2.62	0.65	10.49
8g	Cyclopropyl		77	208	NT	4.17	NT	33.29
9g	4-F-phenyl		81	114	NT	14.55	NT	29.11
10g	*t*-Butyl		71	>250	NT	7.99	NT	31.93
8h	Cyclopropyl		73	172	NT	2.01	4.03	8.08
9h	4-F-phenyl		84	103	70.79	3.53	1.77	14.16
10h	*t*-Butyl		74	108	NT	7.76	NT	30.98
8i	Cyclopropyl		74	173	141.9	0.43	0.43	1.77
9i	4-F-Phenyl		81	108	NT	6.33	NT	25.28
10i	*t*-Butyl		78	156	NT	6.86	NT	27.41

Table 1 (*continued*)

No.	R	R_1	Yield (%)	M.p. (°C)	CC_{50} (μM)	MIC (μM)		
						MTB	MDRTB	MC^2
8j	Cyclopropyl		70	197	129.2	0.81	0.39	3.22
9j	4-F-Phenyl		76	145	NT	5.82	NT	23.24
10j	*t*-Butyl		75	127	NT	6.26	NT	25.00
8k	Cyclopropyl		67	209	NT	1.49	2.97	2.97
9k	4-F-Phenyl		78	181	NT	5.42	NT	43.26
10k	*t*-Butyl		69	>250	NT	5.79	NT	23.11
8l	Cyclopropyl		72	174	NT	3.42	3.42	6.86
9l	4-F-phenyl		73	75	NT	6.13	NT	24.49
10l	*t*-Butyl		70	152	NT	6.62	NT	26.45
8m	Cyclopropyl		64	182	NT	3.76	1.88	15.05
9m	4-F-phenyl		69	110	NT	6.67	NT	26.63
10m	*t*-Butyl		71	136	NT	7.45	NT	28.97
8n	Cyclopropyl		75	206	NT	1.55	1.55	3.09
9n	4-F-phenyl		82	112	NT	5.60	NT	22.38
10n	*t*-Butyl		72	135	NT	12.01	NT	24.01
8o	Cyclopropyl		78	210	142.2	1.78	0.43	7.12
9o	4-F-phenyl		84	179	NT	12.67	NT	50.67
10o	*t*-Butyl		72	>250	NT	6.87	NT	54.89
8p	Cyclopropyl		71	208	NT	3.04	NT	24.34
9p	4-F-phenyl		76	114	NT	5.36	NT	21.42
10p	*t*-Butyl		73	69	NT	5.74	NT	22.91
Gati	–	–	–	–	>155.3	1.04	8.34	2.08
INH	–	–	–	–	>455.8	0.36	45.57	45.57

NT indicates not tested.

the cleaved enzyme–DNA complex. This leads to the change in linking number of DNA in an ATP dependent reaction cycle [14]. DNA gyrase, an essential enzyme in bacteria, is unique in catalyzing the negative supercoiling of DNA and as a result, the enzyme is vital for almost all cellular processes that involve duplex DNA. In all the species of mycobacteria including *M. tuberculosis*, DNA gyrase is the sole type II topoisomerase and topoisomerase IV is not found in these genomes. Our earlier studies have revealed that DNA gyrase from *M. tuberculosis* and *M. smegmatis* are highly similar in antigenic and biochemical properties [15]. The supercoiling assay results with various compounds using *M. smegmatis* DNA gyrase are presented in Fig. 1a and b. The IC_{50} values presented in Table 3 show that compounds **8b**, **8j**, **8i**, **8n**, **8h**, **8k**, **8c**, **8d** and **10f** inhibit DNA gyrase at 30 μg/ml concentration. Compounds **9e**, **9h** and **10b** have IC_{50} of 20 μg/ml and compound **10a** inhibits the enzyme activity with an IC_{50} value as low as 5 μg/ml.

3.3. Phototoxic evaluation

Quinolones in general have favorable safety profiles; phototoxicity has become a significant factor in the clinical use of some. Phototoxicity with the quinolones is generally thought to result from the absorption of light by the parent compound or a metabolite in tissue. Three (**8c**, **8d** and **8i**) compounds were evaluated for potential phototoxicity in a standardized *in vivo* test system that has been used previously to assess quinolone antibiotics. 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

Table 2
In vivo activity data of **8c** 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
8c (50 mg/kg)	5.21 ± 0.17	4.87 ± 0.13

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 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 or not toxic except **8c** (72 h) and **8i** (72 and 96 h) when compared with the negative (vehicle-treated) and positive controls (lomefloxacin hydrochloride). No erythema occurred in mice dosed with 140 mg/kg of **8c** and **8d** throughout the 96 h study, while compound **8i** showed a significant erythema after irradiation at the 72 h time point only while lomefloxacin hydrochloride showed erythema after irradiation throughout the period of testing.

4. Conclusion

Screening of the antimycobacterial activity of these novel series identified newer potent antitubercular 6-nitroquinolone-3-carboxylic acids endowed with high activity toward MDR-TB, with MIC99 ranging from 0.16 to 6.01 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.

5. Experimental protocols

5.1. Chemistry

Melting points were determined in open capillary tubes on a Buchi 530 melting point apparatus and are uncorrected.

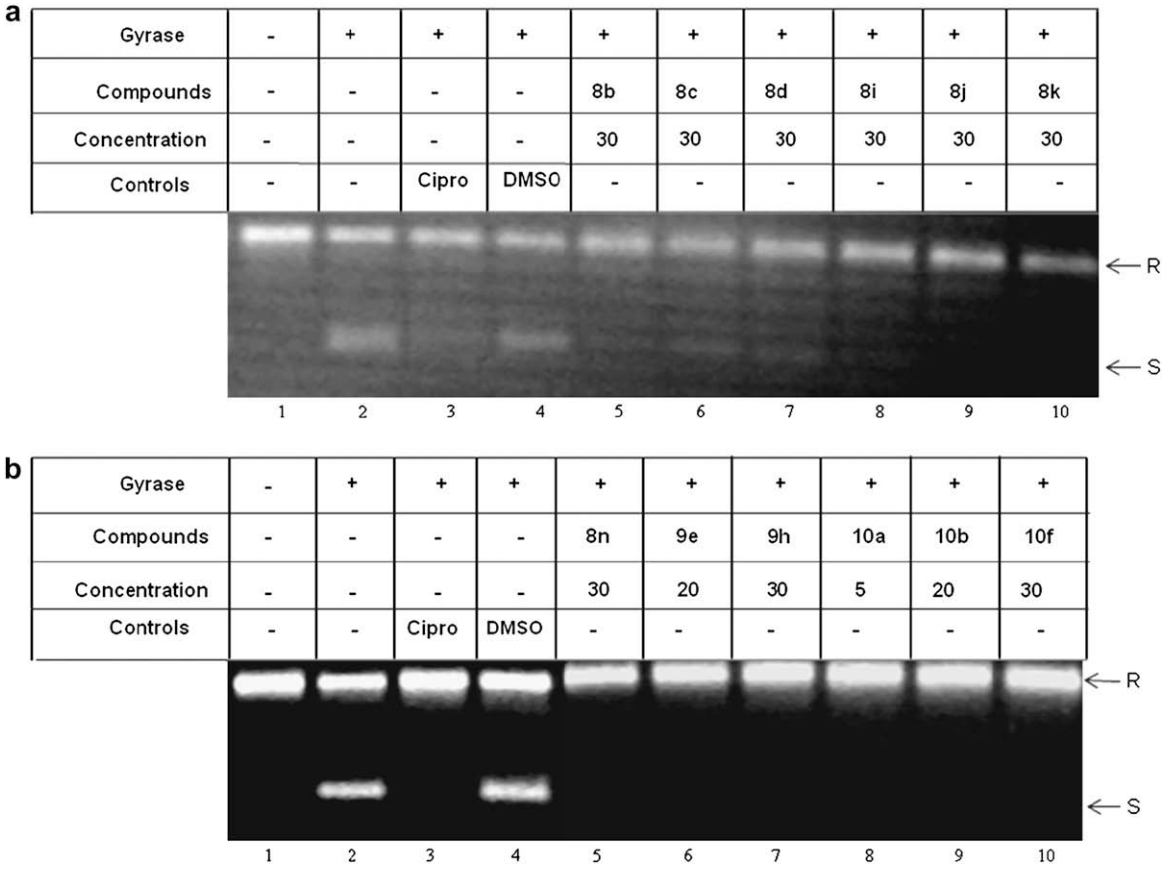


Fig. 1. DNA gyrase supercoiling assay. The assays were performed as described in Section 5. DNA gyrase was incubated with indicated concentrations of the compounds before the addition of rest of the components. (a) Lane 1: relaxed DNA, lane 2: supercoiling reaction, lane 3: supercoiling reaction in the presence of 5% DMSO. Ciprofloxacin at concentration of 10 μg/ml (lane 4) was used as the positive control for inhibition of enzyme. Lanes 5–10 have 30 μg/ml concentrations of compounds **8b–d**, **8i–k**, respectively. (b) Lane 1: relaxed DNA, lane 2: supercoiling reaction, lane 3: supercoiling reaction in the presence of 5% DMSO. Ciprofloxacin at concentration of 10 μg/ml (lane 4) was used as the positive control for inhibition of enzyme. Lane 5: 30 μg/ml **8n**, lane 6: 20 μg/ml **9e**, lane 7: 30 μg/ml **9h**, lane 8: 5 μg/ml **10a**, lane 9: 20 μg/ml **10b** and lane 10: 30 μg/ml **10f**. R and S indicate relaxed and supercoiled pUC18 DNA, respectively.

Table 3
IC_{50} values for DNA gyrase inhibition

Compounds	IC_{50} (μg/ml)
8b	30
8c	30
8d	30
8i	30
8j	30
8k	30
8n	30
9e	20
9h	20
10a	5
10b	20
10f	30

Infrared (IR) and proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded for the compounds on JASCO IR Report 100 (KBr) and Brucker Avance (300 MHz) instruments, respectively. Chemical shifts are reported in parts per million (ppm) using tetramethyl silane (TMS) as an internal standard. All exchangeable protons were confirmed by the addition of D_2O. ^{13}C NMR spectra were recorded on Bruker AC 200/DPX 400 MHz. Elemental analyses (C, H, and N) were undertaken with Perkin–Elmer model 240C analyzer and all analyses were consistent with theoretical values (within ±0.4%) unless indicated. The homogeneity of the compounds was monitored by ascending thin layer chromatography (TLC) on silica gel-G (Merck)-coated aluminium plates, visualized by iodine vapor and UV light. Microwave oven used was Catalyst system, India.

*5.1.1. Synthesis of 2,4-dichloro-5-nitrobenzoic acid (**2**)*

To a solution of 2,4-dichlorobenzoic acid (**1**) (1.00 equiv, 1.91 g) in 96% sulphuric acid (40 ml), 65% nitric acid (1.50 equiv, 0.95 g) was added slowly at 0–5 °C and the resulting mixture was then stirred at room temperature for 1 h. The mixture is then poured into ice-cold water and extracted with ethyl acetate (3 × 50 ml). The combined extracts were dried over magnesium sulphate and evaporated under reduced pressure to yield **2**.

Yield: 98%; m.p.: 138 °C; IR (KBr) cm^{-1}: 2900, 1726, 1470–1370; ^{1}H NMR (DMSO-d_6) δ ppm: 7.80 (s, 1H, ArH), 8.84 (s, 1H, Ar–H), 14.60 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 169.4, 146.9, 141.8, 136.6, 132.0, 130.9, 128.6; Anal ($C_7H_3Cl_2NO_4$) C, H, N.

*5.1.2. Synthesis of ethyl 3-(2,4-dichloro-5-nitrophenyl)-3-oxopropanoate (**3**)*

To a solution of **2** (1.00 equiv, 2.36 g) in tetrahydrofuran, CDI (1.20 equiv, 1.94 g) was added for 30 min at the temperature of 70 °C. The resulting crude imidazolide was used without further purification in the next step. To a solution of potassium salt of ethyl malonate (1.30 equiv, 2.21 g) in acetonitrile was added dropwise magnesium chloride (2.00 equiv, 1.90 g) and tri-ethyl amine (4.0 equiv, 4.04 g) at 0 °C and stirred at room temperature for 2.5 h. To this solution, the imidazolide prepared above was added and the reaction mixture was refluxed at 70 °C for 2.5 h. After completion of reaction, the solvent was distilled off and poured into ice water and acidified to pH 5–6 with 20% HCl, then extracted with ethyl acetate (3 × 25 ml) and dried over magnesium sulphate and distilled the solvent to give **3** (yield: 82%; m.p.: 141 °C).

Yield: 82%; m.p.: 141 °C; IR (KBr) cm^{-1}: 2900, 1726, 1705, 1470–1370; ^{1}H NMR (DMSO-d_6) δ ppm: 1.45 (t, 3H, $-CH_2CH_3$), 3.40 (s, 2H, CH_2), 4.2 (m, 2H, $-CH_2CH_3$), 7.66 (s, 1H, Ar–H), 8.64 (s, 1H, Ar–H); ^{13}C NMR (DMSO-d_6) δ ppm: 194.3, 168.4, 146.9, 141.8, 137.2, 136.6, 134.2, 132.0, 130.9, 128.6, 126.3, 61.3, 50.6, 14.5; Anal ($C_{11}H_9Cl_2NO_5$) C, H, N.

*5.1.3. Synthesis of ethyl 1-(substituted)-7-chloro-6-nitro-4-oxo-1,4-dihydro-quinoloine-3-carboxylates (**6a–c**)*

A mixture of **3** (1.00 equiv, 3.06 g), tri-ethylorthoformate (1.50 equiv, 2.18 g) and acetic anhydride (2.50 equiv, 2.55 g) 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-(2,4-dichloro-5-nitrobenzoyl)-3-ethoxyacrylate (**4**). The above residue was dissolved in a mixture of ether (20 ml) and ethanol (20 ml). Added corresponding primary amine (1.10 equiv) at 0 °C and stirred for 30 min under nitrogen atmosphere, followed by distillation yielded ethyl 2-(2,4-dichloro-5-nitrobenzoyl)-3-(cyclopropyl/4-fluorophenyl/*t*-butyl amino)acrylate (**5a–c**). The above crude solid (1.00 equiv) was dissolved in DMSO (30 ml) and added anhydrous potassium carbonate (1.60 equiv) and refluxed at 60 °C for 3 h. After completion of reaction, the mixture

Table 4
Phototoxic evaluation of the titled compounds

Group	Ear thickness (mm)[a]						Erythema[b]					
	Time (approximately) after start of irradiaton (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
8c	0.25 ± 0.01	0.27 ± 0.01	0.28 ± 0.003	0.28 ± 0.01	0.31 ± 0.01	0.29 ± 0.02	0	0	0	0	0	0
8d	0.30 ± 0.02	0.31 ± 0.02	0.30 ± 0.01	0.32 ± 0.02	0.32 ± 0.01	0.34 ± 0.02	0	0	2[e]	0	0	0
8i	0.24 ± 0.01	0.26 ± 0.02	0.26 ± 0.01	0.26 ± 0.01	0.29 ± 0.01	0.28 ± 0.01	0	0	0	0	3	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 UVA light immediately after dosing); 4 h = end of irradiation period.
[d] Control = 0.5% aqueous solution of sodium carboxymethylcellulose (4 N s/m^2) dosed at 10 ml/kg.
[e] Very slight erythema, not considered to be drug-related.

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 30% ethylacetate:hexane mixture to yield ethyl 1-(substituted)-7-methoxy-6-nitro-4-oxo-1,4-dihydro-quinoloine-3-carboxylates (**6a–c**).

*5.1.3.1. Ethyl 1-(cyclopropyl)-7-chloro-6-nitro-4-oxo-1,4-dihydro-quinoloine-3-carboxylates (**6a**).* Yield: 90%; m.p.: >280 °C; IR (KBr) cm^{-1}: 2900, 1726, 1705, 1616, 1470–1370; ^1H NMR (DMSO-d_6) δ ppm: 0.28–0.44 (m, 4H, cyclopropyl), 1.36 (m, 1H, cyclopropyl), 1.45 (t, 3H, $-CH_2CH_3$), 4.2 (m, 2H, $-CH_2CH_3$), 8.3 (s, 1H, C_8–H), 9.34 (s, 1H, C_5–H), 9.52 (s, 1H, C_2–H); ^{13}C NMR (DMSO-d_6) δ ppm: 177.5, 165.5, 151.6, 145.3, 135.5, 137.7, 127.1, 126.1, 115.3, 110.2, 61.4, 36.2, 14.2, 5.6; Anal ($C_{13}H_9ClN_2O_5$) C, H, N.

*5.1.3.2. Ethyl 1-(4-fluorophenyl)-7-methoxy-6-nitro-4-oxo-1,4-dihydro-quinoloine-3-carboxylates (**6b**).* Yield: 92%; m.p.: >280 °C; IR (KBr) cm^{-1}: 2900, 1726, 1705, 1616, 1470–1370; ^1H NMR (DMSO-d_6) δ ppm: 1.45 (t, 3H, $-CH_2CH_3$), 4.2 (m, 2H, $-CH_2CH_3$), 6.48–6.68 (m, 4H, Ar-H), 8.3 (s, 1H, C_8–H), 9.34 (s, 1H, C_5–H), 9.52 (s, 1H, C_2–H); ^{13}C NMR (DMSO-d_6) δ ppm: 177.5, 165.5, 152.4, 151.6, 145.3, 135.5, 136.3, 137.7, 127.1, 126.1, 120.8, 116.4, 115.3, 110.2, 61.4, 14.2; Anal ($C_{13}H_9ClN_2O_5$) C, H, N.

*5.1.3.3. Ethyl 1-(t-butyl)-7-methoxy-6-nitro-4-oxo-1,4-dihydro-quinoloine-3-carboxylates (**6c**).* Yield: 86%; m.p.: 276 °C; IR (KBr) cm^{-1}: 2900, 1726, 1705, 1616, 1470–1370; ^1H NMR (DMSO-d_6) δ ppm: 1.45 (t, 3H, $-CH_2CH_3$), 1.6 (s, 9H, *t*-butyl), 4.2 (m, 2H, $-CH_2CH_3$), 8.3 (s, 1H, C_8–H), 9.34 (s, 1H, C_5–H), 9.52 (s, 1H, C_2–H); ^{13}C NMR (DMSO-d_6) δ ppm: 177.5, 165.5, 151.6, 145.3, 136.3, 137.7, 127.1, 126.1, 115.3, 110.2, 65.1, 61.4, 28.5, 14.2; Anal ($C_{13}H_9ClN_2O_5$) C, H, N.

*5.1.4. Synthesis of 1-(substituted)-1,4-dihydro-7-chloro-6-nitro-4-oxo-quinoline-3-carboxylic acid (**7a–c**)*

Compound **6a–c** (1.00 equiv) was suspended in 6 N HCl (5 ml) and refluxed for 6 h and then cooled to 0 °C. The precipitate obtained was filtered, washed with water followed by 20% ethyl acetate yielded **7a–c**.

*5.1.4.1. 7-Chloro-1-cyclopropyl-1,4-dihydro-6-nitro-4-oxoquinoline-3-carboxylic acid (**7a**).* Yield: 82%; m.p.: 249 °C; IR (KBr) cm^{-1}: 2900, 1726, 1705, 1616, 1470–1370; ^1H NMR (DMSO-d_6) δ ppm: 0.28–0.44 (m, 4H, cyclopropyl), 1.36 (m, 1H, cyclopropyl), 8.3 (s, 1H, C_8–H), 9.34 (s, 1H, C_5–H), 9.52 (s, 1H, C_2–H), 14.60 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 177.5, 166.3, 151.6, 148.1, 137.7, 135.5, 127.0, 126.1, 115.3, 109.3, 36.3, 5.6; Anal ($C_{13}H_9ClN_2O_5$) C, H, N.

*5.1.4.2. 7-Chloro-1-(4-fluorophenyl)-1,4-dihydro-6-nitro-4-oxoquinoline-3-carboxylic acid (**7b**).* Yield: 86%; m.p.: >280 °C; IR (KBr) cm^{-1}: 2900, 1728, 1710, 1620, 1460–1370; ^1H NMR (DMSO-d_6) δ ppm: 6.48–6.68 (m, 4H, Ar-H), 8.3 (s, 1H, C_8–H), 9.34 (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, 166.3, 152.4, 151.6, 148.1, 145.3, 137.7, 135.5, 127.0, 126.1, 120.8, 116.4, 115.3, 109.3; Anal ($C_{16}H_8ClFN_2O_5$) C, H, N.

*5.1.4.3. 1-tert-Butyl-7-chloro-1,4-dihydro-6-nitro-4-oxoquinoline-3-carboxylic acid (**7c**).* Yield: 85%; m.p.: 245 °C; IR (KBr) cm^{-1}: 2900, 1726, 1705, 1620, 1470–1370; ^1H NMR (DMSO-d_6) δ ppm: 1.6 (s, 9H, *t*-butyl), 8.3 (s, 1H, C_8–H), 9.34 (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, 151.6, 148.1, 145.3, 137.7, 136.5, 127.0, 126.1, 115.3, 109.3, 65.1, 28.5; Anal ($C_{14}H_{13}ClN_2O_5$) C, H, N.

*5.1.5. Synthesis of 1-(substituted)-1,4-dihydro-6-nitro-7-(substituted secondary amines)-4-oxoquinoline-3-carboxylic acid (**8–10a–p**)*

Compound **7a–c** (1.0 equiv) in dimethyl sulphoxide (2.5 ml) and appropriate secondary amines (1.1 equiv) were irradiated in a microwave oven at an intensity of 80% (560 W) 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 isopropanol to give titled products.

*5.1.5.1. 7-(4-((4-Chlorophenyl)(phenyl)methyl)piperazin-1-yl)-1-cyclopropyl-1,4-dihydro-6-nitro-4-oxoquinoline-3-carboxylic acid (**8a**).* Yield: 68%; m.p.: 192 °C; IR (KBr) cm^{-1}: 2900, 1724, 1708, 1620, 1460–1370; ^1H NMR (DMSO-d_6) δ ppm: 0.28–0.48 (m, 4H, cyclopropyl), 1.35 (m, 1H, cyclopropyl), 2.59–3.12 (m, 8H, CH_2 of piperazine), 4.2 (s, 1H, CH of diphenylmethyl), 7.0–7.18 (m, 9H, Ar-H), 8.32 (s, 1H, C_8–H), 9.34 (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, 151.1, 148.1, 146.2, 142.8, 140.9, 131.8, 129.7, 129.3, 128.3, 126.4, 121.9, 117.5, 109.3, 99.3, 73.6, 49.6, 49.3, 36.0, 5.6; Anal ($C_{30}H_{27}ClN_4O_5$) C, H, N.

*5.1.5.2. 1-t-Butyl-7-(4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)-1,4-dihydro-6-nitro-4-oxoquinoline-3-carboxylic acid (**9a**).* Yield: 62%; m.p.: 134 °C; IR (KBr) cm^{-1}: 2900, 1724, 1708, 1620, 1460–1370; ^1H NMR (DMSO-d_6) δ ppm: 2.59–3.12 (m, 8H, CH_2 of piperazine), 4.2 (s, 1H, CH of diphenylmethyl), 6.3–7.18 (m, 13H, Ar-H), 8.32 (s, 1H, C_8–H), 9.34 (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, 152.4, 149.4, 147.7, 146.3, 142.8, 140.9, 131.8, 136.3, 129.7, 129.3, 128.3, 126.6, 126.3, 123.1, 120.7, 116.4, 109.3, 104.9, 73.6, 49.6, 49.3; Anal ($C_{33}H_{26}ClFN_4O_5$) C, H, N.

*5.1.5.3. 7-(4-((4-Chlorophenyl)(phenyl)methyl)piperazin-1-yl)-1,4-dihydro-1-(4-fluorophenyl)-6-nitro-4-oxoquinoline-3-carboxylic acid (**10a**).* Yield: 71%; m.p.: 128 °C; IR (KBr) cm^{-1}: 2900, 1724, 1708, 1620, 1460–1370; ^1H NMR (DMSO-d_6) δ ppm: 1.76 (s, 9H, *t*-butyl), 2.59–3.12 (m, 8H, CH_2 of piperazine), 4.2 (s, 1H, CH of diphenylmethyl), 7.0–7.18 (m, 9H, Ar-H), 8.32 (s, 1H, C_8–H), 9.34 (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, 151.1, 148.1, 146.2, 142.8, 140.9, 131.8, 129.7, 129.3, 128.3, 126.4, 121.9, 117.5, 109.3, 99.3, 73.6, 65.0, 49.6, 49.3, 28.5; Anal ($C_{31}H_{31}ClN_4O_5$) C, H, N.

*5.1.5.4. 1-(Cyclopropyl)-7-(4-(2-furoyl)piperazin-1-yl)-1,4-dihydro-6-nitro-4-oxoquinoline-3-carboxylic acid (**8b**).* Yield: 73%; m.p.: 237 °C; IR (KBr) cm^{-1}: 2890, 1724, 1710, 1624, 1460–1360; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28–0.48 (m, 4H, cyclopropyl), 1.35 (m, 1H, cyclopropyl), 3.16–3.3 (m, 8H, CH_2 of piperazine), 7.1–7.48 (m, 3H, Ar-H), 8.34 (s, 1H, C_8–H), 9.32 (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, 155.2, 151.1, 148.1, 147.1, 146.2, 126.5, 121.9, 117.5, 113.5, 111.7, 109.3, 99.3, 48.5, 47.5, 36.0, 5.6; Anal ($C_{22}H_{20}N_4O_7$) C, H, N.

*5.1.5.5. 1-(4-Fluorophenyl)-7-(4-(2-furoyl)piperazin-1-yl)-1,4-dihydro-6-nitro-4-oxoquinoline-3-carboxylic acid (**9b**).* Yield: 66%; m.p.: 127 °C; IR (KBr) cm^{-1}: 2890, 1724, 1710, 1624, 1460–1360; ^{1}H NMR (DMSO-d_6) δ ppm: 3.16–3.3 (m, 8H, CH_2 of piperazine), 6.5–7.68 (m, 7H, Ar-H), 8.34 (s, 1H, C_8–H), 9.32 (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, 155.2, 152.4, 149.4, 147.1, 147.7, 146.3, 146.0, 136.3, 126.6, 123.1, 120.7, 116.4, 113.5, 111.7, 109.3, 104.9, 48.5, 47.5; Anal ($C_{25}H_{19}FN_4O_7$) C, H, N.

*5.1.5.6. 1-tert-Butyl-7-(4-(2-furoyl)piperazin-1-yl)-1,4-dihydro-6-nitro-4-oxoquinoline-3-carboxylic acid (**10b**).* Yield: 68%; m.p.: 222 °C; IR (KBr) cm^{-1}: 2890, 1724, 1710, 1624, 1460–1360; ^{1}H NMR (DMSO-d_6) δ ppm: 1.76 (s, 9H, *t*-butyl), 3.16–3.3 (m, 8H, CH_2 of piperazine), 7.1–7.48 (m, 3H, Ar-H), 8.34 (s, 1H, C_8–H), 9.32 (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, 155.2, 151.1, 148.1, 147.1, 146.1, 126.5, 121.9, 117.5, 113.5, 111.7, 109.3, 99.3, 65.0, 48.5, 47.5, 28.5; Anal ($C_{23}H_{24}N_4O_7$) C, H, N.

*5.1.5.7. 7-(4-((Benzo[d][1,3]dioxol-5-yl)methyl)piperazin-1-yl)-1-cyclopropyl-1,4-dihydro-6-nitro-4-oxoquinoline-3-carboxylic acid (**8c**).* Yield: 73%; m.p.: 148 °C; IR (KBr) cm^{-1}: 3250, 2890, 1724, 1710, 1624, 1464–1360; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28–0.48 (m, 4H, cyclopropyl), 1.35 (m, 1H, cyclopropyl), 2.82–3.12 (m, 8H, 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), 8.34 (s, 1H, C_8–H), 9.34 (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, 151.1, 148.6, 148.1, 147.8, 146.2, 128.9, 126.5, 122.2, 121.9, 117.5, 115.0, 113.9, 109.3, 101.3, 99.3, 60.4, 52.2, 49.0, 36.0, 5.6; Anal ($C_{25}H_{24}N_4O_7$) C, H, N.

*5.1.5.8. 7-(4-((Benzo[d][1,3]dioxol-5-yl)methyl)piperazin-1-yl)-1,4-dihydro-1-(4-fluorophenyl)-6-nitro-4-oxoquinoline-3-carboxylic acid (**9c**).* Yield: 80%; m.p.: 108 °C; IR (KBr) cm^{-1}: 3250, 2890, 1724, 1710, 1624, 1464–1360; ^{1}H NMR (DMSO-d_6) δ ppm: 2.82–3.12 (m, 8H, CH_2 of piperazine), 3.6 (s, 2H, CH_2 of piperanoyl), 5.86 (s, 2H, –OCH_2O–), 6.42–7.62 (m, 7H, Ar-H), 8.34 (s, 1H, C_8–H), 9.34 (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, 152.4, 149.4, 148.6, 147.7, 146.3, 136.3, 128.9, 126.6, 123.1, 122.2, 120.7, 116.4, 115.0, 113.9, 109.3, 104.9, 101.3, 60.4, 52.2, 49.0; Anal ($C_{28}H_{23}FN_4O_7$) C, H, N.

*5.1.5.9. 7-(4-((Benzo[d][1,3]dioxol-5-yl)methyl)piperazin-1-yl)-1-tert-butyl-1,4-dihydro-6-nitro-4-oxoquinoline-3-carboxylic acid (**10c**).* Yield: 71%; m.p.: 182 °C; IR (KBr) cm^{-1}: 3250, 2890, 1724, 1710, 1624, 1464–1360; ^{1}H NMR (DMSO-d_6) δ ppm: 1.76 (s, 9H, *t*-butyl), 2.82–3.12 (m, 8H, 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), 8.34 (s, 1H, C_8–H), 9.34 (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, 151.1, 148.6, 148.1, 147.8, 146.2, 128.9, 126.5, 121.9, 122.2, 117.5, 115.0, 113.9, 109.3, 101.3, 99.3, 65.0, 60.4, 52.2, 49.0, 28.5; Anal ($C_{26}H_{28}N_4O_7$) C, H, N.

*5.1.5.10. 1-Cyclopropyl-1,4-dihydro-7-(4-methyl-3-phenylpiperazin-1-yl)-6-nitro-4-oxoquinoline-3-carboxylic acid (**8d**).* Yield: 69%; m.p.: 198 °C; IR (KBr) cm^{-1}: 2890, 1724, 1710, 1620, 1460–1368; ^{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), 8.34 (s, 1H, C_8–H), 9.32 (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, 151.1, 148.1, 146.2, 137.4, 128.9, 128.4, 127.5, 126.5, 121.9, 117.5, 109.3, 99.3, 63.4, 59.0, 52.2, 49.0, 40.5, 36.0, 5.6; Anal ($C_{24}H_{24}N_4O_5$) C, H, N.

*5.1.5.11. 1,4-Dihydro-1-(4-fluorophenyl)-7-(4-methyl-3-phenylpiperazin-1-yl)-6-nitro-4-oxoquinoline-3-carboxylic acid (**9d**).* Yield: 80%; m.p.: 126 °C; IR (KBr) cm^{-1}: 2890, 1724, 1710, 1620, 1460–1368; ^{1}H NMR (DMSO-d_6) δ ppm: 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), 6.45–7.4 (m, 9H, Ar-H), 8.34 (s, 1H, C_8–H), 9.32 (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, 152.4, 149.4, 147.7, 146.3, 137.4, 136.3, 128.9, 128.4, 127.5, 126.6, 123.1, 120.7, 116.4, 109.3, 104.9, 63.4, 59.0, 52.2, 49.0, 40.5; Anal ($C_{27}H_{23}FN_4O_5$) C, H, N.

*5.1.5.12. 1-tert-Butyl-1,4-dihydro-7-(4-methyl-3-phenylpiperazin-1-yl)-6-nitro-4-oxoquinoline-3-carboxylic acid (**10d**).* Yield: 67%; m.p.: 147 °C; IR (KBr) cm^{-1}: 2890, 1724, 1710, 1620, 1460–1368; ^{1}H NMR (DMSO-d_6) δ ppm: 1.76 (s, 9H, *t*-butyl), 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), 8.34 (s, 1H, C_8–H), 9.32 (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, 151.1, 148.1, 146.2, 137.4, 128.9, 128.4,

127.5, 126.5, 121.9, 117.5, 109.3, 99.3, 65.0, 63.4, 59.0, 52.2, 49.0, 40.5, 28.5; Anal ($C_{25}H_{28}N_4O_5$) C, H, N.

*5.1.5.13. 1-Cyclopropyl-7-(4-(2,3-dihydrobenzo[b][1,4]dioxin-2-oyl)(piperazin-1-yl))-1,4-dihydro-6-nitro-4-oxoquinoline-3-carboxylic acid (**8e**).* Yield: 70%; m.p.: 134 °C; IR (KBr) cm^{-1}: 2890, 1724, 1710, 1624, 1460–1360; ^1H NMR (DMSO-d_6) δ ppm: 0.28–0.48 (m, 4H, cyclopropyl), 1.35 (m, 1H, cyclopropyl), 3.26–3.4 (m, 8H, CH_2 of piperazine), 4.6 (d, 2H, 3-CH_2 of dihydrobenzodioxinyl), 5.14 (t, 1H, 2-CH of dihydrobenzodioxinyl), 6.62–6.98 (m, 4H, Ar-H), 8.34 (s, 1H, C_8–H), 9.3 (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, 151.1, 148.1, 146.7, 146.2, 126.5, 121.9, 121.0, 117.5, 115.0, 109.3, 99.3, 85.9, 66.2, 48.5, 47.4, 36.0, 5.6; Anal ($C_{26}H_{24}N_4O_8$) C, H, N.

*5.1.5.14. 7-(4-(2,3-Dihydrobenzo[b][1,4]dioxin-2-oyl)(piperazin-1-yl))-1-(4-fluorophenyl)-1,4-dihydro-6-nitro-4-oxoquinoline-3-carboxylic acid (**9e**).* Yield: 83%; m.p.: 70 °C; IR (KBr) cm^{-1}: 2890, 1724, 1710, 1624, 1460–1360; ^1H NMR (DMSO-d_6) δ ppm: 3.26–3.4 (m, 8H, 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), 8.34 (s, 1H, C_8–H), 9.3 (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, 152.4, 149.4, 147.7, 146.7, 146.3, 136.3, 126.6, 123.1, 121.0, 120.7, 116.4, 115.0, 109.3, 104.9, 85.9, 66.2, 48.5, 47.4; Anal ($C_{29}H_{23}FN_4O_8$) C, H, N.

*5.1.5.15. 1-tert-Butyl-7-(4-(2,3-dihydrobenzo[b][1,4]dioxin-2-oyl)(piperazin-1-yl))-1,4-dihydro-6-nitro-4-oxoquinoline-3-carboxylic acid (**10e**).* Yield: 71%; m.p.: 231 °C; IR (KBr) cm^{-1}: 2890, 1724, 1710, 1624, 1460–1360; ^1H NMR (DMSO-d_6) δ ppm: 1.76 (s, 9H, *t*-butyl), 3.26–3.4 (m, 8H, CH_2 of piperazine), 4.6 (d, 2H, 3-CH_2 of dihydrobenzodioxinyl), 5.14 (t, 1H, 2-CH of dihydrobenzodioxinyl), 6.62–6.98 (m, 4H, Ar-H), 8.34 (s, 1H, C_8–H), 9.3 (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, 151.1, 148.1, 146.7, 146.2, 126.5, 121.9, 121.0, 117.5, 115.0, 109.3, 99.3, 85.9, 66.2, 65.0, 48.5, 47.4, 28.5; Anal ($C_{27}H_{28}N_4O_8$) C, H, N.

*5.1.5.16. 1-Cyclopropyl-7-(3-(2,6-difluorophenyl)-5-methylisoxazol-4-oyl)(piperazin-1-yl)-1,4-dihydro-6-nitro-4-oxoquinoline-3-carboxylic acid (**8f**).* Yield: 70%; m.p.: 241 °C; IR (KBr) cm^{-1}: 2890, 1724, 1710, 1624, 1464–1360, 1208; ^1H NMR (DMSO-d_6) δ ppm: 0.28–0.48 (m, 4H, cyclopropyl), 1.35 (m, 1H, cyclopropyl), 2.3 (s, 3H, 5-CH_3 of isoxazolyl), 3.12–3.28 (m, 8H, CH_2 of piperazine), 6.82–7.08 (m, 3H, Ar-H), 8.34 (s, 1H, C_8–H), 9.32 (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, 172.5, 168.9, 166.3, 160.3, 151.1, 148.1, 146.2, 132.0, 126.5, 121.9, 117.5, 111.6, 110.5, 109.3, 99.3, 48.5, 47.1, 36.0, 5.6, 6.0; Anal ($C_{28}H_{23}F_2N_5O_7$) C, H, N.

*5.1.5.17. 7-(3-(2,6-Difluorophenyl)-1-(4-fluorophenyl)-5-methylisoxazol-4-oyl)(piperazin-1-yl)-1,4-dihydro-6-nitro-4-oxoquinoline-3-carboxylic acid (**9f**).* Yield: 84%; m.p.: 223 °C; IR (KBr) cm^{-1}: 2890, 1724, 1710, 1624, 1464–1360, 1208; ^1H NMR (DMSO-d_6) δ ppm: 2.3 (s, 3H, 5-CH_3 of isoxazolyl), 3.12–3.28 (m, 8H, CH_2 of piperazine), 6.58–7.08 (m, 7H, Ar-H), 8.34 (s, 1H, C_8–H), 9.32 (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, 172.5, 168.9, 166.3, 160.3, 152.4, 149.4, 147.7, 146.3, 136.3, 132.0, 126.6, 123.1, 120.7, 116.4, 111.6, 110.5, 109.3, 104.9, 48.5, 47.1, 6.0; Anal ($C_{31}H_{22}F_3N_5O_7$) C, H, N.

*5.1.5.18. 1-tert-Butyl-7-(3-(2,6-difluorophenyl)-5-methylisoxazol-4-oyl)(piperazin-1-yl)-1,4-dihydro-6-nitro-4-oxoquinoline-3-carboxylic acid (**10f**).* Yield: 81%; m.p.: >250 °C; IR (KBr) cm^{-1}: 2890, 1724, 1710, 1624, 1464–1360, 1208; ^1H NMR (DMSO-d_6) δ ppm: 1.74 (s, 9H, *t*-butyl), 2.3 (s, 3H, 5-CH_3 of isoxazolyl), 3.12–3.28 (m, 8H, CH_2 of piperazine), 6.82–7.08 (m, 3H, Ar-H), 8.34 (s, 1H, C_8–H), 9.32 (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, 172.5, 168.9, 166.3, 160.3, 151.1, 148.1, 146.2, 132.0, 126.5, 121.9, 117.5, 111.6, 110.5, 109.3, 99.3, 65.0, 48.5, 47.1, 28.5, 6.0; Anal ($C_{29}H_{27}F_2N_5O_7$) C, H, N.

*5.1.5.19. 1-Cyclopropyl-1,4-dihydro-6-nitro-4-oxo-7-thiomorpholinoquinoline-3-carboxylic acid (**8g**).* Yield: 77%; m.p.: 208 °C; IR (KBr) cm^{-1}: 2890, 1724, 1710, 1620, 1460–1368; ^1H NMR (DMSO-d_6) δ ppm: 0.28–0.54 (m, 4H, cyclopropyl), 1.36 (m, 1H, cyclopropyl), 2.64–3.4 (m, 8H, CH_2 of thiomorpholine), 8.34 (s, 1H, C_8–H), 9.32 (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, 151.1, 148.1, 146.2, 126.5, 121.9, 117.5, 109.3, 99.3, 50.2, 36.0, 28.1, 5.6; Anal ($C_{17}H_{17}N_3O_5S$) C, H, N.

*5.1.5.20. 1,4-Dihydro-1-(4-fluorophenyl)-6-nitro-4-oxo-7-thiomorpholinoquinoline-3-carboxylic acid (**9g**).* Yield: 81%; m.p.: 114 °C; IR (KBr) cm^{-1}: 2890, 1724, 1710, 1620, 1460–1368; ^1H NMR (DMSO-d_6) δ ppm: 2.64–3.4 (m, 8H, CH_2 of thiomorpholine), 8.34 (s, 1H, C_8–H), 6.5–6.98 (m, 4H, Ar-H), 9.32 (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, 152.4, 149.4, 147.7, 146.3, 136.3, 126.6, 123.1, 120.7, 116.4, 109.3, 104.9, 50.2, 28.1; Anal ($C_{20}H_{16}FN_3O_5S$) C, H, N.

*5.1.5.21. 1-tert-Butyl-1,4-dihydro-6-nitro-4-oxo-7-thiomorpholinoquinoline-3-carboxylic acid (**10g**).* Yield: 71%; m.p.: >250 °C; IR (KBr) cm^{-1}: 2890, 1724, 1710, 1620, 1460–1368; ^1H NMR (DMSO-d_6) δ ppm: 1.76 (s, 9H, *t*-butyl), 2.64–3.4 (m, 8H, CH_2 of thiomorpholine), 8.34 (s, 1H, C_8–H), 9.32 (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, 151.1, 148.1, 146.2, 126.5, 121.9, 117.5, 109.3, 99.3, 65.0, 50.2, 28.5, 28.1; Anal ($C_{18}H_{21}N_3O_5S$) C, H, N.

*5.1.5.22. 1-Cyclopropyl-1,4-dihydro-7-(2,6-dimethylmorpholino)-6-nitro-4-oxoquinoline-3-carboxylic acid (**8h**).* Yield: 73%; m.p.: 172 °C; IR (KBr) cm^{-1}: 2892, 1724, 1710, 1628, 1460–1360; ^1H NMR (DMSO-d_6) δ ppm: 0.28–0.48 (m, 4H, cyclopropyl), 1.2 (d, 6H, 2,6-CH_3 of morpholino), 1.35

(m, 1H, cyclopropyl), 3.0 (d, 4H, 2,6-CH_2 of morpholine), 3.9 (m, 2H, 3,5-CH of morpholine), 8.34 (s, 1H, C_8–H), 9.3 (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, 151.1, 148.1, 146.2, 126.5, 121.9, 117.5, 109.3, 99.3, 69.2, 65.1, 36.0, 20.5, 5.6; Anal ($C_{19}H_{21}N_3O_6$) C, H, N.

*5.1.5.23. 1-(4-Fluorophenyl)-1,4-dihydro-7-(2,6-dimethylmorpholino)-6-nitro-4-oxoquinoline-3-carboxylic acid (**9h**).* Yield: 84%; m.p.: 103 °C; IR (KBr) cm^{-1}: 2892, 1724, 1710, 1628, 1460–1360; ^{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), 8.34 (s, 1H, C_8–H), 9.3 (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, 152.4, 149.4, 147.7, 146.3, 136.3, 126.6, 123.1, 120.7, 116.4, 109.3, 104.9, 69.2, 65.1, 20.5; Anal ($C_{22}H_{20}FN_3O_6$) C, H, N.

*5.1.5.24. 1-tert-Butyl-1,4-dihydro-7-(2,6-dimethylmorpholino)-6-nitro-4-oxoquinoline-3-carboxylic acid (**10h**).* Yield: 74%; m.p.: 108 °C; IR (KBr) cm^{-1}: 2892, 1724, 1710, 1628, 1460–1360; ^{1}H NMR (DMSO-d_6) δ ppm: 1.2 (d, 6H, 2,6-CH_3 of morpholino), 1.76 (s, 9H, *t*-butyl), 3.0 (d, 4H, 2,6-CH_2 of morpholine), 3.9 (m, 2H, 3,5-CH of morpholine), 8.34 (s, 1H, C_8–H), 9.3 (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, 151.1, 148.1, 146.2, 126.5, 121.9, 117.5, 109.3, 99.3, 69.2, 65.1, 20.5, 28.5; Anal ($C_{20}H_{25}N_3O_6$) C, H, N.

*5.1.5.25. 1-Cyclopropyl-1,4-dihydro-6-nitro-4-oxo-7-(4-(piperidin-1-yl)piperidin-1-yl)quinoline-3-carboxylic acid (**8i**).* Yield: 74%; m.p.: 173 °C; IR (KBr) cm^{-1}: 2890, 1724, 1710, 1624, 1464–1360; ^{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, 5CH_2), 2.2 (t, 4H, 2CH_2), 2.7 (m, 1H, CH), 2.8 (t, 4H, 2CH_2), 8.34 (s, 1H, C_8–H), 9.32 (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, 151.1, 148.1, 146.2, 126.5, 121.9, 117.5, 109.3, 99.3, 58.2, 52.4, 46.9, 36.0, 28.2, 26.2, 25.9, 5.6; Anal ($C_{23}H_{28}N_4O_5$) C, H, N.

*5.1.5.26. 1,4-Dihydro-1-(4-fluorophenyl)-6-nitro-4-oxo-7-(4-(piperidin-1-yl)piperidin-1-yl)quinoline-3-carboxylic acid (**9i**).* Yield: 81%; m.p.: 108 °C; IR (KBr) cm^{-1}: 2890, 1724, 1710, 1624, 1464–1360; ^{1}H NMR (DMSO-d_6) δ ppm: 1.5–1.6 (m, 10H, 5CH_2), 1.78 (s, 9H, *t*-butyl), 2.2 (t, 4H, 2CH_2), 2.7 (m, 1H, CH), 2.8 (t, 4H, 2CH_2), 6.5–6.88 (m, 4H, Ar-H), 8.34 (s, 1H, C_8–H), 9.32 (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, 152.4, 149.4, 147.7, 146.3, 136.3, 126.6, 123.1, 120.7, 116.4, 109.3, 104.9, 58.2, 52.4, 46.9, 28.2, 26.2, 25.9; Anal ($C_{26}H_{27}FN_4O_5$) C, H, N.

*5.1.5.27. 1-tert-Butyl-1,4-dihydro-6-nitro-4-oxo-7-(4-(piperidin-1-yl)piperidin-1-yl)quinoline-3-carboxylic acid (**10i**).* Yield: 78%; m.p.: 156 °C; IR (KBr) cm^{-1}: 2890, 1724, 1710, 1624, 1464–1360; ^{1}H NMR (DMSO-d_6) δ ppm: 1.5–1.6 (m, 10H, 5CH_2), 1.78 (s, 9H, *t*-butyl), 2.2 (t, 4H, 2CH_2), 2.7 (m, 1H, CH), 2.8 (t, 4H, 2CH_2), 8.34 (s, 1H, C_8–H), 9.32 (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, 151.1, 148.1, 146.2, 126.5, 121.9, 117.5, 109.3, 99.3, 65.0, 58.2, 52.4, 46.9, 28.5, 28.2, 26.2, 25.9; Anal ($C_{24}H_{32}N_4O_5$) C, H, N.

*5.1.5.28. 7-(4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl)-1-cyclopropyl-1,4-dihydro-6-nitro-4-oxoquinoline-3-carboxylic acid (**8j**).* Yield: 70%; m.p.: 197 °C; IR (KBr) cm^{-1}: 2900, 1724, 1708, 1620, 1460–1370; ^{1}H NMR (DMSO-d_6) δ ppm: 0.28–0.46 (m, 4H, cyclopropyl), 1.32 (m, 1H, cyclopropyl), 2.0–2.7 (m, 8H, 3,5-CH_2 of piperidine), 7.1–7.18 (m, 4H, Ar-H), 8.34 (s, 1H, C_8–H), 9.30 (s, 1H, C_5–H), 9.56 (s, 1H, C_2–H), 10.0 (br s, 1H, OH), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 177.5, 166.3, 151.1, 148.1, 146.2, 138.2, 131.5, 129.6, 129.1, 126.5, 121.9, 117.5, 109.3, 99.3, 74.3, 42.6, 38.2, 36.0, 5.6; Anal ($C_{24}H_{22}ClN_3O_6$) C, H, N.

*5.1.5.29. 7-(4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl)-1,4-dihydro-1-(4-fluorophenyl)-6-nitro-4-oxoquinoline-3-carboxylic acid (**9j**).* Yield: 76%; m.p.: 145 °C; IR (KBr) cm^{-1}: 2900, 1724, 1708, 1620, 1460–1370; ^{1}H NMR (DMSO-d_6) δ ppm: 2.0–2.7 (m, 8H, 3,5-CH_2 of piperidine), 6.52–7.18 (m, 8H, Ar-H), 8.34 (s, 1H, C_8–H), 9.30 (s, 1H, C_5–H), 9.56 (s, 1H, C_2–H), 10.0 (br s, 1H, OH), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 177.5, 166.3, 152.4, 149.4, 147.7, 146.3, 138.2, 136.3, 131.5, 129.6, 129.1, 126.6, 123.1, 120.7, 116.4, 109.3, 104.9, 74.3, 42.6, 38.2; Anal ($C_{27}H_{21}ClFN_3O_6$) C, H, N.

*5.1.5.30. 1-tert-Butyl-7-(4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl)-1,4-dihydro-6-nitro-4-oxoquinoline-3-carboxylic acid (**10j**).* Yield: 75%; m.p.: 127 °C; IR (KBr) cm^{-1}: 2900, 1724, 1708, 1620, 1460–1370; ^{1}H NMR (DMSO-d_6) δ ppm: 1.76 (s, 9H, *t*-butyl), 2.0–2.7 (m, 8H, 3,5-CH_2 of piperidine), 7.1–7.18 (m, 4H, Ar-H), 8.34 (s, 1H, C_8–H), 9.30 (s, 1H, C_5–H), 9.56 (s, 1H, C_2–H), 10.0 (br s, 1H, OH), 14.6 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm: 177.5, 166.3, 151.1, 148.1, 146.2, 138.2, 131.5, 129.6, 129.1, 126.5, 121.9, 117.5, 109.3, 99.3, 74.3, 65.0, 42.6, 38.2, 28.5; Anal ($C_{25}H_{26}ClN_3O_6$) C, H, N.

*5.1.5.31. 7-(4-(6-Chloro-1,2-dihydro-2-oxobenzo[d]imidazol-3-yl)piperidin-1-yl)-1-cyclopropyl-1,4-dihydro-6-nitro-4-oxoquinoline-3-carboxylic acid (**8k**).* Yield: 67%; m.p.: 209 °C; IR (KBr) cm^{-1}: 3110, 2896, 1728, 1712, 1710, 1624, 1460–1360; ^{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, 4CH_2 of piperidine), 4.1 (br m, 1H, CH of piperidine), 7.2–7.56 (m, 3H, Ar-H), 8.34 (s, 1H, C_8–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, 166.3, 151.7, 151.1, 148.1, 146.2, 131.4, 130.1, 126.5, 124.7, 123.2, 122.2, 121.9, 117.5, 109.3, 99.3, 50.5, 46.2, 36.0, 26.8, 5.6; Anal ($C_{25}H_{22}ClN_5O_6$) C, H, N.

5.1.5.32. 7-(4-(6-Chloro-1,2-dihydro-2-oxobenzo[d]imidazol-3-yl)piperidin-1-yl)-1-(4-fluorophenyl)-1,4-dihydro-6-nitro-4-

*oxoquinoline-3-carboxylic acid (**9k**)*. Yield: 78%; m.p.: 181 °C; IR (KBr) cm^{-1}: 3110, 2896, 1728, 1712, 1710, 1624, 1460–1360; ^1H NMR (DMSO-d_6) δ ppm: 1.6–2.4 (m, 8H, $4CH_2$ of piperidine), 4.1 (br m, 1H, CH of piperidine), 6.4–6.9 (m, 7H, Ar-H), 8.34 (s, 1H, C_8–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, 166.3, 152.4, 151.7, 149.4, 147.7, 146.3, 136.3, 131.4, 130.1, 126.6, 124.7, 123.1, 122.2, 120.7, 116.4, 109.3, 104.9, 50.5, 46.2, 26.8; Anal ($C_{28}H_{21}ClFN_5O_6$) C, H, N.

*5.1.5.33. 1-tert-Butyl-7-(4-(6-chloro-1,2-dihydro-2-oxobenzo[d]imidazol-3-yl)piperidin-1-yl)-1,4-dihydro-6-nitro-4-oxoquinoline-3-carboxylic acid (**10k**)*. Yield: 69%; m.p.: >250 °C; IR (KBr) cm^{-1}: 3110, 2896, 1728, 1712, 1710, 1624, 1460–1360; ^1H NMR (DMSO-d_6) δ ppm: 1.6–2.4 (m, 8H, $4CH_2$ of piperidine), 1.76 (s, 9H, *t*-butyl), 4.1 (br m, 1H, CH of piperidine), 7.2–7.56 (m, 3H, Ar-H), 8.34 (s, 1H, C_8–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, 166.3, 151.7, 151.1, 148.1, 146.2, 131.4, 130.1, 126.5, 124.7, 123.2, 122.2, 121.9, 117.5, 109.3, 99.3, 65.0, 50.5, 46.2, 26.8, 28.5; Anal ($C_{26}H_{26}ClN_5O_6$) C, H, N.

*5.1.5.34. 1-Cyclopropyl-7-(3-(diethylcarbamoyl)piperidin-1-yl)-1,4-dihydro-6-nitro-4-oxoquinoline-3-carboxylic acid (**8l**)*. Yield: 72%; m.p.: 174 °C; IR (KBr) cm^{-1}: 2890, 1724, 1710, 1624, 1464–1360, 1208; ^1H NMR (DMSO-d_6) δ ppm: 0.28–0.48 (m, 4H, cyclopropyl), 1.2 (t, 6H, 2-CH_3 of ethyl), 1.35 (m, 1H, cyclopropyl), 1.78–2.7 (m, 9H, H of piperidine), 3.24 (q, 4H, 2-CH_2 of ethyl), 8.32 (s, 1H, C_8–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, 175.3, 166.3, 151.1, 148.1, 146.2, 126.5, 121.9, 117.5, 109.3, 99.3, 54.4, 51.0, 42.6, 41.3, 36.0, 28.2, 22.3, 12.9, 5.6; Anal ($C_{23}H_{28}N_4O_6$) C, H, N.

*5.1.5.35. 7-(3-(Diethylcarbamoyl)piperidin-1-yl)-1,4-dihydro-1-(4-fluorophenyl)-6-nitro-4-oxoquinoline-3-carboxylic acid (**9l**)*. Yield: 73%; m.p.: 75 °C; IR (KBr) cm^{-1}: 2890, 1724, 1710, 1624, 1464–1360, 1208; ^1H NMR (DMSO-d_6) δ ppm: 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), 6.5–6.98 (m, 4H, Ar-H), 8.32 (s, 1H, C_8–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, 175.3, 166.3, 152.4, 149.4, 147.7, 146.3, 136.3, 126.6, 123.1, 120.7, 116.4, 109.3, 104.9, 54.4, 51.0, 42.6, 41.3, 28.2, 22.3, 12.9; Anal ($C_{26}H_{27}FN_4O_6$) C, H, N.

*5.1.5.36. 7-(3-(Diethylcarbamoyl)piperidin-1-yl)-1-tert-butyl-1,4-dihydro-6-nitro-4-oxoquinoline-3-carboxylic acid (**10l**)*. Yield: 70%; m.p.: 152 °C; IR (KBr) cm^{-1}: 2890, 1724, 1710, 1624, 1464–1360, 1208; ^1H 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), 8.32 (s, 1H, C_8–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, 175.3, 166.3, 151.1, 148.1, 146.2, 126.5, 121.9, 117.5, 109.3, 99.3, 65.0, 54.4, 51.0, 42.6, 41.3, 28.5, 28.2, 22.3, 12.9; Anal ($C_{24}H_{32}N_4O_6$) C, H, N.

*5.1.5.37. 1-Cyclopropyl-1,4-dihydro-7-(1,4-dioxa-8-azaspiro[4.5]dec-8-yl)-6-nitro-4-oxoquinoline-3-carboxylic acid (**8m**)*. Yield: 78%; m.p.: 126 °C; IR (KBr) cm^{-1}: 2890, 1724, 1712, 1618, 1466–1368; ^1H 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), 8.32 (s, 1H, C_8–H), 9.30 (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, 151.1, 148.1, 146.2, 126.5, 121.9, 117.5, 109.3, 99.3, 64.4, 39.6, 36.0, 34.4, 5.6; Anal ($C_{20}H_{21}N_3O_7$) C, H, N.

*5.1.5.38. 1,4-Dihydro-7-(1,4-dioxa-8-azaspiro[4.5]dec-8-yl)-1-(4-fluorophenyl)-6-nitro-4-oxoquinoline-3-carboxylic acid (**9m**)*. Yield: 69%; m.p.: 110 °C; IR (KBr) cm^{-1}: 2890, 1724, 1712, 1618, 1466–1368; ^1H NMR (DMSO-d_6) δ ppm: 1.78–2.4 (m, 8H, 4-CH_2 of azaspirodecane), 3.96 (m, 4H, 2-CH_2 of azaspirodecane), 6.5–6.98 (m, 4H, Ar-H), 8.32 (s, 1H, C_8–H), 9.30 (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, 152.4, 149.4, 147.7, 146.3, 136.3, 126.6, 123.1, 120.7, 116.4, 109.3, 104.9, 64.4, 39.6, 34.4; Anal ($C_{23}H_{20}FN_3O_7$) C, H, N.

*5.1.5.39. 1-tert-Butyl-1,4-dihydro-7-(1,4-dioxa-8-azaspiro[4.5]dec-8-yl)-6-nitro-4-oxoquinoline-3-carboxylic acid (**10m**)*. Yield: 71%; m.p.: 136 °C; IR (KBr) cm^{-1}: 2890, 1724, 1712, 1618, 1466–1368; ^1H NMR (DMSO-d_6) δ ppm: 1.76 (s, 9H, *t*-butyl), 1.78–2.4 (m, 8H, 4-CH_2 of azaspirodecane), 3.96 (m, 4H, 2-CH_2 of azaspirodecane), 8.32 (s, 1H, C_8–H), 9.30 (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, 151.1, 148.1, 146.2, 126.5, 121.9, 117.5, 109.3, 99.3, 65.0, 64.4, 39.6, 34.4, 28.5; Anal ($C_{21}H_{25}N_3O_7$) C, H, N.

*5.1.5.40. 7-(1-(tert-Butylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-1-cyclopropyl-1,4-dihydro-6-nitro-4-oxoquinoline-3-carboxylic acid (**8n**)*. Yield: 75%; m.p.: 206 °C; IR (KBr) cm^{-1}: 2890, 1724, 1712, 1626, 1468–1360; ^1H NMR (DMSO-d_6) δ ppm: 0.28–0.48 (m, 4H, cyclopropyl), 1.3 (s, 9H, $3CH_3$), 1.35 (m, 1H, cyclopropyl), 2.66–2.9 (m, 4H, $2CH_2$ of isoquinoline), 4.85 (s, 1H, CH of isoquinoline), 6.7–7.1 (m, 4H, Ar-H), 8.32 (s, 1H, C_8–H), 9.28 (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, 168.7, 166.3, 151.1, 148.1, 146.2, 137.5, 135.6, 129.6, 128.3, 127.6, 126.5, 121.9, 117.5, 109.3, 99.3, 65.4, 48.1, 47.3, 36.0, 30.4, 26.3, 5.6; Anal ($C_{27}H_{28}N_4O_6$) C, H, N.

*5.1.5.41. 7-(1-(tert-Butylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-1-(4-fluorophenyl)-1,4-dihydro-6-nitro-4-oxoquinoline-3-carboxylic acid (**9n**)*. Yield: 82%; m.p.: 112 °C; IR (KBr) cm^{-1}: 2890, 1724, 1712, 1626, 1468–1360; ^1H NMR (DMSO-d_6) δ ppm: 1.3 (s, 9H, $3CH_3$), 2.66–2.9 (m, 4H, $2CH_2$ of isoquinoline), 4.85 (s, 1H, CH of isoquinoline), 6.7–7.1 (m, 8H, Ar-H), 8.32 (s, 1H, C_8–H), 9.28 (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, 168.7, 166.3, 152.4, 149.4, 147.7, 146.3, 137.5, 136.3, 135.6, 129.6, 128.3, 127.6, 126.6, 123.1, 120.7, 116.4, 109.3, 104.9, 65.4, 48.1, 47.3, 30.4, 26.3; Anal ($C_{30}H_{27}FN_4O_6$) C, H, N.

*5.1.5.42. 1-tert-Butyl-7-(1-(tert-butylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-1,4-dihydro-6-nitro-4-oxoquinoline-3-carboxylic acid (**10n**).* Yield: 72%; m.p.: 135 °C; IR (KBr) cm^{-1}: 2890, 1724, 1712, 1626, 1468–1360; ^{1}H NMR (DMSO-d_6) δ ppm: 1.3 (s, 9H, 3CH_3), 1.76 (s, 9H, *t*-butyl), 2.66–2.9 (m, 4H, 2CH_2 of isoquinoline), 4.85 (s, 1H, CH of isoquinoline), 6.7–7.1 (m, 4H, Ar-H), 8.32 (s, 1H, C_8–H), 9.28 (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, 168.7, 166.3, 151.1, 148.1, 146.2, 137.5, 135.6, 129.6, 128.3, 127.6, 126.5, 121.9, 117.5, 109.3, 99.3, 65.4, 65.0, 48.1, 47.3, 30.4, 28.5, 26.3; Anal ($C_{28}H_{32}N_4O_6$) C, H, N.

*5.1.5.43. 7-(2-Carboxy-5,6-dihydroimidazo[1,2-a]pyrazin-7(8H)-yl)-1-cyclopropyl-1,4-dihydro-6-nitro-4-oxoquinoline-3-carboxylic acid (**8o**).* Yield: 78%; m.p.: 210 °C; IR (KBr) cm^{-1}: 3200, 2890, 1724, 1710, 1624, 1464–1360, 1208; ^{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), 7.6 (s, 1H, CH), 8.32 (s, 1H, C_8–H), 9.32 (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, 167.5, 166.3, 157.6, 151.1, 148.1, 146.2, 130.3, 129.5, 126.5, 121.9, 117.5, 109.3, 99.3, 57.1, 50.7, 37.1, 36.0, 5.6; Anal ($C_{20}H_{17}N_5O_7$) C, H, N.

*5.1.5.44. 7-(2-Carboxy-5,6-dihydroimidazo[1,2-a]pyrazin-7(8H)-yl)-1,4-dihydro-1-(4-fluorophenyl)-6-nitro-4-oxoquinoline-3-carboxylic acid (**9o**).* Yield: 84%; m.p.: 179 °C; IR (KBr) cm^{-1}: 3200, 2890, 1724, 1710, 1624, 1464–1360, 1208; ^{1}H NMR (DMSO-d_6) δ ppm: 3.1–3.8 (m, 6H, 3-CH_2), 6.5–6.9 (m, 4H, Ar-H), 7.6 (s, 1H, CH), 8.32 (s, 1H, C_8–H), 9.32 (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, 167.5, 166.3, 157.6, 152.4, 149.4, 147.7, 146.3, 136.3, 130.3, 129.5, 126.6, 123.1, 120.7, 116.4, 109.3, 104.9, 57.1, 50.7, 37.1; Anal ($C_{23}H_{16}FN_5O_7$) C, H, N.

*5.1.5.45. 1-tert-Butyl-7-(2-carboxy-5,6-dihydroimidazo[1,2-a]pyrazin-7(8H)-yl)-1,4-dihydro-6-nitro-4-oxoquinoline-3-carboxylic acid (**10o**).* Yield: 72%; m.p.: >250 °C; IR (KBr) cm^{-1}: 3200, 2890, 1724, 1710, 1624, 1464–1360, 1208; ^{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), 8.32 (s, 1H, C_8–H), 9.32 (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, 167.5, 166.3, 157.6, 151.1, 148.1, 146.2, 130.3, 129.5, 126.5, 121.9, 117.5, 109.3, 99.3, 65.0, 57.1, 50.7, 37.1, 28.5; Anal ($C_{21}H_{21}N_6O_7$) C, H, N.

*5.1.5.46. 1-Cyclopropyl-1,4-dihydro-7-(8-(4-methoxybenzyl)-3,4,5,6,7,8-hexahydroisoquinolin-2(1H)-yl)-6-nitro-4-oxoquinoline-3-carboxylic acid (**8p**).* Yield: 71%; m.p.: 208 °C; IR (KBr) cm^{-1}: 3210, 2890, 1724, 1710, 1620, 1460–1368; ^{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), 8.34 (s, 1H, C_8–H), 9.32 (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, 157.9, 151.1, 148.1, 146.2, 131.8, 128.8, 126.5, 123.7, 121.9, 117.5, 114.2, 109.3, 99.3, 61.1, 55.9, 42.8, 37.6, 36.0, 30.2, 28.5, 25.8, 24.2, 5.6; Anal ($C_{30}H_{31}N_3O_6$) C, H, N.

*5.1.5.47. 1,4-Dihydro-1-(4-fluorophenyl)-7-(8-(4-methoxybenzyl)-3,4,5,6,7,8-hexahydroisoquinolin-2(1H)-yl)-6-nitro-4-oxoquinoline-3-carboxylic acid (**9p**).* Yield: 76%; m.p.: 114 °C; IR (KBr) cm^{-1}: 3210, 2890, 1724, 1710, 1620, 1460–1368; ^{1}H NMR (DMSO-d_6) δ ppm: 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), 6.5–7.1 (m, 8H, Ar-H), 8.34 (s, 1H, C_8–H), 9.32 (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, 157.9, 152.4, 149.4, 147.7, 146.3, 136.3, 131.8, 128.8, 126.6, 123.7, 123.1, 120.7, 116.4, 114.2, 109.3, 104.9, 61.1, 55.9, 42.8, 37.6, 30.2, 28.5, 25.8, 24.2; Anal ($C_{33}H_{30}FN_3O_6$) C, H, N.

*5.1.5.48. 1-tert-Butyl-1,4-dihydro-7-(8-(4-methoxybenzyl)-3,4,5,6,7,8-hexahydroisoquinolin-2(1H)-yl)-6-nitro-4-oxoquinoline-3-carboxylic acid (**10p**).* Yield: 73%; m.p.: 69 °C; IR (KBr) cm^{-1}: 3210, 2890, 1724, 1710, 1620, 1460–1368; ^{1}H NMR (DMSO-d_6) δ ppm: 1.6–1.95 (m, 8H, 4-CH_2 of isoquinolinyl), 1.76 (s, 9H, *t*-butyl), 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), 8.34 (s, 1H, C_8–H), 9.32 (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, 157.9, 151.1, 148.1, 146.2, 131.8, 128.8, 126.5, 123.7, 121.9, 117.5, 114.2, 109.3, 99.3, 65.0, 61.1, 55.9, 42.8, 37.6, 30.2, 28.5, 25.8, 24.2; Anal ($C_{31}H_{35}N_3O_6$) C, H, N.

5.2. MIC determination

All compounds were screened for their *in vitro* antimycobacterial activity against MTB, MDR-TB and MC^2 in Middlebrook 7H11 agar 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 [16]. 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.

5.3. Cytotoxicity

Some compounds were further examined for toxicity (CC_{50}) in a mammalian Vero cell line at the concentration of 62.5 μg/ml [17]. 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.

5.4. In vivo studies

One compound was tested for efficacy against MTB at the dose of 50 mg/kg in six-week-old female CD-1 mice six per group. In this model [18,19], 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 spleen and right lung were aseptically removed and ground in a tissue homogenizer, the number of viable organisms were 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.

5.5. DNA gyrase supercoiling assay

In order to carry out gyrase inhibition assays DNA gyrase purified from *M. smegmatis* was used. The enzyme was prepared and stored by standardized procedures [15]. The compounds tested were dissolved in DMSO and pre-incubated with gyrase. Supercoiling assays were carried out as described previously [18], by incubating 400 ng of relaxed circular pUC18 in a 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 at 37 °C. Ciprofloxacin at 10 μg/ml final concentration was used as a control and a reaction having 5% DMSO in the absence of compounds was also performed. The reaction samples were analyzed in agarose gel electrophoresis as described earlier [20].

5.6. 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. The first group received a single dose of screened compound at 140 mg/kg by oral gavage. The second group received a single dose of 140 mg/kg of lomefloxacin HCl. 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 sodium carboxymethylcellulose vehicle only. Test animals were exposed to UVA light in a manner adapted from that described previously [21]. 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, edema or a measurable increase in ear thickness.

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