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ScienceDirect
Bioorganic & Medicinal Chemistry 16 (2008) 3408–3418
Bioorganic & Medicinal Chemistry

Antimycobacterial activities of novel 2-(sub)-3-fluoro/nitro-5, 12-dihydro-5-oxobenzothiazolo[3,2-*a*]quinoline-6-carboxylic acid

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Received 15 September 2007; revised 2 November 2007; accepted 3 November 2007
Available online 9 November 2007

Abstract—Various 2-(sub)-3-fluoro/nitro-5,12-dihydro-5-oxobenzothiazolo[3,2-*a*]quinoline-6-carboxylic acid derivatives were synthesized from 2-aminothiophenol by a five-step reaction, evaluated for in-vitro and in-vivo antimycobacterial activities against *Mycobacterium tuberculosis* H37Rv (MTB), multi-drug resistant *Mycobacterium tuberculosis* (MDR-TB), and *Mycobacterium smegmatis* (MC2), and also tested for the ability to inhibit the supercoiling activity of DNA gyrase from *M. smegmatis*. Among the thirty-four synthesized compounds, 2-(3-(diethylcarbamoyl)piperidin-1-yl)-)-3-fluoro-5,12-dihydro-5-oxobenzothiazolo[3,2-*a*]quinoline-6-carboxylic acid (**7l**) was found to be the most active compound in vitro with MIC of 0.18 and 0.08 μM against MTB and MTR-TB, respectively. Compound **7l** was found to be 2 and 570 times more potent than isoniazid against MTB and MDR-TB, respectively. In the in-vivo animal model **7l** decreased the bacterial load in lung and spleen tissues with 2.78 and 3.12 − log10 protections, respectively, at the dose of 50 mg/kg body weight.

1. Introduction

Tuberculosis (TB) is a worldwide pandemic; about 2 billion people, equal to one-third of the world's total population, are infected with *Mycobacterium tuberculosis* (MTB), the microbes that cause TB. TB is a leading killer among HIV-infected people with weakened immune systems; about 200,000 people living with HIV/AIDS die from TB every year. Multidrug-resistant TB (MDR-TB) is a form of TB that does not respond to the standard treatments using first-line drugs; MDR-TB is present in virtually all countries recently surveyed by World Health Organization (WHO) and partners. 450,000 new MDR-TB cases are estimated to occur every year; the highest rates of MDR-TB are in countries of the former Soviet Union and in China.[1] The WHO declared TB a global health emergency in 1993, and the Stop TB Partnership proposed a Global Plan to stop TB which aims to save 14 million lives between 2006 and 2015.[2] In the course of screening to discover new compounds employed in the chemotherapy of tuberculosis,[3,4] we also identified benzothiazolo[3,2-*a*]quinoline-6-carboxylic acid derivatives which inhibited in-vitro *Mycobacterium tuberculosis* H_{37} Rv (MTB) and multi-drug resistant *Mycobacterium tuberculosis* (MDR-TB). We report herein the results concerning the synthesis of newer benzothiazolo[3,2-*a*]quinoline-6-carboxylic acid derivatives by modifying 2nd position with various unreported bulky secondary amino functions to study the influence of lipophilic character at 2nd position on activity against MTB and also to study the antimycobacterial effect of fluoro/nitro groups at 3rd position, in-vitro and in-vivo antituberculous activity of first representative compounds of this family together with toxicological results.

2. Results and discussion

2.1. Synthesis

Several of the quinolone antibacterials, such as moxifloxacin and gatifloxacin, have been examined as inhibitors of MTB.[5] However, no large systematic study has been undertaken to either optimize the fused quinolone antibacterials against MTB or examine

Keywords: Antimycobacterial activity; Antitubercular activity; Tuberculosis; Benzothiazoloquinolone.
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doi:10.1016/j.bmc.2007.11.016

specific structure–activity relationship of the fused quinolone against MTB. Lipophilic phenyl substituents at N-1 position of simple fluoroquinolone were reported earlier[6] and also substitution of a sulfur atom at the 2 position of 1,4-dihydro-4-oxoquinoline-3-carboxylic acid derivatives can lead to active compounds.[7] Hence, a conformationally restricted rigid analog formed by bridging the phenyl and quinolone rings by a sulfur atom would provide a useful indication of the relevance of the planarity of the ring system to antimycobacterial activity. The titled compounds were synthesized from 2-aminothiophenol by a five-step synthesis. 2-Aminothiophenol (**1**), on reaction with ethyl malonyl chloride (**2**) in presence of triethylamine, yielded ethyl 2-(benzothiazol-2-yl)acetate (**3**) via two intermediates ethyl 2-(2-mercaptophenylcarbamoyl)acetate and its enol tautomer. Compound **3** on reaction with 2,4-dichloro-5-fluoro benzoyl chloride/2,4-dichloro-5-nitro benzoyl chloride in presence of sodium hydride yielded ethyl 2-(benzothiazol-2-yl)-3-(2,4-dichloro-5-fluoro/nitrophenyl)-3-oxopropanoates (**4a**–**b**). Compounds **4a**–**b** undergo cyclization in presence of sodium hydride in ethylene glycol dimethyl ether yielding ethyl 2-Chloro-3-fluoro/nitro-5,12-dihydro-5-oxobenzothiazolo[3,2-*a*]quinoline-6-carboxylates (**5a**–**b**), which on hydrolysis with 2 N sodium hydroxide yielded 2-chloro-3-fluoro/nitro-5,12-dihydro-5-oxobenzothiazolo [3,2-*a*]quinoline-6-carboxylic acids (**6a**–**b**). The titled compounds **7**–**8a**–**s** were prepared by treating **6a**–**b** with appropriate secondary amines in presence of potassium carbonate under microwave irradiation in DMSO. When compared to conventional method[8] of 2–3 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.

2.2. 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 for the determination of MIC in duplicate.[9] 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 Tables 1 and 2.

In the first phase of screening against MTB, all the compounds showed excellent in-vitro activity against MTB with MIC of less than 12.82 μM. Five compounds (**7i**, **7j**, **7l**, **7m**, and **7s**) inhibited MTB with MIC of less than 1 μM and were more potent than standard fluoroquinolone gatifloxacin (MIC: 1.04 μM). When compared to isoniazid (MIC: 0.36 μM), three compounds (**7i**, **7j**, and **7s**) were equally active and one compound (**7l**) was found to be more active against MTB. Compound 2-(3-(diethylcarbamoyl)piperidin-1-yl)-)-3-fluoro-5,12-dihydro-5-oxobenzothiazolo[3,2-*a*]quinoline-6-carboxylic acid (**7l**) was found to be the most active compound in vitro with MIC of 0.18 μM against MTB and was 2 and 5.8 times more potent than isoniazid and gatifloxacin, respectively. Subsequently some of the compounds were evaluated against MDR-TB, and among the twenty-two compounds screened all the compounds inhibited MDR-TB with MIC ranging from 0.08 to 7.55 μM and were found to be more active than isoniazid (MIC: 45.57 μM) and gatifloxacin (MIC: 8.34 μM). Elevan compounds (**7a**, **7i**, **7j**, **7l**, **8l**, **7m**, **8m**, **7o**, **8o**, **7s**, and **8s**) inhibited MDR-TB with MIC of less than 1 μM. Compound **7l** was found to be the most active compound in vitro with MIC of 0.08 μM against MDR-TB and was 104 and 570 times more potent than gatifloxacin and isoniazid, respectively. The compounds were also evaluated against MC^2 in which all the compounds inhibited MC^2 with MIC ranging from 2.79 to 113.26 μM and sixteen compounds were found to be more active than isoniazid (MIC: 45.57 μM).

With respect to structure-MTB activity relationship, at C_2 position we have studied with various substituted piperazines (**7**–**8a**–**f**), (thio) morpholines (**7**–**8g**–**h**), substituted piperidines (**7**–**8i**–**l**), fused piperazines, piperidines, and pyrrolidine (**7**–**8m**–**r**). A comparison of the substitution pattern at C_7 demonstrated that the order of activity was substituted piperidines > fused piperazines, pyrrolidine, and piperidines > substituted piperazines, (thio) morpholines. With respect to C_3 position among the fluoro (**7a**–**r**) and nitro (**8a**–**r**) substituents, in most of the cases compounds with fluoro substituents were found to be more active against MTB.

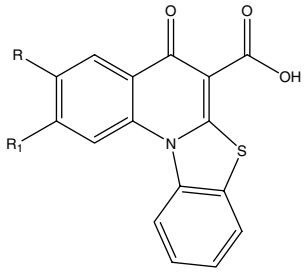
2.3. In-vitro cytotoxicity

Some compounds were further examined for toxicity (IC_{50}) in a mammalian Vero cell line upto 62.5 μg/ml concentrations. 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[10] and the results are reported in Tables 1 and 2. Twenty compounds when tested showed IC_{50} values ranging from 50.0 to 130.3 μM. A comparison of the substitution pattern at C_3 demonstrated that nitro substituted analogs were more cytotoxic than the fluoro substituted analogs. These results are important as the nitro group substituted compounds with their increased cytoliability are much less attractive in the development of a quinolone for the treatment of TB. This is primarily due to the fact that the eradication of TB requires a lengthy course of treatment, and the need for an agent with a high margin of safety becomes a primary concern. The IC_{50} for the compound **7l** was found to be 126.1 μM and showed selectivity index (IC_{50}/MIC) of 1576 against MDR-TB.

2.4. In-vivo antimycobacterial activity

Subsequently, compound **7l** was tested for efficacy against MTB at a dose of 50 mg/kg (Table 3) in CD-1 mice. In this model,[11] the mice were infected intravenously with *M. tuberculosis* ATCC 35801. Drug treatment by intraperitoneal route began after 10 days of

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



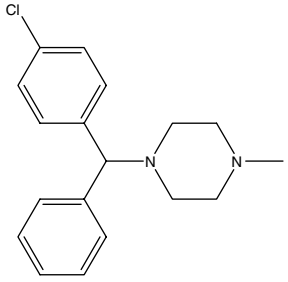
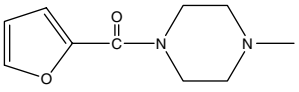
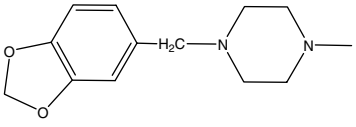
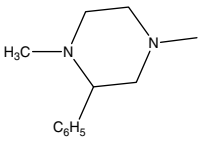
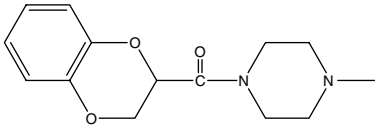
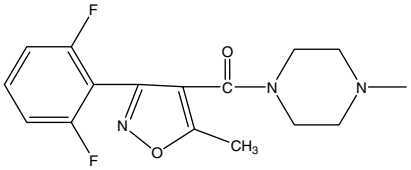
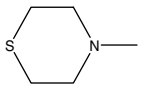
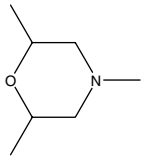
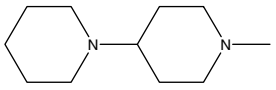
Compound	R	R_1	Yield (%)	Mp (°C)	IC_{50} (μM)	MIC (μM)		
						MTB	MDRTB	MC^2
7a	F		70	253–255	104.5	2.61	0.65	10.45
8a	NO_2		75	209–210	50.0	5.01	NT	39.99
7b	F		77	267–268	NT	6.37	NT	50.87
8b	NO_2		76	207–210	NT	6.04	NT	48.22
7c	F		73	181–182	>117.6	1.47	5.89	5.89
8c	NO_2		72	209–210	111.9	5.60	NT	44.76
7d	F		75	259–261	NT	12.82	NT	102.55
8d	NO_2		73	287–290	NT	12.15	NT	97.17
7e	F		77	237–238	111.7	1.39	5.59	2.79
8e	NO_2		75	233–235	NT	10.66	NT	85.24
7f	F		75	>300	NT	2.52	5.06	40.42
8f	NO_2		72	281–283	NT	4.85	NT	77.45

Table 1 (*continued*)

Compound	R	R_1	Yield (%)	Mp (°C)	IC_{50} (μM)	MIC (μM)		
						MTB	MDRTB	MC^2
7g	F		76	277–278	NT	3.76	7.55	7.55
8g	NO_2		74	268–271	NT	7.09	NT	113.26
7h	F		70	256–260	NT	7.34	NT	29.31
8h	NO_2		79	237–238	NT	6.90	NT	110.26
7i	F		72	260–262	130.3	0.39	0.19	6.53
8i	NO_2		75	294–296	61.7	3.08	1.54	98.71

NT, Not tested.

inoculation of the animal with microorganism and was continued for 10 days. After 35 days post infection the spleens and right lungs were aseptically removed, 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 **7l** decreased the bacterial load in lung and spleen tissues with 2.78 and 3.12 − 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 **7l** decreased the bacterial load with 0.81 and 1.02 − log 10 protections in lung and spleen tissues, respectively. Compound **7l** was found to be less active than isoniazid in the in-vivo study. The reason for this less in-vivo activity might be the low bioavailability of the compound (Scheme 1).

2.5. DNA gyrase inhibition

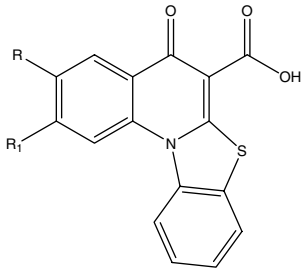
The benzthiazoloquinolone derivatives synthesized and studied in this report were tested for their ability to inhibit supercoiling activity of DNA gyrase. The bacterial targets for quinolones and fluoroquinolones are the type II DNA topoisomerases, DNA gyrase, and topoisomerase IV. These ATP-dependent enzymes act by a transient double-stranded DNA break, followed by strand passage and religation reactions to facilitate DNA transaction processes.[12] DNA gyrase is unique in catalyzing the negative supercoiling of DNA and is essential for DNA replication, transcription, and recombination. In all the species of mycobacteria including MTB, DNA gyrase is the sole type II topoisomerase carrying out the reactions of both the type II topoisomerases. Further, our earlier studies have revealed that DNA gyrase from *M. tuberculosis* and that from *M. smegmatis* are highly similar at protein level, antigenic properties, and catalytic activities.[13] The supercoiling assay results with various compounds using *M. smegmatis* DNA gyrase are presented in Figure 1. The IC_{50} values presented in Table 4 show that the compounds **7j**, **7l**, and **7r** inhibit supercoiling activity with a IC_{50} value of 40 μg/ml. The other two compounds tested, that is, **7e** and **7i**, have IC_{50} values >50 μg/ml, which is much higher than that of the positive control moxifloxacin though these compounds show comparable cell killing activity against MTB, MDR-MTB and MC^2.

2.6. Phototoxic evaluation

Quinolones in general have favorable safety profiles; phototoxicity has become a significant factor in the clinical use of some[14] quinolones. Indeed, the first quinolone, nalidixic acid, caused light-induced dermal effects. This type of response has now been demonstrated for almost all Fluoroquinolones,[15] 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.[16] 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.

Three (**7j**, **7l**, **7m**) compounds were evaluated for potential phototoxicity in a standardized in-vivo test system that has been used previously to assess quinolone antibiotics.[17] 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 sep-

Table 2. Physical constants, in-vitro antimycobacterial activities, and cytotoxicity



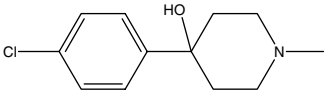
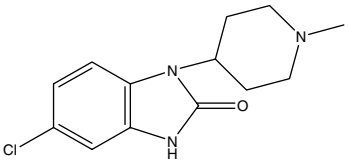
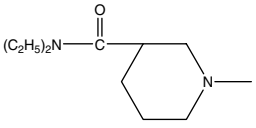
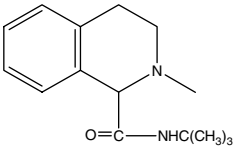
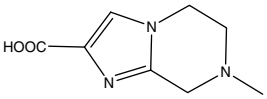
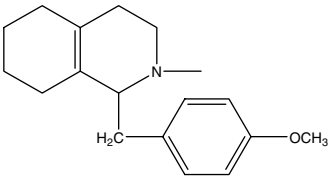
Compound	R	R_1	Yield (%)	Mp (°C)	IC_{50} (μM)	MIC (μM)		
						MTB	MDRTB	MC^2
7j	F		79	229–232	119.5	0.36	0.36	2.98
8j	NO_2		70	214–215	113.6	2.84	2.84	90.91
7k	F		75	233–235	NT	2.77	5.56	44.41
8k	NO_2		74	224–225	NT	5.31	NT	84.75
7l	F		70	256–257	126.1	0.18	0.08	3.15
8l	NO_2		77	227–230	59.8	2.99	0.75	95.68
7m	F		78	280–283	>137.5	0.86	0.86	6.89
8m	NO_2		74	198–199	129.8	1.62	0.39	103.85
7n	F		71	271–272	115.0	2.87	1.43	45.99
8n	NO_2		76	202–205	NT	2.73	5.49	43.81
7o	F		80	263–265	>130.6	3.26	0.39	52.25
8o	NO_2		79	255–258	123.65	1.54	0.77	98.92
7p	F		71	264–266	NT	5.50	NT	43.96
8p	NO_2		79	219–220	NT	10.49	NT	83.94

Table 2 (*continued*)

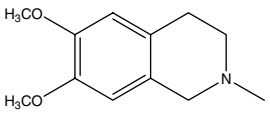
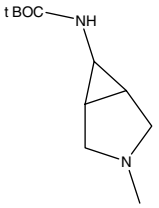
Compound	R	R_1	Yield (%)	Mp (°C)	IC_{50} (μM)	MIC (μM)		
						MTB	MDRTB	MC^2
7q	F		74	>300	>123.9	1.55	3.09	6.20
8q	NO_2		76	237–240	117.6	1.47	2.93	47.04
7r	F			258–259	>122.7	0.37	0.18	6.14
8r	NO_2			239–240	58.24	2.91	0.73	93.19
Gati	—	—	—	—	>155.3	1.04	8.34	2.08
INH	—	—	—	—	>455.8	0.36	45.57	45.57

Table 3. In vivo activity data of **7l**, 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
7l (50 mg/kg)	5.21 ± 0.12	5.9 ± 0.06

arately for each animal and time point and analyzed for statistical significance and are presented in Table 5. The drug and time factors were analyzed by separate univariate methods. Orthogonal contrasts were used to test for both linear and quadratic trends over time in each group by Student's *t*-tests to test whether the change from baseline ear thickness was significantly different from zero. The results indicated that lomefloxacin showed significant increase in ear thickness from 4 to 96 h and 24 to 96 h when compared within time points and with the control, respectively. No significant difference in ear thickness was observed with the test compounds at various time-points when compared with the pre-drug reading (0 h) and were non-toxic when compared with the negative (vehicle-treated) and positive controls (lomefloxacin). No erythema occurred in mice dosed with 140 mg/kg of **7j**, **7l**, and **7m** throughout the 96 h study.

3. Materials and methods

Melting points were taken on an electrothermal melting point apparatus (Buchi BM530) in open capillary tubes and are uncorrected. Infrared spectra (KBr disk) 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. Elemental analyses (C, H, and N) were performed on Perkin-Elmer model 240 C analyzer and the data were within ±0.4% of the theoretical values.

3.1. Synthesis of ethyl 2-(benzothiazol-2-yl)acetate (3)

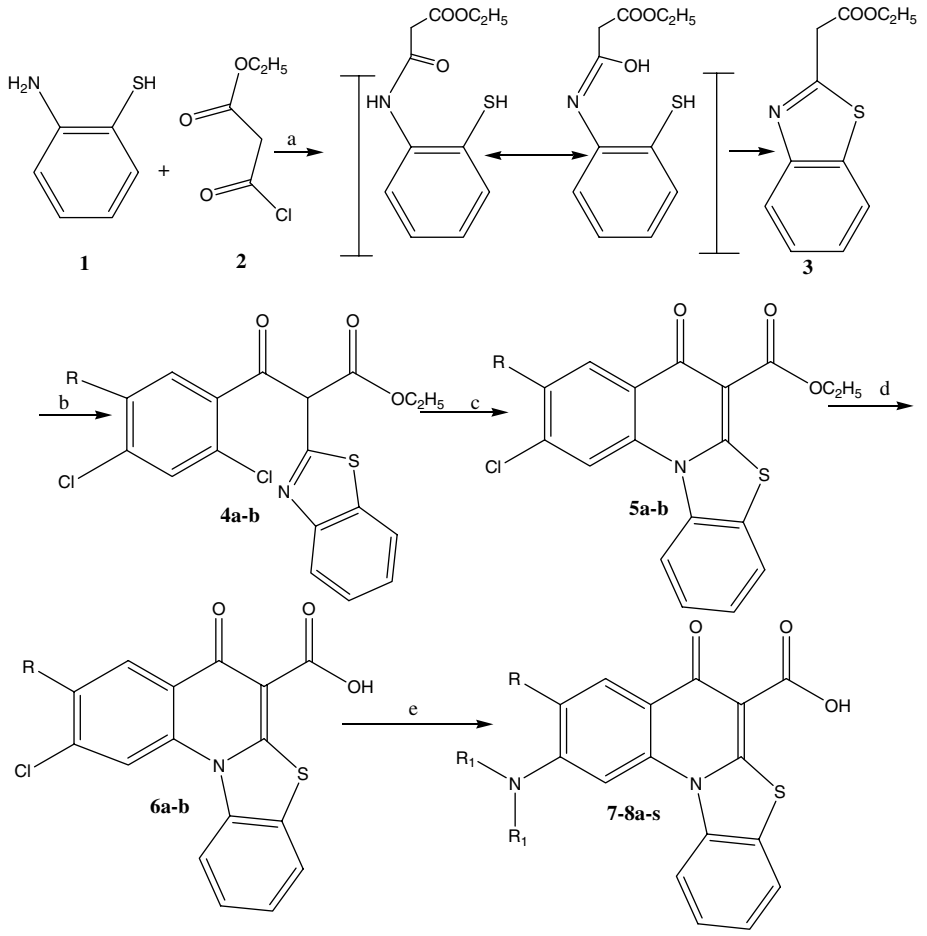
Ethyl malonyl chloride (5.0 g) (**2**) is added to a solution of 2-aminothiophenol (3.6 ml) (**1**) and triethylamine (4.6 ml) in anhydrous ether, and the reaction mixture is stirred for 3 h at room temperature. The triethylamine hydrochloride salt is filtered and washed with diethyl ether. The product is purified by distillation yielding 5.4 g of compound **3** (mp: 190–192 °C).

3.2. Synthesis of ethyl 3-(benzothiazol-2-yl)-4-(2,4-dichloro-5-fluoro/nitrophenyl)-2,4-dioxobutanoate (4a–b)

Compound **3** (5.4 g) dissolved in anhydrous tetrahydrofuran (100 ml) is added slowly to a cold solution of sodium hydride (60% in mineral oil) (1.1 g) suspended in anhydrous tetrahydrofuran. After stirring for 1 h, a solution of 2,4-dichloro-5-fluoro benzoyl chloride/2,4-dichloro-5-nitro benzoyl chloride in anhydrous tetrahydrofuran is added dropwise over a period of 15 min. The reaction mixture is stirred at 25 °C for 2 h. The solvent is removed under reduced pressure, and the residue is redissolved in ethyl acetate. The organic layer is washed with water and saturated aqueous sodium chloride, and then dried over magnesium sulfate. Removal of solvent in vacuo yields a solid which is recrystallized to give compounds **4a**–**b**. (**4a**: Yield: 83%; mp: 210–213 °C, **4b**: Yield: 79%; mp: 219–222 °C).

3.3. Synthesis of ethyl 2-chloro-3-fluoro/nitro-5,12-dihydro-5-oxobenzothiazolo[3,2-*a*]quinoline-6-carboxylic acid (5a–b)

Compounds **4a**–**b** (approximately 4.0 g) dissolved in ethylene glycol dimethyl ether (75 ml) are added slowly to a solution of sodium hydride (60% in mineral oil) (0.42 g)



Scheme 1. Synthetic protocol of compounds. Reagents: (a) $(C_2H_5)_3N$; (b) NaH, 2,4-dichloro-5-fluoro benzoyl chloride/2,4-dichloro-5-nitro benzoyl chloride; (c) NaH; (d) 2N NaOH; (e) MWI.

in ethylene glycol dimethy ether (75 ml). The mixture is heated at 160 °C for 30 min. The reaction mixture is allowed to cool to room temperature and then treated with cold water. The precipitate is collected and washed with water yielding compounds **5a**–**b** (**5a**: Yield: 62%; mp: 220–221 °C, **5b**: Yield: 67%; mp: 230–234 °C).

3.4. Synthesis of 2-chloro-3-fluoro/nitro-5,12-dihydro-5-oxobenzothiazolo[3,2-*a*]quinoline-6-carboxylic acid (6a–b)

Compounds **5a**–**b** (approximately 0.5 g) in tetrahydrofuran (100 ml) were treated with 2 N sodium hydroxide (15 ml) and the mixture was heated at 85 °C for 6 h and was cooled. The precipitate was filtered and washed with ether followed by cold water. The solid was dissolved in trifluoroacetic acid (15 ml) and water (500 ml) was added, and the precipitate was filtered yielding **6a**–**b** (**6a**: Yield: 92%; mp: >300 °C, **6b**: Yield: 94%; mp: >300 °C).

3.5. Synthesis of 2-(sub)-3-fluoro/nitro-5,12-dihydro-5-oxobenzothiazolo[3,2-*a*]quinoline-6-carboxylic acid (7–8a–s)

Compounds **6a**–**b** (1.0 equiv) in dimethylsulfoxide (2.5 ml) and appropriate secondary amines (4 equiv) were irradiated in a microwave oven at an intensity of 80% with 30 s/cycle. The number of cycle 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 **7**–**8a**–**r**.

3.5.1. 2-(4-((4-Chlorophenyl)(phenyl)methyl)piperazin-1-yl)-3-fluoro-5,12-dihydro-5-oxobenzothiazolo[3,2-*a*]quinoline-6-carboxylic acid (7a). Yield: 70%; mp: 253–255 °C; IR (KBr) cm^{-1}: 2910, 1722, 1710, 1620, 1460–1370; ^1H NMR (DMSO-d_6) δ ppm: 2.59–3.12 (m, 8H, CH_2 of piperazine), 4.22 (s, 1H, CH of diphenylmethyl), 5.9 (s, 1H, C_1-H), 6.3–7.15 (m, 13H, Ar-H), 7.26 (s, 1H, C_4-H), 14.2 (s, 1H, COOH); Anal. ($C_{33}H_{25}ClFN_3O_3S$) C, H, N.

3.5.2. 2-(4-(2-Furoyl)piperazin-1-yl)-3-nitro-5,12-dihydro-5-oxobenzothiazolo[3,2-*a*]quinoline-6-carboxylic acid (8b). Yield: 75%; mp: 207–210 °C; IR (KBr) cm^{-1}: 2890, 1710, 1724, 1624, 1460–1360; ^1H NMR (DMSO-d_6) δ ppm: 3.16–3.26 (m, 8H, CH_2 of piperazine), 5.9 (s, 1H, C_1-H), 6.32–7.68 (m, 7H, Ar-H), 7.26 (s, 1H, C_4-H), 14.2 (s, 1H, COOH); Anal. ($C_{25}H_{18}N_4O_7S$) C, H, N.

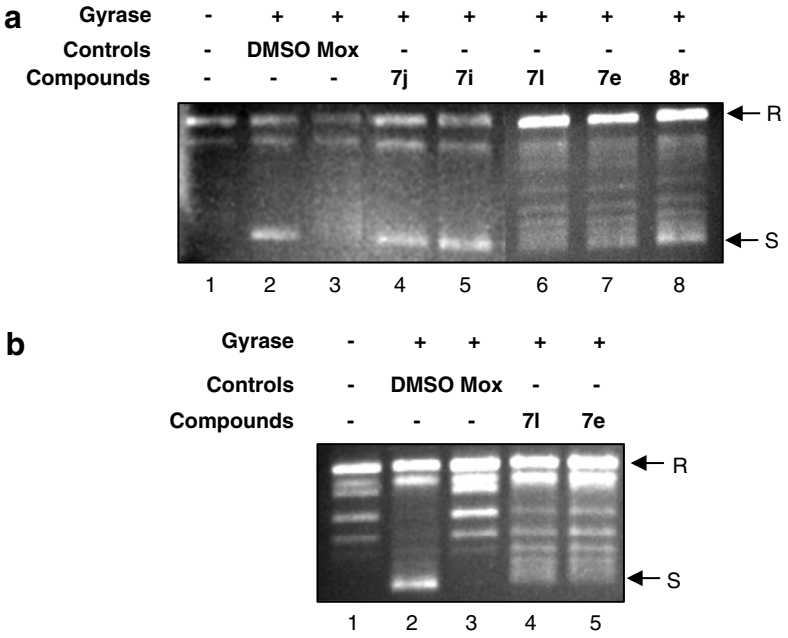


Figure 1. DNA gyrase supercoiling assay. The assays were carried out as described in Section 3. DNA gyrase was pre-incubated with the indicated concentrations of compounds in ice and then rest of the components of the reaction including relaxed DNA was added. (a) Lane 1: relaxed circular DNA, lane 2: supercoiling reaction in presence of 5% DMSO, lane 3: positive control for gyrase inhibition, moxifloxacin at 5 μg/ml concentration. Lanes 4–8: supercoiling reaction in presence of 50 μg/ml of compounds **7j**, **7i**, **7l**, **7e**, and **7r**, respectively. (b) Lane 1: relaxed circular DNA, lane 2: supercoiling reaction in presence of 5% DMSO, lane 3: moxifloxacin at 5 μg/ml concentration. Lanes 4 and 5: supercoiling reaction in presence of 40 μg/ml of compounds **7l** and **7e**, respectively. R and S indicate relaxed and supercoiled DNA, respectively.

Table 4. IC_{50} values for DNA gyrase inhibition

Compounds	IC_{50} (μg/ml)
7e	>50
7i	>50
7j	40
7l	40
7r	50

3.5.3. 2-(4-((Benzo[d][1,3]dioxol-6-yl)methyl)piperazin-1-yl)-3-fluoro-5,12-dihydro-5-oxobenzothiazolo[3,2-*a*]quinoline-6-carboxylic acid (7c). Yield: 75%; mp: 181–182 °C; IR (KBr) cm^{-1}: 3250, 2890, 1710, 1724, 1624, 1464–1360; ^1H 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–), 5.9 (s, 1H, C_1-H), 6.40–7.1 (m, 7H, Ar-H), 7.28 (s, 1H, C_4-H), 14.2 (s, 1H, COOH); Anal. ($C_{28}H_{22}FN_3O_5S$) C, H, N.

3.5.4. 2-(4-Methyl-3-phenylpiperazin-1-yl)-3-nitro-5,12-dihydro-5-oxobenzothiazolo[3,2-*a*]quinoline-6-carboxylic acid (8d). Yield: 73%; mp: 287–290 °C; IR (KBr) cm^{-1}: 2890, 1710, 1724, 1620, 1460–1368; ^1H 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), 5.9 (s, 1H, C_1-H), 6.3–7.2 (m, 9H, Ar-H), 7.3 (s, 1H, C_4-H), 14.4 (s, 1H, COOH); Anal. ($C_{27}H_{22}N_4O_5S$) C, H, N.

3.5.5. 2-(4-(2,3-Dihydrobenzo[b][1,4]dioxin-2-oyl)(piperazin-1-yl)-3-fluoro-5,12-dihydro-5-oxobenzothiazolo[3,2-*a*]quinoline-6-carboxylic acid (7e). Yield: 77%; mp: 237–

Table 5. Phototoxic evaluation of newer 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]	0.29 ± 0.02	0.29 ± 0.01	0.32 ± 0.02	0.31 ± 0.01	0.34 ± 0.03	0.34 ± 0.03	0	0	0	0	0	0
7j	0.29 ± 0.01	0.32 ± 0.03	0.31 ± 0.02	0.29 ± 0.02	0.32 ± 0.02	0.30 ± 0.02	0	0	0	0	0	0
7l	0.27 ± 0.01	0.29 ± 0.02	0.29 ± 0.02	0.29 ± 0.01	0.30 ± 0.01	0.28 ± 0.01	0	2	0	0	0	0
7m	0.29 ± 0.01	0.30 ± 0.02	0.31 ± 0.01	0.30 ± 0.01	0.28 ± 0.02	0.30 ± 0.02	0	0	0	0	0	0
Lomefloxacin	0.31 ± 0.01	0.40 ± 0.02	0.48 ± 0.02	0.53 ± 0.02	0.64 ± 0.04	0.60 ± 0.06	0	6	6	6	6	6

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

238 °C; IR (KBr) cm^{-1}: 2890, 1710, 1724, 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), 5.9 (s, 1H, C_1-H), 6.3–7.12 (m, 8H, Ar-H), 7.3 (s, 1H, C_4-H), 14.2 (s, 1H, COOH); Anal. ($C_{29}H_{22}FN_3O_6S$) C, H, N.

3.5.6. 2-(3-(2,6-Difluorophenyl)-5-methlisoxazol-4-oyl)(piperazin-1-yl)-3-fluoro-5,12-dihydro-5-oxobenzothiazolo[3,2-*a*]-quinoline-6-carboxylic acid (7f). Yield: 75%; mp: >300 °C; IR (KBr) cm^{-1}: 2890, 1710, 1724, 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), 5.9 (s, 1H, C_1-H), 6.32–7.08 (m, 7H, Ar-H), 7.3 (s, 1H, C_4-H), 14.2 (s, 1H, COOH); Anal. ($C_{31}H_{21}F_3N_4O_5s$) C, H, N.

3.5.7. 2-Thiomorpholino-3-nitro-5,12-dihydro-5-oxobenzothiazolo[3,2-*a*]quinoline-6-carboxylic acid (8g). Yield: 74%; mp: 268–271 °C; IR (KBr) cm^{-1}: 2890, 1710, 1724, 1620, 1460–1368; ^1H NMR (DMSO-d_6) δ ppm: 2.64 (t, 4H, 3,5-CH_2 of thiomorpholine), 3.38 (t, 4H, 2,6-CH_2 of thiomorpholine), 5.9 (s, 1H, C_1-H), 6.32–6.88 (m, 4H, Ar-H), 7.3 (s, 1H, C_4-H), 14.2 (s, 1H, COOH); Anal. ($C_{20}H_{15}N_3O_5S_2$) C, H, N.

3.5.8. 2-(2,6-Dimethylmorpholino)-3-fluoro-5,12-dihydro-5-oxobenzothiazolo[3,2-*a*]quinoline-6-carboxylic acid (7h). Yield: 70%; mp: 258–260 °C; IR (KBr) cm^{-1}: 2892, 1710, 1724, 1628, 1460–1360; ^1H 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), 5.9 (s, 1H, C_1-H), 6.4–6.9 (m, 4H, Ar-H), 7.3 (s, 1H, C_4-H), 14.2 (s, 1H, COOH); Anal. ($C_{22}H_{19}FN_2O_4S$) C, H, N.

3.5.9. 2-(4-(Piperidin-1-yl)piperidin-1-yl)-3-nitro-5,12-dihydro-5-oxobenzothiazolo[3,2-*a*]quinoline-6-carboxylic acid (8i). Yield: 75%; mp: 294–296 °C; IR (KBr) cm^{-1}: 2890, 1710, 1724, 1624, 1464–1360; ^1H NMR (DMSO-d_6) δ ppm: 1.5–1.6 (m, 10H, 5 CH_2), 2.2 (t, 4H, 2 CH_2), 2.7 (m, 1H, CH), 2.8 (t, 4H, 2 CH_2), 5.9 (s, 1H, C_1-H), 6.4–6.88 (m, 4H, Ar-H), 7.3 (s, 1H, C_4-H), 14.2 (s, 1H, COOH); Anal. ($C_{26}H_{26}N_4O_5S$) C, H, N.

3.5.10. 2-(4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl)-3-fluoro-5,12-dihydro-5-oxobenzothiazolo[3,2-*a*]quinoline-6-carboxylic acid (7j). Yield: 79%; mp: 229–232 °C; IR (KBr) cm^{-1}: 2900, 1708, 1724, 1620, 1460–1370; ^1H NMR (DMSO-d_6) δ ppm: 2.0 (t, 4H, 3,5-CH_2 of piperidine), 2.7 (t, 4H, 2,6-CH_2 of piperidine), 5.9 (s, 1H, C_1-H), 6.28–7.18 (m, 8H, Ar-H), 7.3 (s, 1H, C_4-H), 10.0 (bs, 1H, OH), 14.62 (s, 1H, COOH); Anal. ($C_{27}H_{20}ClFN_2O_4S$) C, H, N.

3.5.11. 2-(4-(6-Chloro-1,2-dihydro-2-oxobenzo[d]imidazol-3-yl)piperidin-1-yl)-3-nitro-5,12-dihydro-5-oxobenzothiazolo[3,2-*a*]quinoline-6-carboxylic acid (8k). Yield: 74%; mp: 224–225 °C; IR (KBr) cm^{-1}: 3110, 2896, 1712, 1728, 1710, 1624, 1460–1360; ^1H NMR (DMSO-d_6) δ ppm: 1.6–2.4 (m, 8H, 4 CH_2 of piperidine), 4.1 (bm, 1H, CH of piperidine), 5.9 (s, 1H, C_1-H), 6.2–6.9 (m, 7H, Ar-H), 7.3 (s, 1H, C_4-H), 10.8 (s, 1H, NH), 14.2 (s, 1H, COOH); Anal. ($C_{28}H_{20}ClN_5O_6S$) C, H, N.

3.5.12. 2-(3-(Diethylcarbamoyl)piperidin-1-yl)-3-fluoro-5,12-dihydro-5-oxobenzothiazolo[3,2-*a*]quinoline-6-carboxylic acid (7l). Yield: 70%; mp: 256–257 °C; IR (KBr) cm^{-1}: 2890, 1710, 1724, 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), 5.9 (s, 1H, C_1-H), 6.2–6.8 (m, 4H, Ar-H), 7.3 (s, 1H, C_4-H), 14.2 (s, 1H, COOH); Anal. ($C_{26}H_{26}FN_3O_4S$) C, H, N.

3.5.13. 2-(1,4-Dioxa-8-azaspiro[4.5]dec-8-yl)-3-nitro-5,12-dihydro-5-oxobenzothiazolo[3,2-*a*]quinoline-6-carboxylic acid (8m). Yield: 74%; mp: 198–199 °C; IR (KBr) cm^{-1}: 2890, 1712, 1724, 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), 5.9 (s, 1H, C_1-H), 6.2–6.82 (m, 4H, Ar-H), 7.32 (s, 1H, C_4-H), 14.4 (s, 1H, COOH); Anal. ($C_{23}H_{19}N_3O_7S$) C, H, N.

3.5.14. 2-(1-(*tert*-Butylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-fluoro-5,12-dihydro-5-oxobenzothiazolo[3,2-*a*]quinoline-6-carboxylic acid (7n). Yield: 71%; mp: 271–272 °C; IR (KBr) cm^{-1}: 2890, 1712, 1724, 1626, 1468–1360; ^1H 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), 5.88 (s, 1H, C_1-H), 6.28–7.1 (m, 8H, Ar-H), 7.32 (s, 1H, C_4-H), 10.2 (s, 1H, NH), 14.2 (s, 1H, COOH); Anal. ($C_{30}H_{26}FN_3O_4S$) C, H, N.

3.5.15. 2-(2-Carboxy-5,6-dihydroimidazo[1,2-*a*]pyrazin-7(8H)-yl)-3-nitro-5,12-dihydro-5-oxobenzothiazolo[3,2-*a*]quinoline-6-carboxylic acid (8o). Yield: 79%; mp: 255–258 °C; IR (KBr) cm^{-1}: 3200, 2890, 1710, 1724, 1624, 1464–1360, 1208; ^1H NMR (DMSO-d_6) δ ppm: 3.1–3.8 (m, 6H, 3-CH_2), 5.88 (s, 1H, C_1-H), 6.28–7.0 (m, 4H, Ar-H), 7.6 (s, 1H, CH), 7.32 (s, 1H, C_4-H), 12.12 (s, 1H, 2-COOH), 14.6 (s, 1H, 3-COOH); Anal. ($C_{23}H_{15}N_5O_7S$) C, H, N.

3.5.16. 2-(8-(4-Methoxybenzyl)-3,4,5,6,7,8-hexahydroisoquinolin-2(1H)-yl)-3-fluoro-5,12-dihydro-5-oxobenzothiazolo[3,2-*a*]quinoline-6-carboxylic acid (7p). Yield: 79%; mp: 219–220 °C; IR (KBr) cm^{-1}: 3210, 2890, 1710, 1724, 1620, 1460–1368; ^1H 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), 5.88 (s, 1H, C_1-H), 6.28–7.1 (m, 8H, Ar-H), 7.32 (s, 1H, C_4-H), 14.2 (s, 1H, COOH); Anal. ($C_{33}H_{29}FN_2O_4S$) C, H, N.

3.5.17. 2-(3,4-Dihydro-6,7-dimethoxyisoquinolin-2(1H)-yl)-3-nitro-5,12-dihydro-5-oxobenzothiazolo[3,2-*a*]quinoline-6-carboxylic acid (8q). Yield: 76%; mp: 237–240 °C; IR (KBr) cm^{-1}: 3210, 2890, 1710, 1724, 1620, 1460–1368; ^1H NMR (DMSO-d_6) δ ppm: 2.72–3.6 (m, 6H, CH_2 of isoquinolinyl), 3.7 (s, 6H, –OCH_3), 5.88 (s, 1H, C_1–H), 6.3–6.93 (m, 6H, Ar-H), 7.32 (s, 1H, C_4-H), 14.2 (s, 1H, COOH); Anal. ($C_{27}H_{21}N_3O_7S$) C, H, N.

3.5.18. 2-(6-tert-Butoxycarbonylamino-3-azabicyclo[3.1.0]-hex-3-yl)-3-nitro-5,12-dihydro-5-oxobenzothiazolo[3,2-*a*]-quinoline-6-carboxylic acid (8r). Yield: 76%; mp: 239–240 °C; IR (KBr) cm^{-1}: 3210, 2890, 1710, 1724, 1620, 1460–1368; ^1H NMR (DMSO-d_6) δ ppm: 1.43 (s, 9H, *tert*-butyl), 1.78–3.8 (m, 7H, azabicyclohexane ring H), 4.76 (bs, 1H, NH-*t*-Boc), 5.88 (s, 1H, C_1-H), 6.3–6.8 (m, 4H, Ar-H), 7.32 (s, 1H, C_4-H), 14.2 (s, 1H, COOH); Anal. ($C_{26}H_{24}N_4O_7S$) C, H, N.

3.6. In-vitro antimycobacterial activity

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

3.7. Cytotoxicity

Some compounds were further examined for toxicity (IC_{50}) in a mammalian Vero cell line at concentrations of 62.5 μg/ml. After 72 h of exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega Cell Titer 96 non-radioactive cell proliferation assay.

3.8. In-vivo antimycobacterial activity

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 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 was continued for 10 days. After 35 days post infection the spleens and right lungs were aseptically removed and ground in a tissue homogenizer, the number of viable organisms was determined by serial 10-fold dilutions and subsequent inoculation onto 7H10 agar plates. Cultures were incubated at 37 °C in ambient air for 4 weeks prior to counting. Bacterial counts were measured and compared with the counts from negative controls (vehicle treated) in lung and in spleen.

3.9. DNA gyrase supercoiling assay

DNA gyrase was purified from *M. smegmatis* cells using the method described by Manjunatha et al.[18] The supercoiling assays were carried out by the procedure already described.[11] Briefly, supercoiling assays were carried out by incubating 400 ng of relaxed circular pUC18 in supercoiling buffer (35 mM Tris–HCl, pH 7.5, 5 mM $MgCl_2$, 25 mM potassium glutamate, 2 mM spermidine, 2 mM ATP, 50 μg/ml bovine serum albumin, and 90 μg/ml yeast *t*-RNA in 5% (v/v) glycerol) for 30 min. The compounds tested were dissolved in DMSO and pre-incubated with gyrase in reaction buffer prior to the addition of DNA. Moxifloxacin at final concentration of 5 μg/ml was used as a positive control and another control reaction having 5% DMSO in absence of compounds was also performed. The reaction samples were heat-inactivated at 65 °C for 15 min and applied onto 1% agarose gel for electrophoresis in Tris–acetate–EDTA buffer for 12 h. The gels were stained with ethidium bromide.

3.10. Phototoxicity evaluation

Female swiss albino mice, approximately 2 months old and weighing 20–25 g, were used in this study. Before oral dosing, they were fasted overnight for at least 18 h. Food was returned at the end of the 4 h photo-irradiation period. Eighteen mice were randomly distributed into three dosing groups. First group received a single dose of screened compound at 140 mg/kg by oral gavage. A second group received a single dose of 140 mg of lomefloxacin HCl/kg. This lomefloxacin dose is one that, in preliminary experiments in this test system, produced a consistent erythema and ear thickening response. The final group served as a vehicle control and received 10 ml/kg of the methylcellulose vehicle only. Test animals were exposed to UV light in a manner adapted from that described previously. Animals were irradiated for 4 h, equal to a total UV light irradiation of approximately 18 J/cm^2. Before dosing, at the end of the irradiation period, and at approximately 24, 48, 72, and 96 h after dosing, both ears of each mouse were evaluated for changes indicative of a positive response: erythema, edema or a measurable increase in ear thickness.

Acknowledgments

The authors are thankful to Department of Biotechnology (BT/01/COE/05/06/01), Government of India, for their financial assistances. The authors are also thankful to Dr. Vanaja Kumar, Deputy Director, Tuberculosis Research Center, Chennai, India, for their assistance in biological screening.

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