Synthesis, Antimycobacterial Activities and Phototoxic Evaluation of 5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylic Acid Derivatives

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Abstract: Thirty four novel 7-fluoro/nitro-1,2-dihydro-5-oxo-8-(sub)-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylic acids were synthesized from 2,4-dichlorobenzoic acid and 2,4-dichloro-5-fluoroacetophenone by multi 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* (MC^2) and also tested for the ability to inhibit the supercoiling activity of DNA gyrase from *M. smegmatis*. Among the synthesized compounds, 8-[6-[[(1,1-dimethylethoxy)carbonyl]amino]-3-azabicyclo[3.1.0]hex-3-yl]-1,2-dihydro-7-nitro-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylic acid (**10q**) was found to be the most active compound *in vitro* with MIC of 0.08 μM and <0.08 μM against MTB and MDR-TB respectively. Compound **10q** was found to be 4.5 and >570 times more potent than isoniazid against MTB and MDR-TB respectively. In the *in vivo* animal model **10q** decreased the bacterial load in lung and spleen tissues with 2.51 and 3.71-log10 protections respectively at the dose of 50 mg/kg body weight.

Key Words: Antimycobacterial activity, antitubercular activity, tuberculosis, thiazoloquinolone.

INTRODUCTION

Tuberculosis is a global health problem, currently infecting over one-third of the world's population and resulting in over 2-million deaths annually [1,2]. Although the incidence of tuberculosis (TB) declined for decades due to effective drug treatment, the emergence of drug-resistant strains of *Mycobacterium tuberculosis* (MTB) and the advent of HIV-TB co-infection has led to a global increase in TB-related morbidity and mortality [2]. A short-course treatment regimen for TB includes at least three drugs taken over a period of 6–9 months [1]. Failure to adhere to the treatment regimen due to the long duration and complexity of treatment is associated with development of antibiotic-resistant strains of MTB [3,4]. New antitubercular drugs are needed to treat antibiotic resistant strains of MTB and simplify the current treatment regimen [5]. The scope and severity of TB infection warrants the development of antimicrobials specifically for use against MTB. Fluoroquinolones are used for the clinical control of multi-drug resistant tuberculosis (MDR-TB) i.e. tuberculosis due to bacilli that are resistant to both isoniazid and rifampin. Several of the quinolone antibacterials such as gatifloxacin, moxifloxacin, sitafloxacin, have been examined as inhibitors of *Mycobacterium tuberculosis* (MTB), as well as other mycobacterial infections [6]. Quinolones inhibit bacterial type II topoisomerase, DNA gyrase and topoisomerase IV [7], which are essential enzymes that maintain the supercoils in DNA. The incidence of mycobacterial resistance to fluoroquinolones is relatively low at the present time, and there are no reports of cross-resistance or antagonism with other classes of antimycobacterial agents [8]. As a part of the study attempting to further optimize the quinolone antibacterials against MTB [9,10], we have identified novel 5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylic acid derivatives with potent antimycobacterial activities. Herewith we report results that describe concerning the synthesis, *in vitro* and *in vivo* antimycobacterial activity of first representative compounds of this family together with toxicological results. One of the compounds in the series i.e. **10q** was found to be very effective by inhibiting cell growth at very low concentrations.

CHEMISTRY

The synthesis of the titled compounds **9**, **10a-q** was accomplished as outlined in Scheme **1**. The 2,4-dichlorobenzoic acid (**1**) was converted to 2,4-dichloro-5-nitrobenzoic acid (**2**) by treatment with nitric acid in presence of sulphuric acid at 0-5 ºC. 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 presence of triethylamine to yield ethyl 3-(2,4-dichloro-5-nitrophenyl)-3-oxopropanoate (**4a**). Compound ethyl 2,4-dichloro-5-fluorobenzoylacetate **(4b)** was prepared by condensation of 2,4-dichloro-5-fluoroacetophenone (**3**) with diethyl carbonate in the presence of sodium hydride. The reaction of β-ketoesters ethyl 3-(2,4-dichloro-5-fluoro/nitrophenyl)-3-oxopropanoate (**4a-b**) with sodium hydride, carbon disulfide and methyl iodide in N, N-dimethylacetamide produces methyl sulfanyl ethyl α-[bis(methylthio)methylene]-2,4-dichloro-5-fluoro-β-oxo-benzenepropanoic acids (**5a-b**). The reactions of the dithioacetal with cysteamine in toluene afford ethyl 2,4-dichloro-α-[dihydro-2(3H)-thiazolyliden]-5-fluoro/nitro-β-oxo-benzenepropanoic acid (**6a-b**). Their intramolecular

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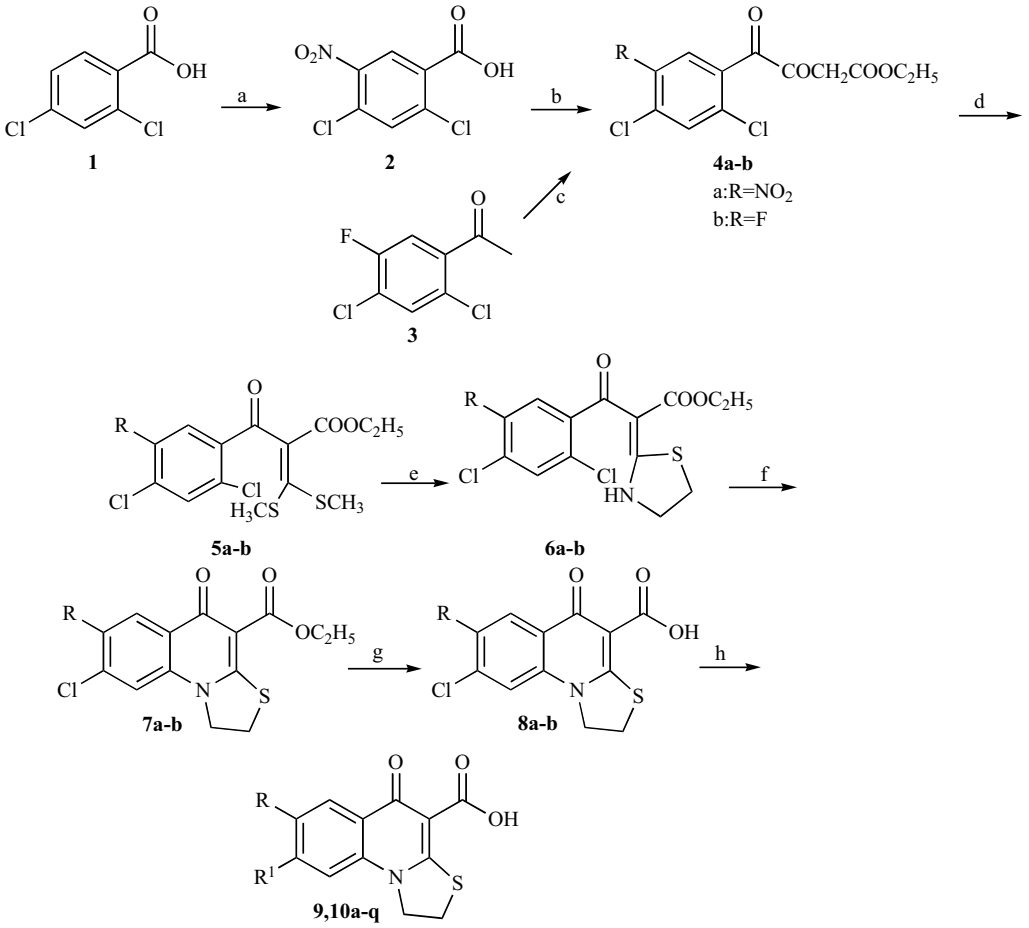


Scheme 1. Synthetic protocol of the compounds.

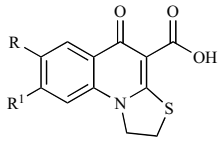
cyclisation in the presence of potassium carbonate gives rise to ethyl 8-chloro-7-fluoro/nitro-1,2-dihydro-5-oxo-5H-thiazolo[3,2-a]quinoline-4-carboxylic acid (**7a-b**). Acid hydrolysis of esters with 6M HCl, **7a-b** gives the corresponding carboxylic acids **8a-b**. The titled compounds **9, 10a-q** were prepared by treating **8a-b** with appropriate secondary amines under microwave irradiation in DMSO. When compared to conventional method [11] of 4 hours process, microwave assisted synthesis was performed with short reaction times (2-3 minutes), 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.

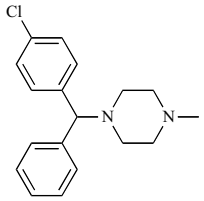
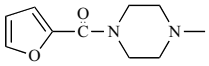
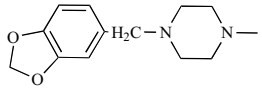
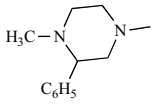
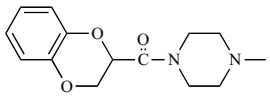
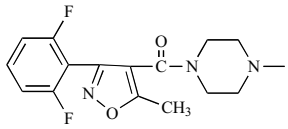
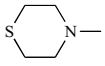
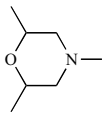
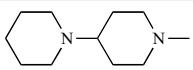
IN VITRO ANTIMYCOBACTERIAL ACTIVITY

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 duplicates [12]. 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 MIC's of the synthesized compounds along with the standard drugs for comparison are reported in Tables **1** & **2**.

In the first phase of screening against MTB, all the compounds showed excellent *in vitro* activity against MTB with MIC ranging from 0.08-11.61μM. Eleven compounds (**9f**, **9h, 10h, 9k, 10k, 9m, 10m, 10n, 9o, 10o,** and **10q**) 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), six compounds (**9h, 10h, 8k, 10m, 9o,** and **10q**) were found to be more active against MTB. Compound 8-[6-[[(1,1-dimethylethoxy)carbonyl]amino]-3-azabicyclo[3.1.0]hex-3-yl]-1,2-dihydro-7-nitro-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylic acid (**10q**) was found to be the most active compound *in vitro* with MIC of 0.08 μM against MTB and was 4.5 and 13 times more potent than isoniazid and gatifloxacin respectively. Subsequently some of the compounds were evaluated against MDR-TB, and among the twenty six compounds screened; all the compounds inhibited MDR-TB with MIC ranging from <0.08-8.54 μM and were found to be more active than isoniazid (MIC: 45.57 μM), and gatifloxacin (MIC: 8.34 μM) except compound **9g**. Ten compounds (**9f**, **10h, 10k, 9m, 10m, 10n, 9o, 10o, 9q** and **10q**) inhibited MDR-TB with MIC of less than 1 μM. Compound **10q** 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

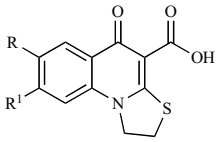
Table 1. Physical Constants, *In Vitro* Antimycobacterial Activities and Cytotoxicity

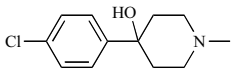
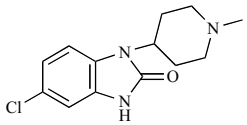
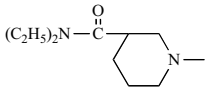
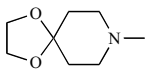
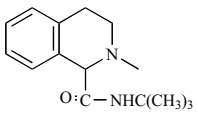
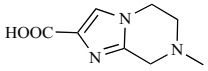
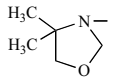
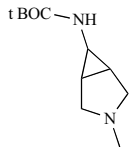


No	R	R^1	Yield (%)	M.P. (°C)	IC_{50} (µM)	MIC (µM)		
						MTB	MDRTB	MC[2]
9a	- F		82.1	271-273	NT	5.69	NT	22.73
10a	- NO_2	- do -	78.6	250-252	NT	10.83	NT	43.32
9b	- F		75.4	218-220	>140.9	1.76	1.76	14.09
10b	- NO_2	- do -	69.0	188-190	132.8	3.32	1.66	13.28
9c	- F		81.8	215-217	>129.3	1.61	1.61	3.23
10c	- NO_2	- do -	84.3	210-212	NT	6.13	NT	24.48
9d	- F		76.5	212-214	142.2	3.55	1.77	14.22
10d	- NO_2	- do -	72.1	251-253	NT	6.71	NT	26.79
9e	- F		69.5	224-226	NT	6.12	NT	12.22
10e	- NO_2	- do -	70.6	210-212	NT	11.61	NT	11.61
9f	- F		64.2	208-210	>109.5	0.68	0.34	1.37
10f	- NO_2	- do -	69.8	260-262	NT	10.46	NT	83.68
9g	- F		84.2	280-282	NT	4.26	8.54	17.06
10g	- NO_2	- do -	74.6	191-193	NT	3.97	7.96	31.77
9h	- F		63.2	277-279	>165.1	0.24	1.03	2.06
10h	- NO_2	- do -	74.3	194-196	154.2	0.22	0.96	15.42
9i	- F		84.7	263-265	NT	3.62	3.62	14.48
10i	- NO_2	- do -	69.5	240-242	136.3	3.40	1.70	27.26

NT indicates not tested.

Table 2. Physical Constants, *In Vitro* Antimycobacterial Activities and Cytotoxicity



No	R	R^1	Yield (%)	M.P. (°C)	IC_{50} (μM)	MIC (μM)		
						MTB	MDRTB	MC^2
9j	- F		75.6	220-222	NT	6.59	NT	26.32
10j	- NO_2	- do -	80.2	190-192	62.2	1.55	1.55	12.45
9k	- F		69.5	205-207	121.4	0.76	1.51	3.03
10k	- NO_2	- do -	70.2	205-207	57.6	0.35	0.72	46.13
9l	- F		63.4	>300	139.7	3.49	1.74	13.97
10l	- NO_2	- do -	74.6	228-230	65.8	1.64	1.64	26.34
9m	- F		63.2	225-227	>153.8	0.47	0.22	1.92
10m	- NO_2	- do -	86.2	198-200	144.2	0.09	0.09	28.84
9n	- F		69.4	291-293	NT	3.15	1.57	12.61
10n	- NO_2	- do -	60.8	175-177	119.6	0.73	0.36	47.84
9o	- F		68.8	258-260	>145.2	0.21	0.09	1.81
10o	- NO_2	- do -	65.6	210-212	136.6	0.42	0.20	27.33
9p	- F		75.4	250-252	NT	2.14	2.14	34.30
10p	- NO_2	- do -	61.2	200-202	159.7	1.99	3.99	15.97
9q	- F		74.6	241-243	>135.4	3.38	0.41	27.09
10q	- NO_2	- do -	78.6	281-283	>127.9	0.08	<0.08	1.59
Gati	-	-	-	-	>155.3	1.04	8.34	2.08
INH	-	-	-	-	>455.8	0.36	45.57	45.57

NT indicates not tested.

were also evaluated against MC^2 in which all the compounds inhibited MC^2 with MIC ranging from 1.59-83.68 μM and thirty one compounds were found to be more active than isoniazid (MIC: 45.57 μM).

With respect to structure-MTB activity relationship, at C_8 position we have studied with various substituted piperazines (**9-10a-f**), (thio) morpholines (**9-10g-h**), substituted piperidines (**9-10i-l**), fused piperazines, piperidines, and pyrrolidine

(**8-10m-o** and **q**) and oxazolidine (**9-10p**). The results demonstrated that the contribution of the C_8 position to antimycobacterial activity was dependent on the substituent at C_7 and was in the order of fused piperazines, piperidines, and pyrrolidine > (thio) morpholines > substituted piperazines > substituted piperidines when C_7 was fluoro substituent; fused piperazines, piperidines, and pyrrolidine > substituted piperidines > (thio) morpholines > sub piperazines when C_7 was nitro group. Some compounds (**10b, 9f, 9m, 9n, 9o, 10o, 9q** and **10q**) endowed with high activity towards MDR-TB rather than MTB.

IN VITRO CYTOTOXICITY

Some compounds were further examined for toxicity (IC_{50}) in mammalian Vero cell line till 62.5 μg/mL concentration. 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 [13] and the results are reported in Tables **1** & **2**. Twenty one compounds when tested showed IC_{50} values ranging from 57.6->165.1 μM. A comparison of the substitution pattern at C_8 demonstrated that piperidine-substituted analogs were more cytotoxic than the substituted piperazines. These results are important as the piperdine substituted compounds with their increased cytoliability, are much less attractive in the development of a quinolone for the treatment of TB. This is primarily 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 compound **10q** was found to be non-toxic up to 62.5 μg/mL (127.9μM) and showed selectivity index (IC_{50}/MIC) of more than 1598.

IN VIVO ANTIMYCOBACTERIAL ACTIVITY

Subsequently, compound **10q** was tested for efficacy against MTB at a dose of 50 mg/kg (Table **3**) in CD-1 mice [10]. In this model, the mice were infected intravenously with *M. tuberculosis* ATCC 35801. Drug treatment by intra peritoneal route began after 10 days of inoculation of the animal with microorganism and continued for 10 days. After 35 days post infection the spleens and right lungs were aseptically removed, the number of viable organisms were determined and compared with the counts from negative (vehicle treated) controls (Mean culture forming units (CFU) in lung: 7.99 ± 0.16 and in spleen: 9.02 ± 0.21). Compound **10q** decreased the bacterial load in lung and spleen tissues with 2.51 and 3.71-log10 protections respectively and was considered to be promising in reducing bacterial count in lung and spleen tissues. When compared to gatifloxacin at the same dose level **10q** decreased the bacterial load with 0.54 and 1.61-log10 protections in lung and spleen tissues respectively. Compound **10q** was found to be less active than isoniazid in the *in vivo* study. The reason for this less *in vivo* activity might be due to the low bioavailability of the compound.

DNA GYRASE INHIBITION

The thiazolo quinolone 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 transactions processes [14]. 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. Earlier studies have revealed that DNA gyrase from *M. tuberculosis* and *M. smegmatis* are highly similar at protein level, antigenic properties and catalytic activities [15]. The supercoiling assay results with various compounds using MC^2 DNA gyrase is presented in Fig. **1a** and **1b**. The IC_{50} values are presented in Table **4**. Amongst the tested compounds, compound **10q** inhibits supercoiling reaction with an IC_{50} value of 30 μg/ml. The compound **10q** also showed highest *in vitro* efficacy against MTB, MDR-TB and MC^2 of all the compounds tested in this report. The other three compounds tested for supercoiling inhibition, i.e., **7c, 9k** and **9o** have lower IC_{50} and are somewhat less effective inhibitors of the supercoiling reaction. Since selected compounds showed only weak inhibition activity against target enzyme, it is supposed that the mode of action of these compounds is different from inhibition of DNA gyrase.

PHOTOTOXIC EVALUATION

Quinolones in general have favorable safety profiles; phototoxicity has become a significant factor in the clinical use of some [16] quinolones. Indeed, the first quinolone, nalidixic acid, caused light-induced dermal effects. This type of response has now been demonstrated for almost all fluoroquinolones [17], although the relative phototoxic potential varies greatly among compounds. Phototoxicity is

Table 3. ***In Vivo*** **Activity Data of 10q, Gatifloxacin and Isoniazid Against M.** ***tuberculosis*** **ATCC 35801 in Mice**

Compound	Lungs (log CFU ± SEM)	Spleen (log CFU ± SEM)
Control	7.99 ± 0.16	9.02 ± 0.21
Gatifloxacin (50 mg/kg)	6.02 ± 0.23	6.92 ± 0.07
Isoniazid (25 mg/kg)	5.86 ± 0.23	4.71 ± 0.10
10q (50 mg/kg)	5.48± 0.19	5.31± 0.13

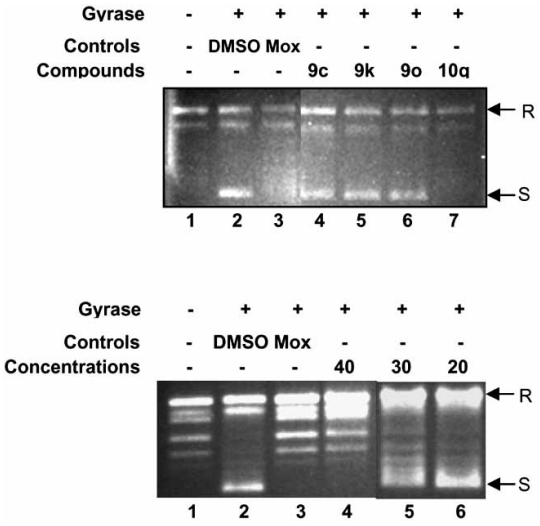


Fig. (1). DNA gyrase supercoiling assay. The assays were carried out as described in Materials and Methods. DNA gyrase was pre incubated with the indicated concentrations of compounds in ice and then rest of the components of the reaction including relaxed DNA was added. (a) Lane1: relaxed circular DNA, lane 2: supercoiling reaction in presence of 5% DMSO, Lane 3: Moxifloxacin at 5μg/ml concentration is used as a positive control. Lanes 4 to 7: reactions in presence of 50 μg/ml of compounds **9c, 9k, 9o and 10q** respectively. (b) Lane1: relaxed circular DNA, lane 2: supercoiling reaction in presence of 5% DMSO, Lane 3: Moxifloxacin at 5μg/ml concentration. Lanes 4 to 6: reactions in presence of 40, 30 and 20 μg/ml of compound **10q**, respectively. R and S indicate relaxed and supercoiled DNA respectively.

considered to be an acute, light-induced irritation response characterized by dermal inflammation, with erythema and edema as primary clinical end points. Phototoxicity with the quinolones is generally thought to result from the absorption of light by the parent compound or a metabolite in tissue [18]. 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 (**9c, 9k, 9o**) compounds were evaluated for potential phototoxicity in a standardized *in vivo* test system that has been used previously to assess quinolone antibiotics [19]. 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 **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 4-96 h and 24-96 h when compared within time points and with the control respectively. The test compounds were found to show a significant difference in ear thickness at various time-points when compared with the pre-drug reading (0 h) but were less or non toxic when compared with the negative (vehicle-treated) and positive controls (lomefloxacin). No erythema occurred in mice dosed with 140 mg/kg of **9k** throughout the 96 h study, while compound **9c** showed a significant erythema after irradiation till 24 h only. Significant erythema was observed in the ears of mice dosed with compound **9o** and this response was maximal after 24 hrs but gradually subsided over the next 2–3 days.

Table 4. IC_{50} Values for DNA Gyrase Inhibition

Compounds	IC_{50} (μM)
9c	>103.41
9k	>97.09
9o	>116.17
10q	61.41

EXPERIMENTAL SECTION

Melting points were taken on an electrothermal melting point apparatus (Buchi BM530) in open capillary tubes and are uncorrected. Infrared spectra (KBr disc) were run on Jasco IR Report 100 spectrometer. ^{1}H-NMR spectra were scanned on a JEOL Fx 300MHz 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 240C analyzer and the data were within ± 0.4% of the theoretical values. Microwave oven used was catalyst, India.

Synthesis of 2,4-dichloro-5-nitrobenzoic Acid (2)

To a solution of 2,4-dichlorobenzoic acid (**1**) (1.0 M equiv) in 96% sulfuric acid (40 ml), 65% nitric acid (1.5 M equiv) was added slowly at 0-5 °C and the resulting mixture was then stirred at room temperature for 1 hour. The mixture is then poured into ice-cold water and extracted with ethyl acetate (3x50 ml). The combined extracts were dried over magnesium sulphate and evaporated under reduced pressure to yield **2** (Yield: 98% M.P.: 138-140°C).

Synthesis of ethyl 3-(2,4-dichloro-5-nitrophenyl)-3-oxo-propanoate (4a)

To a solution of **2** (1.0 M equiv) in tetrahydrofuran, CDI (1.2 M equiv) was added for 30 minutes at a 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 (1.3 M equiv) in acetonitrile was added drop wise magnesium chloride (2.0 M equiv) and triethylamine (4.0 M equiv) at 0 °C and stirred at room temperature for 2.5 hrs. To this solution, the imidazolide prepared above was added and the reaction mixture was refluxed at 70 °C for 2.5 hrs. 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 x 25ml) and dried over magnesium sulphate and distilled the solvent to give **4a** (Yield: 82 %; M.P.: 141-143°C).

Table 5. Phototoxic Evaluation of 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
9c	0.26 ± 0.01	0.25 ± 0.01	0.27 ± 0.01	0.30 ± 0.02	0.29 ± 0.02	0.35 ± 0.02	0	4	2	0	0	0
9k	0.25 ± 0.01	0.29 ± 0.01	0.26 ± 0.01	0.27 ± 0.02	0.27 ± 0.01	0.26 ± 0.01	0	0	0	0	0	0
9o	0.31 ± 0.02	0.31 ± 0.01	0.31 ± 0.02	0.31 ± 0.01	0.31 ± 0.01	0.33 ± 0.01	0	0	6	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 UVA light immediately after dosing); 4 h = end of irradiation period.

[d] Control = 0.5% aqueous solution of sodium carboxymethylcellulose (4 Ns/m2) dosed at 10 mL/kg.

Synthesis of 3-(2,4-dichloro-5-fluorophenyl)-3-oxopropanoate (4b)

A 60% sodium hydride-in-oil suspension (2 M equiv) was added slowly at room temperature to a cold solution of 2,4-dichloro-5-fluoroacetophenone (**3**) (1 M equiv) in diethyl carbonate. The mixture was then heated at 80 °C for 1.5 h. It was poured into ice-cold water (700 mL) containing acetic acid. The mixture was extracted with three portions of ether. The organic phase was dried and evaporated, and the residual oil was distilled at 111 °C (0.7 mmHg) to give **4b** (Yield: 80%).

Synthesis of ethyl α-[bis(methylthio)methylene]-2,4-dichloro-5-fluoro-β-oxo-benzenepropanoic Acids (5a-b)

A 60% suspension of sodium hydride in oil (1 M equiv) was slowly added at room temperature to a solution of carbon disulfide (1 M equiv) and methyl iodide (1.2 M equiv) in N, N-dimethylacetamide (DMA), and the mixture was stirred for 1 h and to this a solution of **4a**/**4b** (0.5 M equiv) in DMA was added drop wise. The reaction mixture was stirred for 3 h, poured into cold water and the mixture was extracted with ethyl acetate. The organic phase was washed with water and dried over anhydrous sodium sulfate and the solvent was evaporated. The residue was chromatographed on a column of silica gel with n-hexane/ethyl acetate (50:1) as eluent under medium pressure to give **5a-b** (58 & 64% yield respectively) as yellow oil.

Synthesis of ethyl 2,4-dichloro-α-[dihydro-2(3H)-thiazolyliden]-5-fluoro/nitro-β-oxo-benzenepropanoic Acid (6a-b)

A solution of **5a-b** (1.0 M equiv) in toluene was added to a solution of cysteamine HCl (1.0 M equiv) and triethylamine (1.0 M equiv) in toluene. The mixture was heated under reflux for 2 h and the solvent was evaporated. Water was added to the residue and the mixture extracted with EtOAc (3x25ml). The organic layer dried over anhydrous $MgSO_4$. The solvent was evaporated, and the residue was recrystallized from diethyl ether to give **6a-b** as yellow crystal (yield: 80 & 76%; M.P.: 182-184 & 192-194°C respectively).

Synthesis of ethyl 8-chloro-7-fluoro/nitro-1,2-dihydro-5-oxo-5H-thiazolo[3,2-a]quinoline-4-carboxylic Acid (7a-b)

To a solution of **6a-b** (1.0 M equiv) in dry DMSO (30ml) was added K_2CO_3 (1.6 M equiv) under ice cooling. The reaction mixture was heated at 60 – 80 °C for 3h and then diluted with ice cold water and 20% HCl (to adjust pH 5-6). The resulting precipitate was collected by filtration, washed with water successively and the resulting solid was crystallized from a mixture of dichloromethane/ether to afford **7a-b** (85 & 79% yield; M.P: 205-207 & 210-212 °C respectively).

Synthesis of 8-chloro-7-fluoro/nitro-1,2-dihydro-5-oxo-5H-thiazolo[3,2-a]quinoline-4-carboxylic Acid (8a-b)

A solution of **7a-b** (1.0 M equiv) and 6M HCl (100ml) was refluxed for 3 h. The reaction mixture was then poured into ice water. The precipitate was washed with water, ethanol and ether and dried to yield **8a-b** (96 & 92% yield; M.P: 250-252 & 280-282 °C respectively).

General method for synthesis of 7-fluoro/nitro-1,2-dihydro-5-oxo-8-(sub)-5H-thiazolo[3,2-a]quinoline-4-carboxylic Acids (9, 10a-q)

A solution of **8a-b** (1.0 M equiv) and various secondary amines (4.0 M equiv) in dry DMSO (3ml) to be placed in microwave oven, and irradiated with 60% intensity for 3min. The reaction mixture was cooled to room temperature and poured into crushed ice and acidified with 1M HCl, kept to overnight to afford a precipitate which was filtered and washed with water successively and dried to give a pure solid **9, 10a-q**.

8-(4-((4-Chlorophenyl)(phenyl)methyl)piperazin-1-yl)-7-fluoro-2,5-dihydro-5-oxo-1H-thiazolo[3,2-a]quinoline-4-carboxylic Acid (9a)

Yield: 82.1%; M.P.: 271-273°C; ^{1}H NMR ($CDCl_3$): δ ppm: 2.59 (t, 4H, 3,5-CH_2 of piperazine), 3.12 (t, 4H, 2,6-CH_2 of piperazine), 3.4 (t, 2H, 2-CH_2 of thiazolidine), 4.2 (s,

1H, CH of diphenylmethyl), 4.43 (t, 2H, 3-CH_2 of thiazolidine), 7.0-7.9 (m, 11H, Ar-H), 15.2 (bs, 1H, COOH); Anal ($C_{29}H_{25}ClFN_3O_3S$) C, H, N.

8-[4-(2-Furanylcarbonyl)-1-piperazinyl]-1,2-dihydro-7-nitro-5-oxo-5H-thiazolo[3,2-a]quinoline-4-carboxylic Acid (10b)

Yield: 69.0%; M.P.: 188-190 °C; ^{1}H NMR ($CDCl_3$) δ ppm: 3.16 (t, 4H, 3,5-CH_2 of piperazine), 3.26 (t, 4H, 2,6-CH_2 of piperazine), 3.4 (t, 2H, 2-CH_2 of thiazolidine), 4.43 (t, 2H, 3-CH_2 of thiazolidine), 6.5-7.88 (m, 5H, Ar-H), 15.2 (bs, 1H, COOH); Anal ($C_{21}H_{18}N_4O_7S$) C, H, N.

8-(4-((Benzo[d][1,3]dioxol-5-yl)methyl)piperazin-1-yl)-7-fluoro-2,5-dihydro-5-oxo-1H-thiazolo[3,2-a]quinoline-4-carboxylic Acid (9c)

Yield: 81.8%; M.P.: 215-217 °C; ^{1}H NMR ($CDCl_3$) δ ppm: 2.82 (t, 4H, 3,5-CH_2 of piperazine), 3.1 (t, 4H, 2,6-CH_2 of piperazine), 3.43 (t, 2H, 2-CH_2 of thiazolidine), 3.6 (s, 2H, CH_2 of piperanoyl), 4.4 (t, 2H, 3-CH_2 of thiazolidine), 5.86 (s, 2H, -OCH_2O-), 6.82-7.8 (m, 5H, Ar-H), 15.2 (bs, 1H, COOH); Anal ($C_{24}H_{22}FN_3O_5S$) C, H, N.

2,5-Dihydro-8-(4-methyl-3-phenylpiperazin-1-yl)-7-nitro-5-oxo-1H-thiazolo[3,2-a]quinoline-4-carboxylic Acid (10d)

Yield: 72.1%; M.P.: 251-253 °C; ^{1}H NMR ($CDCl_3$) 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), 3.38 (d, 2H, 2-CH_2 of piperazine), 3.43 (t, 2H, 2-CH_2 of thiazolidine), 4.12 (t, 1H, 3-CH of piperazine), 4.4 (t, 2H, 3-CH_2 of thiazolidine), 7.0-7.82 (m, 7H, Ar-H), 15.2 (bs, 1H, COOH); Anal ($C_{23}H_{22}N_4O_5S$) C, H, N.

7-Fluoro-2,5-dihydro-8-(4-(2,3-dihydrobenzo[b][1,4]dioxin-2-yl)piperazin-1-yl)-5-oxo-1H-thiazolo[3,2-a]quinoline-4-carboxylic Acid (9e)

Yield: 69.5%; M.P.: 224-226 °C; ^{1}H NMR ($CDCl_3$) δ ppm: 3.26 (t, 4H, 2,6-CH_2 of piperazine), 3.4 (t, 4H, 3,5-CH_2 of piperazine), 3.46 (t, 2H, 2-CH_2 of thiazolidine), 4.4 (t, 2H, 3-CH_2 of thiazolidine), 4.6 (d, 2H, 3-CH_2 of dihydrobenzodioxinyl), 5.14 (t, 1H, 2-CH of dihydrobenzodioxinyl), 6.8-7.98 (m, 6H, Ar-H), 15.2 (bs, 1H, COOH); Anal ($C_{24}H_{22}FN_3O_5S$) C, H, N.

8-[4-[[3-(2,6-Difluorophenyl)-5-methyl-4-isoxazolyl]carbonyl]-1-piperazinyl]-7-nitro-1,2-dihydro-5-oxo-5H-thiazolo [3,2-a]quinoline-4-carboxylic Acid (10f)

Yield: 69.8%; M.P.: 260-262 °C; ^{1}H NMR ($CDCl_3$) δ ppm: 2.3 (s, 3H, 5-CH_3 of isoxazolyl), 3.12 (t, 4H, 3,5-CH_2 of piperazine), 3.28 (t, 4H, 2,6-CH_2 of piperazine), 3.46 (t, 2H, 2-CH_2 of thiazolidine), 4.4 (t, 2H, 3-CH_2 of thiazolidine), 6.82-7.88 (m, 5H, Ar-H), 15.2 (bs, 1H, COOH); Anal ($C_{27}H_{21}F_2N_5O_7S$) C, H, N.

7-Fluoro-2,5-dihydro-5-oxo-8-thiomorpholino-1H-thiazolo [3,2-a]quinoline-4-carboxylic Acid (9g)

Yield: 84.2%; M.P.: 280-282 °C; ^{1}H NMR ($CDCl_3$) δ ppm: 2.64 (t, 4H, 3,5-CH_2 of thiomorpholine), 3.38 (t, 4H, 2,6-CH_2 of thiomorpholine), 3.43 (t, 2H, 2-CH_2 of thiazolidine), 4.4 (t, 2H, 3-CH_2 of thiazolidine), 7.22-7.88 (m, 2H, Ar-H), 15.2 (bs, 1H, COOH); Anal ($C_{16}H_{15}FN_2O_3S_2$) C, H, N.

2,5-Dihydro-8-(2,6-dimethylmorpholino)-7-nitro-5-oxo-1H-thiazolo[3,2-a]quinoline-4-carboxylic Acid (10h)

Yield: 74.3%; M.P.: 194-196 °C; ^{1}H NMR ($CDCl_3$) δ ppm: 1.2 (d, 6H, 2,6 CH_3 of morpholino), 3.0 (d, 4H, 2,6-CH_2 of morpholine), 3.43 (t, 2H, 2-CH_2 of thiazolidine), 3.9 (m, 2H, 3,5-CH of morpholine), 4.4 (t, 2H, 3-CH_2 of thiazolidine), 7.22-7.88 (m, 2H, Ar-H), 15.2 (bs, 1H, COOH); Anal ($C_{18}H_{19}N_3O_6S$) C, H, N.

2,5-Dihydro-7-fluoro-5-oxo-8-(4-(piperidin-1-yl)piperidin-1-yl)-1H-thiazolo[3,2-a]quinoline-4-carboxylic Acid (9i)

Yield: 84.7%; M.P.: 263-265 °C; ^{1}H NMR ($CDCl_3$) 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), 3.43 (t, 2H, 2-CH_2 of thiazolidine), 4.4 (t, 2H, 3-CH_2 of thiazolidine), 7.22-7.86 (m, 2H, Ar-H), 15.2 (bs, 1H, COOH); Anal ($C_{22}H_{26}FNO_3S$) C, H, N.

8-(4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl)-2,5-dihydro-7-nitro-5-oxo-1H-thiazolo[3,2-a]quinoline-4-carboxylic Acid (10j)

Yield: 80.2%; M.P.: 190-192 °C; ^{1}H NMR ($CDCl_3$) δ ppm: 2.0 (t, 4H, 3,5-CH_2 of piperidine), 2.7 (t, 4H, 2,6-CH_2 of piperidine), 3.43 (t, 2H, 2-CH_2 of thiazolidine), 4.4 (t, 2H, 3-CH_2 of thiazolidine), 7.1-7.88 (m, 6H, Ar-H), 15.2 (bs, 1H, COOH); Anal ($C_{23}H_{20}ClN_3O_6S$) C, H, N.

8-(4-(6-Chloro-1,2-dihydro-2-oxobenzo[d]imidazol-3-yl) piperidin-1-yl)-2,5-dihydro-7-fluoro-5-oxo-1H-thiazolo[3, 2-a]quinoline-4-carboxylic Acid (9k)

Yield: 69.5%; M.P.: 205-207 °C; ^{1}H NMR ($CDCl_3$) δ ppm: 1.6-2.4 (m, 8H, 4 CH_2 of piperidine), 3.43 (t, 2H, 2-CH_2 of thiazolidine), 4.1 (bm, 1H, CH of piperidine), 4.4 (t, 2H, 3-CH_2 of thiazolidine), 6.4-6.9 (m, 6H, Ar-H), 15.2 (bs, 1H, COOH); Anal ($C_{24}H_{20}FClN_4O_4S$) C, H, N.

8-(3-(Diethylcarbamoyl)piperidin-1-yl)-2,5-dihydro-7-nitro-5-oxo-1H-thiazolo[3,2-a]quinoline-4-carboxylic Acid (10l)

Yield: 74.6%; M.P.: 228-230 °C; ^{1}H NMR ($CDCl_3$) 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), 3.43 (t, 2H, 2-CH_2 of thiazolidine), 4.4 (t, 2H, 3-CH_2 of thiazolidine), 7.2-7,86 (m, 2H, Ar-H), 15.2 (bs, 1H, COOH); Anal ($C_{22}H_{26}FN_4O_6S$) C, H, N.

8-(1,4-Dioxa-8-azaspiro[4.5]dec-8-yl)-7-fluoro-1,2-dihydro-5-oxo-5H-thiazolo[3,2-a]quinoline-4-carboxylic Acid (9m)

Yield: 63.2%; M.P.: 225-227 °C; ^{1}H NMR ($CDCl_3$) δ ppm: 1.78-2.4 (m, 8H, 4-CH_2 of azaspirodecane), 3.43 (t, 2H, 2-CH_2 of thiazolidine), 3.96 (m, 4H, 2-CH_2 of azaspirodecane), 4.4 (t, 2H, 3-CH_2 of thiazolidine), 7.2-7,86 (m, 2H, Ar-H), 15.2 (bs, 1H, COOH); Anal ($C_{19}H_{19}FN_2O_5S$) C, H, N.

8-(1-(tert-Butylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-2,5-dihydro-7-nitro-5-oxo-1H-thiazolo[3,2-a]quinoline-4-carboxylic Acid (10n)

Yield: 60.8%; M.P.: 175-177 °C; ^{1}H NMR ($CDCl_3$) δ ppm: 1.3 (s, 9H, 3 CH_3), 2.66- 2.9 (m, 4H, 2 CH_2 of isoqui-

noline), 3.43 (t, 2H, 2-CH_2 of thiazolidine), 4.4 (t, 2H, 3-CH_2 of thiazolidine), 4.85 (s, 1H, CH of isoquinoline), 6.7-7.81 (m, 6H, Ar-H), 10.2 (s, 1H, NH), 15.2 (bs, 1H, COOH); Anal ($C_{26}H_{26}N_4O_6S$) C, H, N.

8-(2-Carboxy-5,6-dihydroimidazo[1,2-a]pyrazin-7(8H)-yl)-7-fluoro-2,5-dihydro-5-oxo-1H-thiazolo[3,2-a]quinoline-4-carboxylic Acid (9o)

Yield: 68.8%; M.P.: 258-260 °C; ^{1}H NMR ($CDCl_3$) δ ppm: 3.1-3.8 (m, 8H, 4-CH_2 of piperazino and thiazolidine), 4.4 (t, 2H, 3-CH_2 of thiazolidine), 7.2 (s, 1H, CH), 7.28-7.81 (m, 2H, Ar-H), 12.12 (s, 1H, 2-COOH), 15.2 (bs, 1H, CO-OH); Anal ($C_{19}H_{15}FN_4O_5S$) C, H, N.

2,5-Dihydro-8-(4,4-dimethyloxazolidin-3-yl)-7-nitro-5-oxo-1H-thiazolo[3,2-a]quinoline-4-carboxylic Acid (10p)

Yield: 61.2%; M.P.: 200-202 °C; ^{1}H NMR ($CDCl_3$) δ ppm: 1.16 (s, 6H, 2-CH_3), 3.34 (s, 2H, 5-CH_2 of oxazolidinyl), 3.43 (t, 2H, 2-CH_2 of thiazolidine), 4.4 (t, 2H, 3-CH_2 of thiazolidine), 4.6 (s, 2H, 2-CH of oxazolidinyl), 7.28-7.81 (m, 2H, Ar-H), 15.2 (bs, 1H, COOH); Anal ($C_{17}H_{17}N_3O_6S$) C, H, N.

8-[6-[[(1,1-dimethylethoxy)carbonyl]amino]-3-azabicyclo [3.1.0]hex-3-yl]-7-fluoro-1,2-dihydro-5-oxo-5H-thiazolo[3, 2-a]quinoline-4-carboxylic Acid (9q)

Yield: 74.6%; M.P.: 241-243 °C; ^{1}H NMR ($CDCl_3$) δ ppm: 1.43 (s, 9H, *t*-butyl), 1.78-3.28 (m, 7H, azabicylcohexane ring H), 3.43 (t, 2H, 2-CH_2 of thiazolidine), 4.4 (t, 2H, 3-CH_2 of thiazolidine), 4.76 (bs, 1H, NH-*t*-Boc), 7.28-7.81 (m, 2H, Ar-H), 15.2 (bs, 1H, COOH); Anal ($C_{22}H_{24}FN_3O_5S$) C, H, N.

8-[6-[[(1,1-dimethylethoxy)carbonyl]amino]-3-azabicyclo [3.1.0]hex-3-yl]-7-nitro-1,2-dihydro-5-oxo-5H-thiazolo[3, 2-a]quinoline-4-carboxylic Acid (10q)

Yield: 78.6%; M.P.: 281-283 °C; ^{1}H NMR ($CDCl_3$) δ ppm: 1.43 (s, 9H, *t*-butyl), 1.78-3.28 (m, 7H, azabicylcohexane ring H), 3.43 (t, 2H, 2-CH_2 of thiazolidine), 4.4 (t, 2H, 3-CH_2 of thiazolidine), 4.76 (bs, 1H, NH-*t*-Boc), 7.28-7.81 (m, 2H, Ar-H), 15.2 (bs, 1H, COOH); Anal ($C_{22}H_{24}N_4O_7S$) C, H, N.

***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 duplicates. The MDR-TB clinical isolate was obtained from Tuberculosis Research Center, Chennai, India, and was resistant to isoniazid, rifampicin, ethambutol and ofloxacin. The minimum inhibitory concentration (MIC) is defined as the minimum concentration of compound required to give complete inhibition of bacterial growth.

Cytotoxicity

Some compounds were further examined for toxicity (IC_{50}) in mammalian Vero cell line at concentration 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.

***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 intra-peritoneal route began after 10 days of inoculation of the animal with microorganism and continued for 10 days. After 35 days post infection the spleens and right lungs were aseptically removed and 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.

DNA Gyrase Supercoiling Assay

The source of the enzyme and the purification procedure has been described before [20]. The compounds tested were dissolved in DMSO and pre-incubated with the enzyme. The assays were carried out as described previously, by incubating 400 ng of relaxed pUC18 at 37°C in supercoiling buffer [35 mM Tris-HCl pH 7.5, 5 mM $MgCl_2$, 25 mM potassium glutamate, 2 mM spermidine, 2mM ATP, 50μg/ml bovine serum albumin, and 90 μg/ml yeast t-RNA in 5% (v/v) glycerol] for 30 minutes. Moxifloxacin at 5 μg/ml final concentration was used as a fluoroquinolone positive control. A reaction having 5% DMSO in absence of compounds was also performed as solvent control. The reaction samples were heat inactivated and applied on to 1% agarose gel and electrophoresis was carried out in Tris - acetate - EDTA buffer for 12 hours. The gels were stained with ethidium bromide to visualize the topoisomers of the DNA.

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 groups received a single dose of screened compound at 140 mg/kg by oral gavage. A second group received a single dose of 140 mg/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 methylcellulose vehicle only. Test animals were exposed to UVA 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/cm2. Before dosing, at the end of the irradiation period and at approximately 24, 48, 72 and 96 h after dosing, both ears of each mouse were evaluated for changes indicative of a positive response: erythema, edema or a measurable increase in ear thickness.

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