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Gatifloxacin derivatives: Synthesis, antimycobacterial activities, and inhibition of *Mycobacterium tuberculosis* DNA gyrase

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Abstract—Sixteen 7-substituted gatifloxacin derivatives were synthesized and evaluated for antimycobacterial activity in vitro and in vivo against *Mycobacterium tuberculosis* H37Rv (MTB) and multi-drug resistant *M. tuberculosis* (MDR-TB), and also tested for the ability to inhibit the supercoiling activity of DNA gyrase from *M. tuberculosis*. Among the synthesized compounds, 1-cyclopropyl-6-fluoro-8-methoxy-7-[[[N^4-[1′-(5-isatinyl-β-semicarbazo)]methyl]3-methyl]N^1-piperazinyl]-4-oxo-1,4-dihydro-3-quinoline carboxylic acid (**3d**) was found to be the most active compound in vitro with an MIC of 0.0125 μg/mL against MTB and MTR-TB. In the in vivo animal model **3d** decreased the bacterial load in lung and spleen tissues with 3.62- and 3.76-log10 protections, respectively. Compound **3d** was also found to be equally active as gatifloxacin in the inhibition of the supercoiling activity of wild-type *M. tuberculosis* DNA gyrase with an IC_{50} of 3.0 μg/mL. The results demonstrate the potential and importance of developing new quinolone derivatives against mycobacterial infections.

Tuberculosis (TB) remains the leading cause of mortality due to a bacterial pathogen, *Mycobacterium tuberculosis*. The interruption of centuries of decline in case rates of TB occurred, in most cases, in the late 1980s and involved the USA and some European countries due to increased poverty in urban settings and the immigration from TB high-burden countries.[1] Thus, no sustainable control of TB epidemics can be reached in any country without properly addressing the global epidemic. It is estimated that 8.2 million new TB cases occurred worldwide in the year 2000, with approximately 1.8 million deaths in the same year, and more than 95% of those were in developing countries.[2] Approximately, 2 billion individuals are believed to harbor latent TB based on tuberculin skin test surveys,[3] which represents a considerable reservoir of bacilli. Possible factors underlying the resurgence of TB worldwide include the HIV epidemic, increase in the homeless population, and decline in health care structures and national surveillance.[4] Another contributing factor is the evolution of multi-drug resistant strains, defined as resistant to isoniazid and rifampicin, which are the most effective first-line drugs.[5] According to the 2004 Global TB Control Report of the World Health Organization, there are 300,000 new cases per year of multi-drug resistant strains worldwide, and 79% of multi-drug resistant strain cases are now 'super strains,' resistant to at least three of the four main drugs used to treat TB.[6] The factors that most influence the emergence of drug-resistant strains include inappropriate treatment regimens, and patient non-compliance in completing the prescribed courses of therapy due to the lengthy standard 'short-course' treatment or when the side effects become unbearable.[7] Hence, faster acting and effective new drugs to better combat TB, including multi-drug resistant tuberculosis, are needed. Several of the quinolone antibacterials such as gatifloxacin, moxifloxacin, and levofloxacin have been examined as inhibitors of *M. tuberculosis*, as well as other mycobacterial infections.[8]

Quinolones inhibit bacterial type II topoisomerases, deoxyribonucleic acid (DNA) gyrase and topoisomerase

Keywords: Gatifloxacin derivatives; Antimycobacterial; DNA gyrase inhibition.
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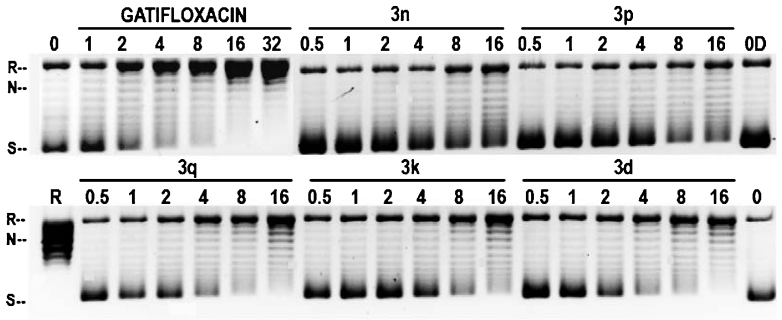
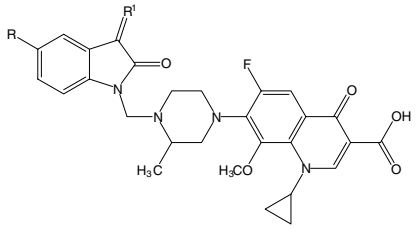


Figure 1. Inhibitory effects of gatifloxacin and its derivatives on the supercoiling activity of wild-type *Mycobacterium tuberculosis* DNA gyrase. Relaxed pBR322 DNA (0.4 μg) was incubated with gyrase activity (2U) reconstituted from WT GyrA and WT GyrB in the presence of 1 mM ATP and in the absence or presence of the indicated amounts (in μg/ml) of quinolones. Reactions were stopped and the DNA was examined by electrophoresis in 1% agarose. 0, 0D, N, R, and S denote control without drug, control without drug with the highest amount of DMSO used in the assay, nicked, relaxed, and supercoiled DNA, respectively.

IV,[9] 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.[10] One major factor relevant to the design of new antitubercular agents is the transport of compounds through the cell wall of mycobacteria. This is difficult since it is well known that mycolic acids and surface associated lipids of these organisms form a transport barrier when compared to the cell wall of other eubacteria.[11] As a part of the study attempting to further optimize the quinolone antibacterials against *M. tuberculosis*,[12] we have explored the effect of increasing the lipophilic character at 7th position of gatifloxacin on activity against MTB. In the present report, we describe the synthesis, antimycobacterial evaluation in vitro and in vivo against *M. tuberculosis* H37Rv (MTB), and multi-drug resistant *M. tuberculosis* (MDR-TB), and tested the ability to inhibit the supercoiling activity of wild-type *M. tuberculosis* DNA gyrase (see Fig. 1).

The general procedures for the preparation[12] of target compounds **3a-q** (Tables 1 and 2) are described in Scheme 1. Isatin and its derivatives (**1**) react with formaldehyde and secondary amino (piperazino) function of Gatifloxacin (**2**) to form the required Mannich bases of Gatifloxacin in 62–79% yield. The purity of the synthesized compounds was checked by thin-layer chromatography (TLC) and elemental analyses, and the structures were identified by spectral data.[13] In general, infrared spectra (IR) showed C=N (azomethine) peak at 1640 cm^{-1} and CH_2 (Mannich methylene) peak at 2860 and 2846 cm^{-1}. In the Nuclear Magnetic resonance spectra (^1H NMR), the signals of the respective protons of the prepared gatifloxacin derivatives were verified on the basis of their chemical shifts, multiplicities, and coupling constants. The spectra showed a singlet at δ 4.2–4.8 ppm corresponding to $-NCH_2N-$ group; multiplet at δ 2.8–3.6 ppm for piperazine proton; multiplet at δ 0.28–1.32 ppm for cyclopropyl proton; singlet at 3.52 for C-8 methoxy group and singlet at δ 8.1 ppm for C_2–H. The elemental analysis results were within ±0.4% of the theoretical values.

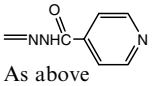
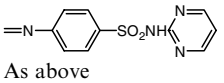
Table 1. Physical constants, in vitro antimycobacterial activity, and DNA gyrase inhibition of **3a**–**3k**



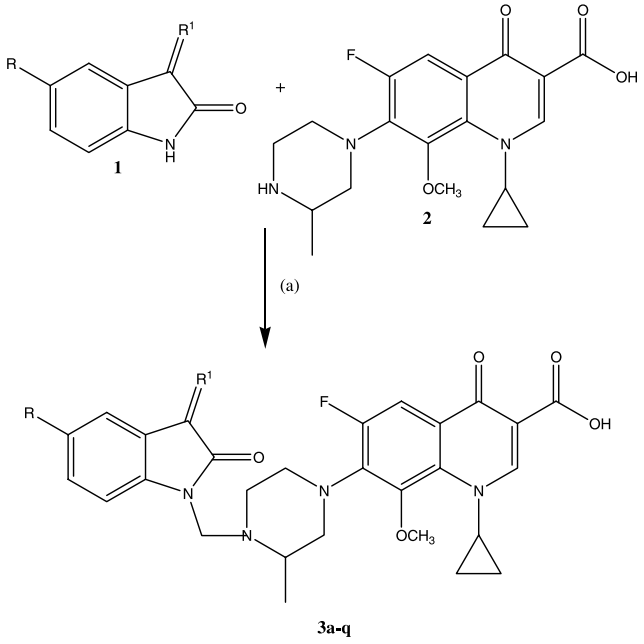
Compound	R	R^1	Yield (%)	Mp (°C)	log P^a	MIC MTB[b]	MIC MDR-TB[c]	IC_{50}[d]
3a	F	$=NNHCONH_2$	64.8	233	2.02	0.2	0.2	4.0
3b	CH_3	$=NNHCONH_2$	71.1	242	2.34	0.39	0.78	4.5
3c	Cl	$=NNHCONH_2$	69.6	221	2.42	0.39	0.78	4.0
3d	H	$=NNHCONH_2$	74.2	228	1.86	0.0125	0.05	3.0
3e	F	$=NNHCSNH_2$	62.6	193	2.58	0.1	0.1	8.0
3f	CH_3	$=NNHCSNH_2$	78.8	217	2.9	0.78	0.78	10.0
3g	Cl	$=NNHCSNH_2$	62.9	189	2.98	0.39	0.78	4.0
3h	H	$=NNHCSNH_2$	70.3	205	2.42	0.2	0.2	5.0

[a] log *P* values calculated with Chem office 2004 software.
[b] Minimum inhibitory concentration (in μg/mL) required to inhibit 90% inhibition against *M. tuberculosis*.
[c] MIC in μg/mL against multi-drug resistant *M. tuberculosis*.
[d] IC_{50} of drugs in μg/mL, that inhibit *M. tuberculosis* DNA gyrase activity.

Table 2. Physical constants, in vitro antimycobacterial activity, and DNA gyrase inhibition of **3l–3s**

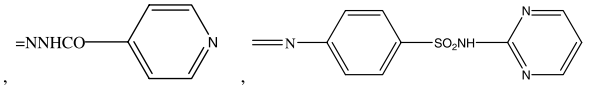
Compound	R	R^1	Yield (%)	Mp (°C)	log P^a	MIC MTB[b]	MIC MDR-TB[c]	IC_{50}[d]
3j	F		62.1	242	2.9	0.78	0.78	NT
3k	CH_3	As above	79.0	231	3.23	0.78	0.78	5.5
3l	Cl	As above	68.3	209	3.3	0.39	0.78	5.0
3m	H	As above	72.0	216	2.75	0.2	0.78	4.0
3n	Cl		64.8	178	4.16	0.1	0.1	6.0
3o	F	As above	65.7	192	3.76	0.2	0.1	6.0
3p	H	As above	71.9	203	3.6	0.2	0.1	8.0
3q	CH_3	As above	71.8	214	4.09	0.1	0.78	3.0
Gatifloxacin	—	—	—	—	1.51	0.2	3.12	4.0

For footnotes see Table 1. NT indicates not tested.



R= H, F, Cl, CH_3

R^1= =O, =NNHCONH$_2$, =NNHCSNH$_2$,



(a) HCHO, C_2H_5OH, reflux, 26-32h

Scheme 1. Synthesis of gatifloxacin derivatives.

All compounds were screened for their antimycobacterial activity against MTB and MDR-TB in BACTEC 12B medium using the microplate alamar blue assay using serial double dilution technique[14] in duplicate. The MDR-TB clinical isolate was obtained from Tuberculosis Research Center, Chennai, India, and was resistant to isoniazide, rifampicin, ethambutol, and ofloxacin. The minimum inhibitory concentration (MIC) is defined as the minimum concentration of compound required to give 90% inhibition of bacterial growth and MICs of the compounds are reported in (Tables 1 and 2). Among the synthesized compounds, four compounds (**3d**, **3e**, **3n**, and **3q**) (MIC < 0.2 μg/mL) were more active and five compounds (**3a**, **3h**, **3m**, **3o**, and **3p**) were equipotent (MIC: 0.2 μg/mL) to that of gatifloxacin against MTB. Compound 1-cyclopropyl-6-fluoro-8-methoxy-7-[[[N^4-[1′-(5-isatinyl-β-semicarbazo)]methyl]3-methyl]N^1-piperazinyl]-4-oxo-1,4-dihydro-3-quinoline carboxylic acid (**3d**) was found to be the most active compound in vitro with an MIC of 0.0125 μg/mL against MTB and was 16 times more potent than gatifloxacin. Against MDR-TB, when compared to gatifloxacin (MIC 3.12 μg/mL), all the compounds were more active with an MIC of ⩽0.78 μg/mL, Compound **3d** was found to be the most potent (MIC 0.05 μg/mL) and was 64 times more potent against MDR-TB when compared to the parent drug gatifloxacin.

The lipophilicity of the fluoroquinolones is well known to play an important role in the penetration of these compounds into bacterial cells.[15] Assuming that the issue of penetration is even more crucial for quinolone activity against mycobacteria,[16] our results demonstrated that simply increasing the lipophilic character at C-7 increased the activity, as shown with log P values of the synthesized compounds (1.86–4.16, statistically significant at $p < 0.0001$ using t test) (Tables 1 and 2) which were much more than that of the parent compound (1.51), these results were consistent with our earlier work on ciprofloxacin derivatives.[12]

All the compounds were further examined for toxicity (IC_{50}) in a mammalian VERO cell line at concentrations of 62.5 μg/mL. After 72 h 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.[17] The compounds were found to be non-toxic until 62.5 μg/mL. Compound **3d** showed selectivity index (IC_{50}/MIC) of more than 1250.

Subsequently, compound **3d** was tested for efficacy against MTB at a dose of 50 mg/kg (Table 3) in six-week-old female CD-1 mice. In this model,[12] the mice were infected intravenously through caudal vein approximately 10^7 viable *M. tuberculosis* ATCC 35801. Drug treatment began after inoculation of the animal with microorganism for 10 days by intraperitoneal route. After 35 days postinfection, 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

Table 3. In vivo activity data of **3d** and gatifloxacin against *Mycobacterium tuberculosis* in mice

Compound	Lungs (log CFU ± SEM)	Spleen (log CFU ± SEM)
Control	7.88 ± 0.22	8.84 ± 0.21
3d (50 mg/kg)	4.26 ± 0.19	5.08 ± 0.31
Gatifloxacin (50 mg/kg)	5.82 ± 0.18	6.14 ± 0.12

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 (untreated) controls (Mean culture-forming units (CFU) in lung: 7.88 and in spleen: 8.84). Compound **3d** decreased the bacterial load in lung and spleen tissues with 3.62- and 3.76-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 **3d** decreases the bacterial load with 1.56- and 1.06-log10 protections in lung and spleen tissues, respectively.

The bacterial targets of quinolones are the type II DNA topoisomerases, DNA gyrase and topoisomerase IV. These ATP-dependent enzymes act by a transient double-stranded DNA break and cooperate to facilitate DNA replication and other key DNA transactions.[17] DNA gyrase is unique in catalyzing the negative supercoiling of DNA and is essential for efficient DNA replication, transcription, and recombination, and appears that DNA gyrase is the sole topoisomerase target for quinolones in MTB.[18] The ability of 16 synthesized quinolones to inhibit DNA supercoiling by *M. tuberculosis* gyrase was studied [19] and compared with gatifloxacin and their IC_{50} data are summarized in Tables 1and 2. Each of the new compound tested showed dose-dependent inhibition. From the data it is clear that the synthesized compounds inhibit the DNA gyrase activity. When compared to gatifloxacin, four compounds (**3a**, **3c**, **3g**, and **3m**) were found to be equally active with an IC_{50} of 4 μg/mL and two compounds were slightly more active with an IC_{50} of 3 μg/mL. The results demonstrate that lipophilic quinolones retain their inhibitory property on DNA gyrase from wild-type MTB.

The results revealed that the compound **3d** is more active than gatifloxacin against TB and MDR-TB. This study also has revealed that increasing the lipophilic side chain at C-7 had improved the antimycobacterial activity in vitro. The investigation on further structure–activity relationships on various quinolones and emergence of other drug resistance is now in progress. The present results highlight the importance of increasing lipophilicity of the compounds to overcome transport barrier into the cells. Appropriate modification of other quinolones such as moxifloxacin, sparfloxacin, and sitafloxacin which are more effective than gatifloxacin is likely to provide more effective inhibitors of the enzyme with improved efficacy.

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