Expression and purification of an active form of the

*Mycobacterium leprae* DNA gyrase and its inhibition by quinolones

Running title: *Mycobacterium leprae* DNA gyrase and quinolones

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Abstract

*Mycobacterium leprae*, the causative agent of leprosy, is non-cultivable in vitro, therefore evaluation of antibiotic activity against *M. leprae* relies mainly upon mouse footpad system that requires at least 12 months before the results become available. We have developed an in vitro assay for studying the activity of quinolones against the DNA gyrase of *M. leprae*. We overexpressed, in *Escherichia coli*, the *M. leprae* GyrA and GyrB subunits, separately, as His-Tagged proteins by using a pET plasmid carrying the *gyrA* and *gyrB* genes. The soluble 97.5-kDa GyrA and 74.5-kDa GyrB subunits were purified by nickel chelate chromatography, and reconstituted an enzyme with a DNA supercoiling activity. Based on the drug concentrations that inhibited DNA supercoiling by 50% (IC\(_{50}\)) or induced DNA cleavage by 25% (CC\(_{25}\)), the 13 tested quinolones may cluster into three groups. Analysis of the quinolone structure-activity relationship demonstrates that the most active quinolones against *M. leprae* DNA gyrase share the following structural features: a substituted carbon at position 8, a cyclopropyl substituent at N-1, a fluorine at C-6, and a substituent ring at C-7. We conclude that the assays based on DNA supercoiling inhibition and drug-induced DNA cleavage on purified *M. leprae* DNA gyrase are rapid, efficient and safe methods for screening quinolone derivatives with potential in vivo activity against *M. leprae*.
INTRODUCTION

Leprosy is a chronic infectious disease which may cause severe disabilities due to damage of the peripheral nerves (29). Since 1980s, tremendous progress has been made in controlling the disease by intensively implementation of multidrug therapy (MDT), nevertheless, the latest data indicates that around 300 000 new cases of leprosy are still detected every year, mostly in Asia, Latin America and Africa (35).

By the end of 1970s, due to long-term monotherapy, dapsone resistance had become a widespread phenomenon (20). In 1981, the World Health Organization (WHO) introduced the multidrug therapy (MDT), which composed of rifampin, dapsone and clofazimine (34). Although the duration of MDT has been reduced to 6 or 12 months, respectively, for patients with paucibacillary or multibacillary leprosy (36), the durations remain too long, which cause a number of operational difficulties, particularly for direct supervision of drug administration by the patients. Also due to operational reasons, MDT did not completely prevent the emergence of drug-resistance; by the end of 1990s, a few multidrug resistant isolates of *M. leprae* began to occur (5, 24, 25). In order to short the duration of MDT treatment, new antimicrobial agents with powerful bactericidal activity against *M. leprae* must be incorporated into the MDT regimens for significantly improving its efficacy. Within the last 20 years, a number of newer antimicrobial agents, such as quinolones (e.g. pefloxacin, ofloxacin, sparfloxacin and moxifloxacin), macrolides (e.g., clarithromycin) and tetracyclines (e.g., minocycline), had been identified to possess various degree of bactericidal activities against *M. leprae* in mouse experiments and in human trials (18, 19). Among these newer antimicrobial agents, moxifloxacin has been demonstrated to display very powerful bactericidal activity against *M. leprae* (8), virtually identical to that of rifampin (the key component of
the current MDT regimens), and is well tolerated in human trials (26). Therefore moxifloxacin may become an important component of the future generation MDT regimen. Because many quinolone derivatives are synthesized every year, it is possible to identify quinolones with anti-
*M. leprae* activity more potent than moxifloxacin if keep on screening of new derivatives for activity against *M. leprae*.

Because *M. leprae*, the causative agent of leprosy, is non-cultivable in vitro despite of hundred years of efforts (29), the only way for cultivation of leprosy bacillus is the mouse footpad system (22, 31), which remains to be the main system for testing the activities of antimicrobial agents against *M. leprae*. Since *M. leprae* has the longest doubling time (≈ 14 days) of all known bacteria, the mouse experiment is time-consuming, which requires at least 12 months before the results become available. Therefore, it would be highly desirable to develop an in vitro method which would greatly accelerate the progress of identifying more potent quinolones for their activities against *M. leprae*.

The targets of quinolones are type 2 topoisomerases, which consist of DNA gyrase and topoisomerase IV. These enzymes play a crucial role in the DNA topology regulation by facilitating DNA replication and chromosome segregation during cell division (6). The active form of DNA gyrase is a heterotetramer GyrA$_2$GyrB$_2$ that catalyzes the negative supercoiling of DNA by wrapping a double-stranded segment around itself, cleaving this DNA in both strands, passing the wrapped DNA through the break, and then resealing the DNA (6). Quinolones inhibit DNA supercoiling by stabilizing the complex between gyrase and the cleaved DNA, interrupting the replication fork propagation. This and the permanent breakdown of DNA lead to the
death of the bacteria (17). After the complete genome of *M. leprae* was sequenced (7), interestingly, no gene of topoisomerase IV was found. It is therefore very likely that DNA gyrase is the sole target of quinolones in *M. leprae*, as in *Mycobacterium tuberculosis*. Another particularity of *M. leprae* is the presence of a protein splicing element, termed intein in the gyrA gene encoding the GyrA subunit of DNA gyrase.

In order to measure the activity of new quinolones against *M. leprae*, we reconstituted, in vitro, the *M. leprae* DNA gyrase by expressing the two subunits separately in *Escherichia coli* as recombinant proteins carrying a poly-His-Tag, and then measured the supercoiling inhibition of *M. leprae* DNA gyrase and the induction of DNA cleavage arising from stabilization of the cleavage complex by quinolones. We demonstrated that the in vitro activities of quinolones were consistent with the corresponding in vivo activities determined in the mouse footpad model. Therefore, determination of quinolone interaction with purified *M. leprae* DNA gyrase may provide a rapid, efficient and safe in vitro test for screening new quinolones with putative antileprosy activity.
MATERIALS AND METHODS

Reagents.

The following 13 quinolones were provided by their corresponding manufacturers: levofloxacin (Roussel Uclaf), gatifloxacin (Grünenthal, Levallois-Perret, France), ciprofloxacin and moxifloxacin (Bayer Pharma, Puteaux, France), sparfloxacin and pefloxacin (Aventis, Paris, France), nalidixic acid, oxolinic acid, ofloxacin (Sigma-Aldrich Chimie, Saint Quentin Fallavier, France), temafloxacin (Abbott, Saint Rémy sur Avre, France), sitafloxacin (Daiichi Pharmaceutical, Co., Ltd. Tokyo, Japan), garenoxacin (Bristol-Meyers Squibb, Saint Nazaire, France) and clinafloxacin (Anakena Pharma Marketing, Japan). Oligonucleotide primers were synthesized by MWG (Ebersberg, Germany).

Bacterial strains and plasmids.

*gyrA* and *gyrB* genes were amplified from the cosmid B1770 of *M. leprae* genome bank, which was kindly provided by S. Cole (7). *E. coli* DH5α was used as host for cloning purposes and *E. coli* BL21 codon plus (λDE3) pLysS (Stratagene) was used for protein expression. The pMOS Blue plasmid kit (Amersham Biosciences Europe, Orsay, France) was used to clone amplified DNA fragments. Plasmid pET-29a (Novagen, Merck Eurolab, Fontenay Sous Bois, France) was used to construct vectors for overexpression of *M. leprae* GyrA and GyrB proteins. Supercoiled plasmid pBR322 DNA was provided by Roche (Roche Diagnostics, Meylan Cedex, France) and relaxed plasmid pBR322 DNA was obtained from John Innes Enterprises Ltd (Norwich Research Park, Colney, Norwich, UK).
**Construction of GyrA- and GyrB-expression vectors.**

The intein-containing *gyrA* and the *gyrB* genes were directly amplified from the cosmid B1770, cloned into pMOS Blue, and inserted into pET-29a as described hereafter. In each of the forward primers used for the amplification step, *NdeI* sites (CATATG) were engineered overlapping the ATG initiation codon of *gyrA* and *gyrB*. Into each of the reverse primers, a *XhoI* site was introduced before the stop codon of *gyrA* and *gyrB*.

The *gyrA* gene was amplified by using the forward primer (LEPSnew) 5'-GCAAATGAGGAACATGACTGATATCACG-3' (underlined bases correspond to the *NdeI* site, the ATG codon is indicated in bold), and the reverse primer (LEPASnew) 5'-ATCACTTTACTCGAGACCGCGTCGGCGCT (*XhoI* site underlined). The *gyrB* gene was amplified by using the forward primer (MLB2NdeI) 5'-CAAGGAGAGCATTCAGCATATGCTGCCCAGAGG (*NdeI* site underlined, ATG initiation codon in bold), and the reverse primer (MLBSPXhoI) 5'-CGTCGAAAAATGTCTCGAGTAAGTAGACATCCAGGA (*XhoI* site underlined). Cosmid B1770 DNA, containing the *gyrB* and *gyrA* genes of *M. leprae* was used as template in the amplification reaction carried out using the Expand Long Template PCR System kit (Boehringer Mannheim, Meylan, France) in the presence of 5 mM dNTPs and 2.25 mM MgCl₂. Amplification conditions were as follows: for *gyrA*, 10 min of denaturation at 94°C, 30 amplification cycles of 1 min of denaturation at 94°C, 1 min of annealing at 55°C and 1 min of extension at 68°C; for *gyrB*, 2 min of denaturation at 94°C, 10 amplification cycles of denaturation at 94°C for 15 seconds, annealing at 55°C for 30 seconds and extension at 68°C for 3 min, followed by 30 cycles of denaturation at 94°C for 15 seconds, annealing at 60°C for 30 seconds and extension at 68°C for 4 min, with
a final extension step for 7 min at 68°C. The PCR products obtained (3.7-kb for gyrA-
intein and 2-kb for gyrB), were ligated into the pMOSBlue plasmid, and transformed into
E. coli MosBlue competent cells according to the manufacturer’s instructions.
Recombinant plasmids were recovered from the white colonies, digested with Ndel and
Xhol, and the DNA fragments obtained were ligated into Ndel-Xhol-cut pET-29a, and
transformed into E. coli DH5α. Recombinant clones were screened among the resistant
colonies selected on plates containing kanamycin (50 µg/ml). The plasmid containing
the M. leprae gyrA gene without the intein (MLA) was obtained by using the Quick
Change® Site Directed Mutagenesis kit (Stratagene), according to the manufacturer’s
instructions. Two primers were employed: DELINTEINS 5’
ACCGAGGCTCCTTCTGACTCCATTGGCGATG and DELINTEINAS 5’
ATAACGCATCGCTGCCGGTGGGTCATTACC. The absence of intein was checked by
DNA sequencing.

Protein overexpression and purification of GyrA and GyrB subunits.
GyrA (MLA) and GyrB (MLB) were overexpressed and purified as previously described
with minor modifications (1, 2). In order to increase the yield of soluble proteins, the
induction temperature was reduced to 14°C with an induction time of 20 h. Since dialysis
led to the loss of most of the purified proteins by precipitation, two amino acids (arginine
and glutamic acid) were added in the dialysis buffer to prevent the precipitation of MLA
and MLB (13). Protein concentrations were measured with a Nanodrop® ND-1000 and
the protein fractions were examined by sodium dodecyl sulfate-polyacrylamide gel
electrophoresis (SDS-PAGE).
DNA supercoiling assays.

DNA supercoiling activity was tested with 1U of purified DNA gyrase (defined as the amount of DNA gyrase that converted 400 ng of relaxed pBR322 to the supercoiled form in 1 h at 30°C). The reaction mixture (total volume, 30 µl) contained the DNA gyrase assay buffer (40 mM Tris-HCl [pH 7.5], 25 mM KCl, 20 mM magnesium acetate, 2 mM spermidine, 4 mM dithiothreitol, 0.1 mg/ml of yeast tRNA, bovine serum albumin [0.36 mg/ml], 3 mM ATP) (pH 8.0) and relaxed pBR322 DNA (0.4 µg) as substrate. Gyrase proteins were added, and the reaction mixtures were incubated at 30°C for 1h. The reactions were stopped by the addition of glycerol (50%) containing 0.25% bromophenol blue. The total reaction mixture was subjected to 5h of electrophoresis at 50V in a 1% agarose gel in 0.5X TBE (Tris-borate-EDTA, pH 8.3) buffer. The gel was stained with ethidium bromide (0.7 mg/ml). The supercoiling activity was assessed by quantifying the brightness of the bands corresponding to the supercoiled pBR322 DNA with the Molecular Analyst software (Bio-Rad).

The inhibitory effect of quinolones on the recombinant *M. leprae* DNA gyrase was assessed by determining the concentration of drug required to inhibit the supercoiling activity of the enzyme by 50% (IC$_{50}$), following the method previously described (1, 2).

DNA cleavage assays.

DNA cleavage assays were carried out in the buffer used for DNA supercoiling except that supercoiled pBR322 DNA was used as substrate instead of relaxed pBR322 DNA. Three hundred ng of GyrA and 250 ng of GyrB were mixed in the presence of increasing concentrations of quinolones for 1h at 30°C. Three µl of 2% SDS were added to
separate free DNA from cleaved DNA covalently linked to DNA gyrase, and 3 µl of a
1 mg/ml solution of proteinase K were added to remove covalently bound GyrA protein.
Incubation was continued for 30 min at 37°C. Reactions were stopped as described
above for supercoiling. DNA products were examined by agarose gel electrophoresis
and the drug concentration allowing the obtention of 25% of DNA cleavage (CC\textsubscript{25}) was
determined.
RESULTS

Purification and DNA supercoiling activity of the recombinant His-Tagged GyrA and GyrB proteins from *M. leprae*.

The *M. leprae gyrA* and *gyrB* genes were amplified from the cosmid B1770 that contains a *gyrB-gyrA* contig of *M. leprae*. The amplified genes were separately inserted in frame downstream of a T7 promoter in a pET29a expression vector, thus obtained two recombinant plasmids MLA (*gyrA* without the intein region) and MLB (*gyrB*), which allow the production of proteins with a hexahistidine tag at the C-terminal. After expression in *E. coli* and purification, a 74.5 kDa protein from MLB and a 91.5 kDa protein from MLA were obtained at a concentration of around 0.5 mg/ml (Fig. 1).

Combinations of GyrA (MLA) and GyrB (MLB) subunits were tested for DNA supercoiling activity. As expected, either GyrA or GyrB subunit alone did not induce any detectable activity (Fig. 2). In contrast, combination of GyrA and GyrB allowed the supercoiling of plasmid pBR322 in the presence of ATP, demonstrating that a functional DNA gyrase was reconstituted (Fig. 2). The specific supercoiling activity of the recombinant *M. leprae* DNA gyrase was $3.2 \times 10^3$ U/mg for GyrA and $2.8 \times 10^3$ U/mg for GyrB.

Inhibition of DNA supercoiling by quinolones

The ability of 13 quinolones to inhibit 50% of the DNA supercoiling of the *M. leprae* DNA gyrase was investigated by using 2 U of MLA combined with 2 U of MLB in each test. Each of the quinolones showed a dose-dependent inhibition, and their IC$_{50}$ ranged from 1 µg/ml (sitafloxacin) to 300 µg/ml (nalidixic acid). The results are summarized in Table 1, and a set of representative data (IC$_{50}$s: 2, 5 and 7 µg/ml obtained respectively with
gatifloxacin, sparfloxacin and levofloxacin) is shown in Figure 3. The 13 quinolones are clustered into three groups according to their IC\textsubscript{50} values. Quinolones of the first group had IC\textsubscript{50}s \(\leq 10\) µg/ml and included sitafloxacin, gatifloxacin, moxifloxacin, clinafloxacin, ciprofloxacin, garenoxacin, sparfloxacin, levofloxacin and ofloxacin. Quinolones of the second group, pefloxacin and temafloxacin, showed values >10 µg/ml but <100 µg/ml. Quinolones of the third group, characterized by IC\textsubscript{50}s \(\geq 100\) µg/ml, included oxolinic acid and nalidixic acid.

Analysis of the structure-activity relationship indicated that the most active quinolones shared several structural features: (i) a substituted carbon at position 8; (ii) a cyclopropyl group at N-1; (iii) a fluorine at C-6, except for garenoxacin which has a difluoromethoxy substituent in C-8; and (iv) a substituent ring at C-7. None of the two quinolones of the third group displayed any of the 4 above-mentioned structural features. As expected, ofloxacin was 2-fold less active than levofloxacin, because ofloxacin is a racemic mixture of the inactive R-isomer and the active L-isomer levofloxacin.

**DNA cleavage activity of *M. leprae* DNA gyrase in the presence of quinolones.**

The enhancement of supercoiled pBR322 DNA cleavage by the *M. leprae* gyrase in the presence of each of the 13 quinolones was measured. A representative set of results obtained with moxifloxacin, gatifloxacin and ofloxacin is presented in Figure 4. As shown in Table 1, the drug concentration producing 25% of DNA cleavage (CC\textsubscript{25}) was \(\leq 10\) µg/ml for sitafloxacin, gatifloxacin, moxifloxacin, clinafloxacin, ciprofloxacin, garenoxacin, sparfloxacin, levofloxacin and ofloxacin. The CC\textsubscript{25} of temafloxacin was 11 µg/ml and that of pefloxacin was 40 µg/ml. Little or no enhancement of DNA cleavage was observed in
presence of nalidixic acid or oxolinic acid. From structure-activity point of view, the
cluster of quinolones based on DNA cleavage test was very similar to those based on
the DNA supercoiling test. In addition, the results indicate that the presence of a fluorine
at position 6 or a piperazinyl ring at C-7 is important for DNA cleavage since classical
quinolones were unable to stimulate it.

DISCUSSION

Unable to cultivate *M. leprae* in cell-free medium is a serious handicap for leprosy
research, particularly for screening of new drugs with anti-*M. leprae* activity. The results
of the present study indicate that the tests using purified *M. leprae* DNA gyrase as the
target are useful in vitro systems to evaluate the activity of quinolones against *M. leprae*.
Based on our previous study on expression and purification of *M. tuberculosis* DNA
gyrase (1), we overproduced, purified the His-Tagged GyrA and GyrB subunits of
*M. leprae* and obtained a functional *M. leprae* DNA gyrase after reconstitution. It must
be pointed out that one characteristic of the gyrA gene of *M. leprae* is the presence of an
intein inserted close to the tyrosine residue of the active site (Tyr$^{130}$). Inteins are proteins
removed from host protein sequences during the post-translational maturation process
(23), their role remains unclear. Heterologous expression and physiological studies of
intein-containing genes require either the post-translational splicing in the heterologous
host, or the accurate excision of the intein coding region of the gene before expression.
Such technical difficulties explain the rarity of the investigations on such genes (12, 16,
21, 32). After numerous failures to obtain efficient *M. leprae* intein splicing in *E. coli*
GyrA (data not shown), we have successfully excised the intein encoding region of gyrA
before expression and obtained an homogeneous purified protein, i.e., GyrA without
intein, showing an expected size of 91.5 kDa. Association of this protein with GyrB permitted to reconstitute an active gyrase protein. As shown recently in *M. tuberculosis* (1) and also in various other bacteria (4, 27), addition of poly His-Tag to GyrA and GyrB subunits did not hamper the DNA gyrase activity or interaction with quinolones.

The quinolone activity was evaluated by two different assays, inhibition of DNA supercoiling and induction of DNA cleavage. We observed that both the DNA supercoiling inhibition and the DNA cleaved complex formation occurred in a dose dependent manner, and 9 out of 13 tested quinolones displayed high activities against *M. leprae* DNA gyrase, with $IC_{50}$s and $CC_{25}$s ≤ 10 µg/ml. Results of quinolone structure-activity relationship analysis based on *M. leprae* DNA gyrase were highly concordant with those based on *M. tuberculosis* DNA gyrase (1). In brief, a substituted carbon at position 8, substitution of C-1 by a cyclopropyl, a fluor at position 6, and a substituent ring at C-7 of the quinolone are associated with stronger activities against DNA gyrase of both *M. tuberculosis* and *M. leprae*.

DNA cleavage assay was thought to be more relevant than supercoiling inhibition in measuring the activity of gyrase inhibitor (3, 33). However, it seems the conclusion does not apply for *M. tuberculosis*, the effective quinolone concentrations measured by the DNA cleavage assay were slightly different from those measured by supercoiling assay, (ranged from 2-fold lower to 2-fold higher), and were less correlated with the concentrations inhibiting *M. tuberculosis* growth (1). In the case of *M. leprae*, except for pefloxacin, the effective quinolone concentrations inducing the DNA cleavage were found to be 2- to 6-fold lower than those inhibiting the supercoiling activity, a ratio close to that found for *E. coli* DNA gyrase (3, 33). Among the 13 quinolones tested in the present study, the anti-*M. leprae* activities of sitafloxacin (9), moxifloxacin (8), pefloxa...
ciprofloxacin (15), sparfloxacin (10, 11), ofloxacin (10, 14, 28), pefloxacin (11, 14, 15) and temafloxacin (11) had been tested either in the mouse foot pad system or in various in vitro systems. Ranking the activities of these quinolones based on inhibiting DNA supercoiling and inducing DNA cleavage were in similar order as those based on activities against *M. leprae* in the mouse foot pad system or in in vitro assays (Table 1). For example, moxifloxacin is highly active in inhibiting *M. leprae* DNA gyrase, and is also by far the most active quinolone against *M. leprae* in the mouse foot pad system (8); on the other hand, ofloxacin is less active in inhibiting the DNA gyrase, and also significantly less bactericidal than moxifloxacin in the mouse (8). Despite good in vitro activity on purified enzyme, ciprofloxacin is poorly active in vivo probably due to unfavorable pharmacokinetic (15), and lack of intracellular killing (30).

In conclusion, we have succeeded in purification of *M. leprae* DNA gyrase, which is the sole target of quinolones in this particular microorganism. Based on measuring the interaction between quinolones and the purified *M. leprae* DNA gyrase, we have developed two simple assays for rapidly investigate the quinolone structure-activity relationship and screening of new quinolone derivatives for their anti-*M. leprae* activities, thus avoid of testing a great number of quinolone derivatives by the mouse foot pad system, which is time-consuming, labor intensive and expensive. We propose that only those quinolones with IC₅₀s below 10 µg/ml should be considered for further testing in the mouse footpad system.

**ACKNOWLEDGMENTS**

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FIG. 1. SDS-PAGE analysis of purified *M. leprae* GyrA (MLA) and GyrB (MLB) proteins. The His-Tagged proteins were overexpressed in *E. coli*, purified by nickel resin chromatography, and approximately 8 ng of each protein sample were loaded on a SDS-9% polyacrylamide gel. Following electrophoresis, proteins were revealed by staining with Coomassie blue. Lane M, marker (sizes in kilodaltons are indicated to the right).

FIG. 2. *M. leprae* GyrA and GyrB proteins generate an ATP-dependent DNA supercoiling activity. Relaxed pBR322 (0.4 µg) was incubated with DNA gyrase reconstituted from GyrA (1 U, i.e. 320 ng) and GyrB (1 U, i.e. 300 ng) in the presence of 3 mM ATP. Reactions were stopped, and the DNA products were separated by electrophoresis in 1% agarose gel. DNA was stained with ethidium bromide and photographed under UV illumination. Lanes: a, supercoiled pBR322 DNA; b, relaxed pBR322 DNA; A, relaxed pBR322 DNA and GyrA (1U) protein; B, relaxed pBR322 DNA and GyrB (1U) protein; AB, relaxed pBR322 DNA and both GyrA (1U) and GyrB (1U). R and S denote relaxed and supercoiled DNA, respectively.

FIG. 3. DNA supercoiling activity of *M. leprae* DNA gyrase is sensitive to inhibition by gatifloxacin (GAT), sparfloxacin (SPX) and levofloxacin (LVX). Relaxed pBR322 (0.4 µg) was incubated with DNA gyrase reconstituted from GyrA (2 U, i.e. 600 ng) and GyrB (2 U, i.e. 700 ng) in the absence or in the presence of GAT, SPX or LVX. Reactions were stopped, and the DNA products were analyzed by electrophoresis in 1% agarose gel.
Lane a represents relaxed pBR322 DNA. R and S denote relaxed and supercoiled DNA, respectively.

FIG. 4: Quinolone mediated DNA cleavage by *M. leprae* DNA gyrase. Supercoiled pBR322 DNA (0.4 µg) was incubated with *M. leprae* GyrA (300 ng) and GyrB (250 ng) proteins in presence of ATP and of moxifloxacin (MXF), gatifloxacin (GAT) or ofloxacin (OFX) at the concentration indicated on the figure (in µg/ml). After addition of SDS and proteinase K, DNA samples were analyzed by electrophoresis in 1% agarose. Lane a: supercoiled pBR322 DNA. R, N and S denote relaxed, nicked, and supercoiled DNA, respectively.
FIG 1:
FIG 2:
FIG 3:
FIG 4:

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Table 1: Structural features and concentrations of quinolones inhibiting *M. leprae* DNA gyrase activity and *M. leprae* growth

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<th>Quinolones</th>
<th>R1</th>
<th>R6</th>
<th>R7</th>
<th>N- or C-8</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (µg/ml)</th>
<th>CC&lt;sub&gt;25&lt;/sub&gt; (µg/ml)</th>
<th>MGI&lt;sup&gt;a&lt;/sup&gt; (5 µg/ml)</th>
<th>Mice MIC (mg/kg/j)&lt;sup&gt;b&lt;/sup&gt;</th>
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<td>nd</td>
<td>150&lt;sup&gt;5&lt;/sup&gt;</td>
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<td>F</td>
<td>pyrrolidine</td>
<td>C-Cl</td>
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<td>piperazine</td>
<td>C-H</td>
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<td>Side Chain</td>
<td>Ring 1</td>
<td>Ring 2</td>
<td>Cycles</td>
<td>Growth Index</td>
<td>Dosage (µg/ml)</td>
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<tr>
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<td>C-F</td>
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<td>F</td>
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<td>F</td>
<td>piperazine</td>
<td>C-H</td>
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<td>C-H</td>
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<td>CH₃</td>
<td>N</td>
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1 ethyl = C₂H₅, cyclopropyl = c-C₃H₅, difluorophenyl = 2',4'-F-C₃H₅, no = not observed, nd = not determined

2 Mean Growth Index from days 11 to 18 at 5 µg/ml of drug determined in the BACTEC 460 system against *M. leprae* (10)

3 Dosage of quinolones given to mice infected with leprosy leading to a fully bactericidal effect (>99% of *M. leprae*)
organisms killed) even 9 months after completed therapy expressed as mg of drug per kg of mouse body weight per day

References


34. **WHO.** 1982. Chemotherapy of leprosy for control programmes. WHO.
