

# Gatifloxacin Activity against Quinolone-Resistant Gyrase: Allele-Specific Enhancement of Bacteriostatic and Bactericidal Activities by the C-8-Methoxy Group

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**Antibacterial activities of gatifloxacin (AM1155), a new C-8-methoxy fluoroquinolone, and two structurally related compounds, AM1121 and ciprofloxacin, were studied with an isogenic set of ten quinolone-resistant, *gyrA* (gyrase) mutants of *Escherichia coli*. To compare the effect of each mutation on resistance, the mutant responses were normalized to those of wild-type cells. Alleles exhibiting the most resistance to growth inhibition mapped in  $\alpha$ -helix 4, which is thought to lie on a GyrA dimer surface that interacts with DNA. The C-8-methoxy group lowered the resistance due to these mutations more than it lowered resistance arising from several *gyrA* alleles located outside  $\alpha$ -helix 4. These data are consistent with  $\alpha$ -helix 4 being a distinct portion of the quinolone-binding site of GyrA. A helix change to proline behaved more like nonhelix alleles, indicating that helix perturbation differs from the other changes at helix residues. Addition of a *parC* (topoisomerase IV) resistance allele revealed that the C-8-methoxy group also facilitated attack of topoisomerase IV. When lethal effects were measured at a constant multiple of the minimum inhibitory concentration for each fluoroquinolone to normalize for differences in bacteriostatic action, gatifloxacin was more potent than the C-8-H compounds, both in the presence and absence of protein synthesis (an exception was observed when alanine was substituted for aspartic acid at position 82). Collectively, these data show that the C-8-methoxy group contributes to the enhanced activity of gatifloxacin against resistant gyrase and wild-type topoisomerase IV.**

Fluoroquinolones are potent antibacterial agents that poison cells by trapping DNA gyrase and DNA topoisomerase IV on chromosomes as quinolone-enzyme-DNA complexes (reviewed in references 4 and 5). Occasionally the compounds are rendered ineffective through the development of bacterial resistance (reviewed in reference 15). To identify fluoroquinolones that more avidly attack resistant bacteria, *gyrA* (gyrase) mutants have been used to screen new fluoroquinolone derivatives. By this test, particularly potent compounds contain a halogen or methoxy group at the C-8 position (2, 7, 12, 23–25). Avid attack of resistant mutants allows the C-8-methoxy fluoroquinolones to restrict the selection of resistant mutants more effectively than C-8-H derivatives (3). Why the C-8-methoxy fluoroquinolones are particularly active against gyrase (25) and topoisomerase IV mutants (24) is not known.

One way to examine the action of C-8-methoxy fluoroquinolones is to compare the effectiveness of structural variants against a series of resistant gyrase mutants. Since the crystal structure of the breakage-reunion fragment of the GyrA protein from *Escherichia coli* has been determined (14), a starting point exists for spatially orienting resistance alleles. When the GyrA dimer is modeled, two helices (one  $\alpha$ -helix 4 on each GyrA monomer) are found on a surface where DNA is thought to interact. The most commonly selected resistance alleles map on the same surface of  $\alpha$ -helix 4. Whether the C-8-methoxy group preferentially enhances attack of mutant gyrase encoded by particular alleles has not been studied.

In the present work we examined the effect of fluoroquinolone structure by comparing the action of gatifloxacin (C-8-methoxy), AM1121 (C-8-H), and ciprofloxacin (C-8-H) against a set of ten quinolone-resistant *gyrA* mutants of *E. coli*. Data

were normalized to the wild-type value to cancel differences among the compounds with respect to potencies against wild-type strains, and a *parC* quinolone-resistance allele was included in some mutant strains to minimize effects due to topoisomerase IV. Under these conditions, the highly resistant alleles in  $\alpha$ -helix 4 were more sensitive to the presence of a C-8-methoxy group than mutations mapping outside the helix. An exception to the pattern was a helix mutation that placed a proline between the major resistance positions (amino acids 83 and 87). This mutant behaved more like those outside the helix. The helix-disrupting proline substitution probably perturbed the helix as a whole, while the other helix mutations modified portions.

During formation of quinolone-gyrase-DNA complexes, gyrase undergoes a conformational change and DNA is broken (8, 9). Release of double-strand DNA breaks is thought to be the lethal event (1). To estimate lethal effects separately from complex formation, survival of the *E. coli* mutants was measured at a constant multiple of the minimum inhibitory concentration (MIC) for the three fluoroquinolones. The relative resistance contributed by the alleles differed for lethal and bacteriostatic effects, emphasizing that blocking growth and killing cells are separate events. For most of the mutants, gatifloxacin was more lethal than the two C-8-H compounds. Thus, a C-8-methoxy moiety improves fluoroquinolone action against gyrase at both the bacteriostatic and bactericidal levels.

## MATERIALS AND METHODS

**Bacterial strains.** Bacterial strains, listed in Table 1, were derivatives of *E. coli* K-12. The *gyrA* mutations were obtained from a variety of sources (Table 1), and P1-mediated transduction was used to place them in the DM4100 background. For transduction, we first introduced into each *gyrA* (Nal<sup>r</sup>) strain *zfa*-3145::Tn10Kan or *zeg*::Tn10 that cotransduced with *gyrA* at frequencies of 70 and 50%, respectively. The *gyrA* mutations were then transduced into DM4100 by selection for Kan<sup>r</sup> or Tet<sup>r</sup> with scoring for resistance to nalidixic acid (10 or 20  $\mu$ g/ml, depending on the allele). Each *gyrA* mutant was also introduced into a derivative of DM4100 (KD1373) containing the S80L *parC* quinolone resistance allele. For

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TABLE 1. Bacterial strains

Strain	Relevant genotype <sup>a</sup>	Source or reference
DM4100	Wild type	20
KD66	DM4100 <i>gyrA</i> (Nal <sup>r</sup> S83L)	17
KD1366	<i>gyrA</i> (Nal <sup>r</sup> S83L) <i>parC</i> (Cip <sup>r</sup> S80L) <sup>b</sup>	25
KD1373	DM4100 <i>parC</i> (Cip <sup>r</sup> S80L)	25
KD1525	<i>gyrA</i> (Nal <sup>r</sup> D82A) <i>parC</i> (Cip <sup>r</sup> S80L)	This work, as described in reference 25
KD1721	DM4100 <i>gyrA</i> (Nal <sup>r</sup> A51V)	This work, by P1-mediated transduction from T/r (6)
KD1911	DM4100 <i>gyrA</i> (Nal <sup>r</sup> A67S)	This work, by P1-mediated transduction from P-10 (22)
KD1915	DM4100 <i>gyrA</i> (Nal <sup>r</sup> G81C)	This work, by P1-mediated transduction from N-97 (22)
KD1909	DM4100 <i>gyrA</i> (Nal <sup>r</sup> S83W)	This work, by P1-mediated transduction from P-18 (22)
KD1913	DM4100 <i>gyrA</i> (Nal <sup>r</sup> D87N)	This work, by P1-mediated transduction from N-113 (22)
KD1917	DM4100 <i>gyrA</i> (Nal <sup>r</sup> Q106H)	This work, by P1-mediated transduction from N-89 (22)
KD1957	<i>gyrA</i> (Nal <sup>r</sup> A51V) <i>parC</i> (Cip <sup>r</sup> S80L)	This work, by P1-mediated transduction from KD1721 <i>zeg::Tn10<sup>r</sup></i> into KD1373
KD1959	<i>gyrA</i> (Nal <sup>r</sup> A67S) <i>parC</i> (Cip <sup>r</sup> S80L)	This work, by P1-mediated transduction from KD1911 <i>zeg::Tn10</i> into KD1373
KD1961	<i>gyrA</i> (Nal <sup>r</sup> G81C) <i>parC</i> (Cip <sup>r</sup> S80L)	This work, by P1-mediated transduction from KD1915 <i>zeg::Tn10</i> into KD1373
KD1963	<i>gyrA</i> (Nal <sup>r</sup> S83W) <i>parC</i> (Cip <sup>r</sup> S80L)	This work, by P1-mediated transduction from KD1909 <i>zeg::Tn10</i> into KD1373
KD1965	<i>gyrA</i> (Nal <sup>r</sup> A84P) <i>parC</i> (Cip <sup>r</sup> S80L)	This work, by P1-mediated transduction from KD1975 <i>zeg::Tn10</i> into KD1373
KD1967	<i>gyrA</i> (Nal <sup>r</sup> D87Y) <i>parC</i> (Cip <sup>r</sup> S80L)	This work, by P1-mediated transduction from KD1977 <i>zeg::Tn10</i> into KD1373
KD1969	<i>gyrA</i> (Nal <sup>r</sup> Q106H) <i>parC</i> (Cip <sup>r</sup> S80L)	This work, by P1-mediated transduction from KD1917 <i>zeg::Tn10</i> into KD1373
KD1971	<i>gyrA</i> (Nal <sup>r</sup> D87N) <i>parC</i> (Cip <sup>r</sup> S80L)	This work, by P1-mediated transduction from KD1913 <i>zeg::Tn10</i> into KD1373
KD1973	DM4100 <i>gyrA</i> (Nal <sup>r</sup> D82A)	This work, by P1-mediated transduction from KD1525 into DM4100 (25)
KD1975	DM4100 <i>gyrA</i> (Nal <sup>r</sup> A84P)	This work, by P1-mediated transduction from P-5 (22)
KD1977	DM4100 <i>gyrA</i> (Nal <sup>r</sup> D87Y)	This work, by P1-mediated transduction from KD1677 <sup>d</sup> (M. Friedman)

<sup>a</sup> Amino acid abbreviations: A, alanine; D, aspartic acid; C, cysteine; H, histidine; G, glycine; L, leucine; N, asparagine; P, proline; Q, glutamine; S, serine; V, valine; W, tryptophan; Y, tyrosine. The first letter represents the wild-type amino acid at the position indicated by the number. The second letter indicates the identity of the amino acid which was substituted for the original at that position.

<sup>b</sup> By itself this *parC* allele confers no resistance because topoisomerase IV is a secondary target.

<sup>c</sup> *zeg::Tn10* encodes a tetracycline resistance gene that is 50% cotransducible with *gyrA*.

<sup>d</sup> Obtained by selection on agar containing nalidixic acid (20 μg/ml).

this construction, *zeg::Tn10* was introduced into each *gyrA* (Nal<sup>r</sup>) mutant. P1-mediated transduction, with selection for tetracycline resistance followed by scoring for nalidixic acid resistance, was then used to introduce each *gyrA* allele into the *parC* mutant (in *gyrA*<sup>+</sup> cells, the *parC* allele confers no quinolone resistance).

**Fluoroquinolones and measurement of potency.** Ciprofloxacin was purchased from Miles Pharmaceutical Co.; gatifloxacin (C-8-methoxy; AM1155) and its C-8-H control AM1121 were obtained from Bristol-Myers Squibb. Stock solutions (10 mg of fluoroquinolone per ml in 0.1 M NaOH) were stored at -70°C.

The MIC at which 99% of the isolates tested were inhibited (MIC<sub>99</sub>) was determined for each fluoroquinolone by spotting 10-μl aliquots of diluted, late log-phase cultures on Luria-Bertani (LB) agar plates (13) containing various concentrations of the fluoroquinolone. After overnight incubation at 37°C, the number of colonies appearing on the plates was counted and plotted against the fluoroquinolone concentration. The MIC<sub>99</sub> of the fluoroquinolone was determined by interpolation.

Bactericidal effects of the fluoroquinolones were measured by incubating exponentially growing cultures with the fluoroquinolones in the presence or absence of chloramphenicol (20 μg/ml) for 2 h. Aliquots were then removed, diluted, and plated on drug-free LB agar for determination of viable cell numbers.

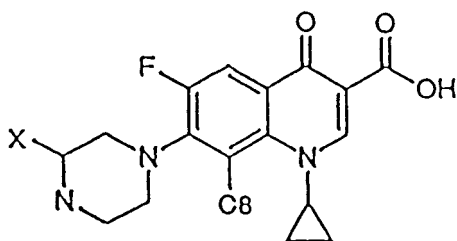
## RESULTS

**Bacteriostatic effect of fluoroquinolone C-8-methoxy group against resistant gyrase.** To examine the effects of fluoroquinolone structure on the attack of mutant gyrase, we obtained a variety of *E. coli gyrA* mutants and prepared an isogenic collection by P1-mediated transduction (Table 1). We then measured the ability of gatifloxacin, AM1121, and ciprofloxacin (Fig. 1) to block the growth of mutant and wild-type strains through determination of MIC<sub>99</sub>s. Data analysis included normalization of mutant MICs to wild-type values to minimize possible differences among the compounds with respect to nontopoisomerase factors such as drug uptake, efflux, etc. As shown in Fig. 2A, the effect of individual resistance alleles varied considerably, with mutations at codons 83 and 87 in *gyrA* conferring the most resistance. By this assay gatifloxacin was more active than its C-8-H derivative (AM1121) against all mutations except A84P and Q106H, which showed little dif-

ference. Ciprofloxacin exhibited the same general pattern of activity as AM1121 (Fig. 2A).

In *E. coli*, topoisomerase IV is attacked after gyrase has become resistant (1, 11, 16). Thus some of the effects described above could have arisen from differences in fluoroquinolone action against topoisomerase IV. To minimize these effects we transduced each *gyrA* allele into an *E. coli* strain that carried a quinolone-resistance allele of *parC* (S80L), which alone confers no resistance. The MIC<sub>99</sub> of each of the three fluoroquinolones was then determined for each double mutant and normalized to the MIC<sub>99</sub> obtained with the *gyrA*<sup>+</sup> *parC* (S80L) strain to estimate the increase in resistance due to each *gyrA* allele. Introduction of resistant topoisomerase IV changed the relative resistance contributed by the alleles (compare Fig. 2A and B), indicating that wild-type topoisomerase IV can be a quinolone target when gyrase is resistant. Gatifloxacin was more active than its C-8-H control or ciprofloxacin (Fig. 2B). As described above, mutants carrying the A84P and Q106H alleles showed little difference among the compounds. With respect to absolute values of MIC, gatifloxacin was 60% more effective than ciprofloxacin for the most resistant mutant (S83W).

For each of the three quinolones, the effect of introducing a *parC* resistance allele into *gyrA* mutants is shown in Fig. 3. In Fig. 3, the abscissa represents the protective effect of the various *gyrA* mutations in the presence of a wild-type *parC* allele; the ordinate represents their protective effect in the presence of a *parC* resistance allele. Resistance due to the less protective *gyrA* mutations was unaffected by the *parC* resistance allele, presumably because mutant gyrase was more susceptible than wild-type topoisomerase IV. The curves sharply increase at the point where wild-type topoisomerase IV becomes more susceptible than mutant gyrase—at that point, the *parC* resistance allele contributes to resistance. The magnitude of protection provided by the *parC* resistance allele should depend on how well each fluoroquinolone attacks resistant gyrase, since mu-



Fluoroquinolone	Structure variation	
	C8	X
Ciprofloxacin	H	H
AM1121	H	CH <sub>3</sub>
Gatifloxacin	OCH <sub>3</sub>	CH <sub>3</sub>

FIG. 1. Fluoroquinolone structures.

tant gyrase was the more sensitive target in the double mutant, as indicated by selection of a triple mutant in which the third mutation mapped in the quinolone resistance-determining region of *GyrA* (data not shown). By this criterion gatifloxacin was more potent than the two other compounds (the *parC* resistance allele protected least against gatifloxacin [Fig. 3]).

The point (*gyrA* allele) in Fig. 3 at which the *parC* resistance allele began to be protective, i.e., the point where *parC*-dependent resistance increased sharply, depended on how effectively each compound attacked wild-type topoisomerase IV. Thus, gatifloxacin was more active against wild-type topoisomerase IV than AM1121 or ciprofloxacin. The mutation at position 82 showed anomalous behavior (Fig. 3): for each fluoroquinolone tested, the addition of a *parC* allele was exceptionally protective (see Discussion).

To assess the effect of the C-8-methoxy group on resistance due to each *gyrA* allele, we divided the normalized MIC<sub>99</sub> for gatifloxacin by that for AM1121, obtained with *gyrA parC* double mutants to minimize the contribution of topoisomerase IV. As shown in Fig. 4, the C-8-methoxy group facilitated attack of alleles in the core of the quinolone-resistance-determining region (positions 81-87). Those lying outside the core were affected little by the methoxy group (Fig. 4). The A84P allele, an  $\alpha$ -helix 4 mutation, was unresponsive to the methoxy group (Fig. 4). Thus, specific changes in fluoroquinolone structure elicit effects that depend on *GyrA* structure.

**Bactericidal effects of fluoroquinolone C-8-methoxy group against resistant gyrase.** To compare lethal activity among the three compounds for the set of mutants, we measured survival rates at specific multiples of the MIC<sub>99</sub>s to minimize differences in bacteriostatic activity, which probably reflects formation of quinolone-gyrase-DNA complexes (4). Pilot experiments (data not shown) indicated that the three compounds killed wild-type (strain DM4100) and mutant (strains KD66 and KD1366) cells extensively within 2 h at fluoroquinolone concentrations 10 times higher than the MIC<sub>99</sub>s. Under these conditions the paradoxical increase in survival rates at high concentrations (reviewed in reference 19) was not observed. Gatifloxacin killed a greater fraction of cells than its C-8-H derivative or ciprofloxacin (Fig. 5A) for all mutants except strains containing A51V and D82A gyrase mutations. By introducing a *parC* resistance allele we minimized attack of topoisomerase IV, and again gatifloxacin was the most lethal of

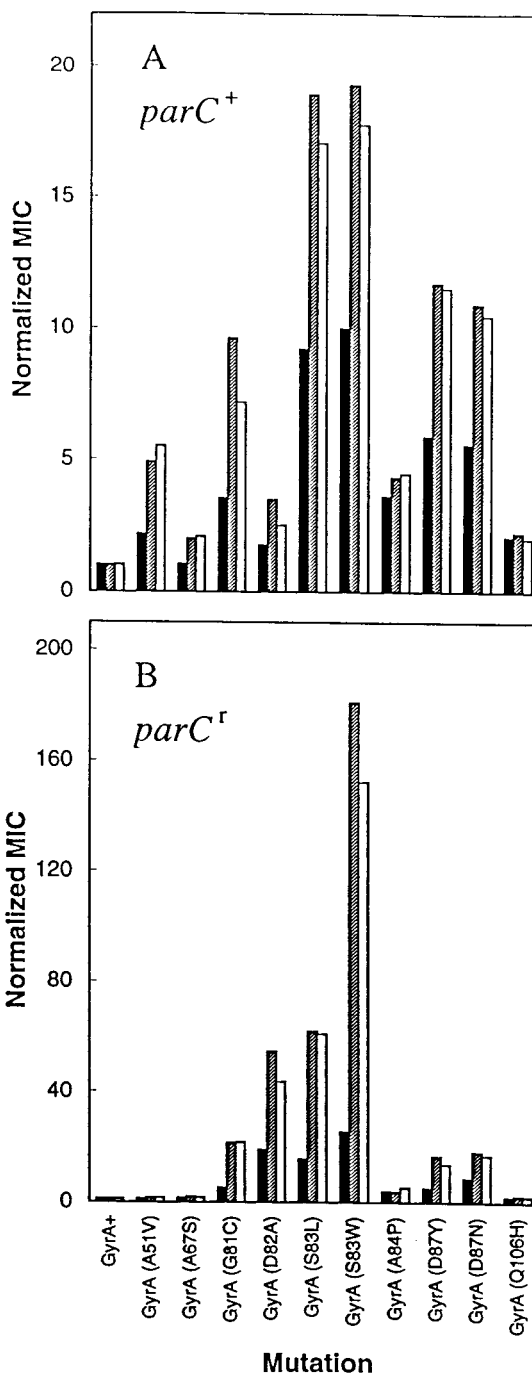


FIG. 2. Bacteriostatic action of fluoroquinolones against resistant mutants. Isogenic strains of *E. coli* containing the indicated alleles of *gyrA* (A) or *gyrA parC* (B) were applied to LB agar plates containing various concentrations of the fluoroquinolones for determination of the MIC<sub>99</sub>s. The MIC<sub>99</sub> for each mutant was then divided by the MIC<sub>99</sub> of wild-type or *parC* (S80L) cells to generate the normalized MIC<sub>99</sub> (the MIC<sub>99</sub>s with wild-type cells were 0.038, 0.013, and 0.011  $\mu$ g/ml for gatifloxacin [C-8-methoxy; solid bars], AM1121 [C-8-H; shaded bars], and ciprofloxacin [C-8-H; open bars], respectively. The data shown are the averages of two determinations.

the three compounds (Fig. 5B). The A51V and D82A alleles proved to be exceptions, with AM1121 showing more activity than gatifloxacin. With some mutants ciprofloxacin was more lethal than AM1121, while the reverse was true with others.

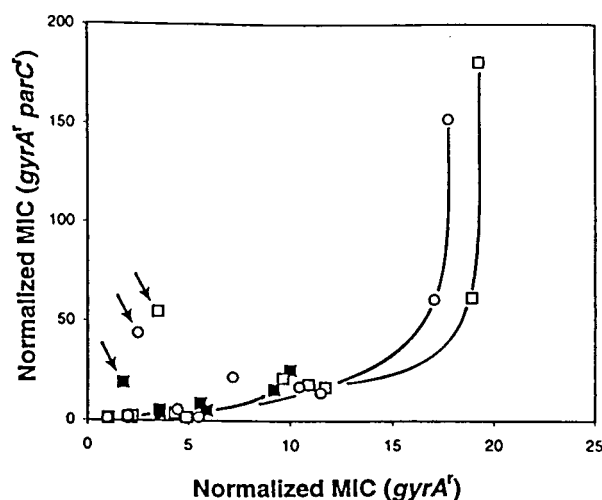


FIG. 3. Relationship between bacteriostatic activities of fluoroquinolones against *gyrA* mutants and *gyrA parC* mutants. The  $MIC_{99.5}$  for *gyrA* (Fig. 2A) and *gyrA parC* (Fig. 2B) mutants, normalized to the  $MIC_{99.5}$  of wild-type and *parC* mutant cells, respectively, as described in the legend for Fig. 2, were plotted such that each data point represents one *gyrA* allele with wild-type and mutant *parC* alleles. Symbols: ■, gatifloxacin; □, AM1121; and ○, ciprofloxacin. Arrows indicate values for the D82A *gyrA* allele.

Survival after treatment with gatifloxacin was only 2 to 50% of that with AM1121 and 10 to 60% of that with ciprofloxacin.

**Effect of chloramphenicol on bactericidal activity of fluoroquinolones.** Fluoroquinolones cause two types of lethal activity, one that requires protein synthesis and one that does not (reviewed in reference 5). With single *gyrA* mutants gatifloxacin was from 2- to 40-fold more lethal than AM1121 or ciprofloxacin when chloramphenicol was included to block protein synthesis (Fig. 6A). Exceptions were seen with the A67S and S83W alleles; for these there was little difference among the compounds. When the *gyrA parC* double mutants were examined, gatifloxacin was about twice as effective as the two other compounds, except in the case of the S83W mutation where not much difference was seen (Fig. 6B). Thus, the C-8-methoxy group improved lethal action against most of the *gyrA* mutants, as was the case when inhibition of growth was measured.

## DISCUSSION

The work described above compared gatifloxacin, a C-8-methoxy fluoroquinolone, with two structurally related C-8-H compounds for the ability to attack a wide variety of resistant *gyrA* mutants of *E. coli*. Since C-8-methoxy and C-8-H compounds are not equally active against wild-type gyrase or wild-type *E. coli* (18, 21, 25), we normalized all mutant MICs to wild-type values. In some experiments we also included a resistant *parC* allele to minimize the contribution of a secondary target, DNA topoisomerase IV. For 9 out of 10 *gyrA* alleles, gatifloxacin exhibited more normalized bacteriostatic activity than the two C-8-H compounds (Fig. 2). Differences caused by the introduction of the *parC* resistance allele (compare Fig. 2A and B) indicate that wild-type topoisomerase IV can become a target when the gyrase allele provides sufficient resistance. The least protective *gyrA* mutant with which resistant *parC* had a substantial effect reflected the susceptibility of wild-type topoisomerase IV to each compound. That value was lowest for gatifloxacin (Fig. 3), which indicated that the C-8-methoxy group enhances attack of wild-type topoisomerase IV. Once gyrase became resistant enough to make wild-type topoisomerase IV the main target, the extent of protection afforded by

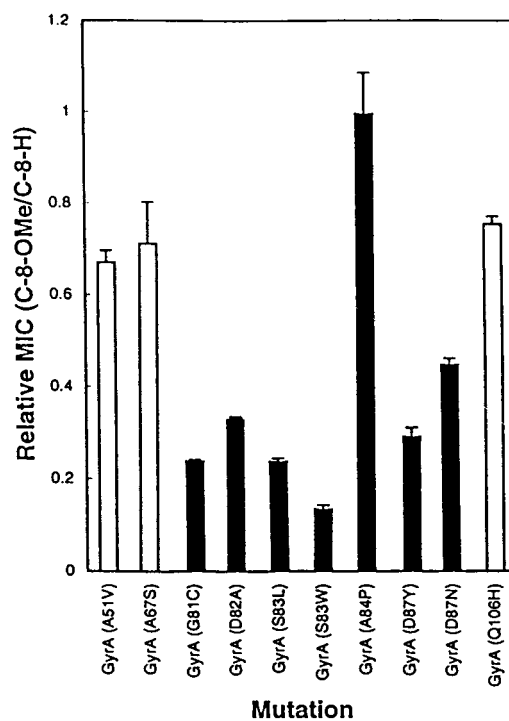


FIG. 4. Effect of fluoroquinolone structure on bacteriostatic activity against resistant gyrase. Normalized  $MIC_{99.5}$  of *gyrA parC* mutants (Fig. 2B) are expressed as gatifloxacin/AM1121  $MIC_{99.5}$  ratios. Filled bars represent alleles located in  $\alpha$ -helix 4 according to reference 14; open bars represent alleles outside  $\alpha$ -helix 4. The values shown are the averages of two independent determinations. Error bars indicate the upper ranges of the determinations.

the *parC* mutation depended on the *gyrA* allele and the fluoroquinolone. The least protection was observed with gatifloxacin (Fig. 3).

The response of individual mutations to the presence of a C-8-methoxy group can be rationalized by separating the alleles into two categories, those that lie in  $\alpha$ -helix 4 of the GyrA protein and those that do not (Fig. 7). For mutations mapping in the helix, resistance to bacteriostatic action was decreased substantially by a C-8-methoxy group, while those mapping outside the helix were relatively insensitive to the group (Fig. 4). These data, plus the observation that mutations in  $\alpha$ -helix-4 confer the highest levels of resistance, suggest that  $\alpha$ -helix-4 may be part of a quinolone-binding site. Substitution of Leu or Trp for Ser-83 and Asn or Tyr for Asp-87 may confer quinolone resistance by making the microenvironment of  $\alpha$ -helix 4 less electron-rich and less able to bind quinolone. C-8-methoxy or C-8-halogenated fluoroquinolones may reduce resistance by partially restoring electron richness to the region.

Substitution of proline for alanine at codon 84 generated a helix mutant that behaved like the nonhelix alleles (Fig. 4). Unlike other substitutions that confer resistance, a proline is expected to disrupt helix structure. Thus, it is not surprising that the effect of A84P differs from the mutations that probably change local affinity for a quinolone. Perturbation of the helix between positions 83 and 87 might alter the alignment between these putative quinolone-binding sites. According to this idea, placing a proline at position 85 or 86, two positions that lie on the bottom surface of the helix and are not generally associated with quinolone resistance, should have the same effect as seen at position 84.

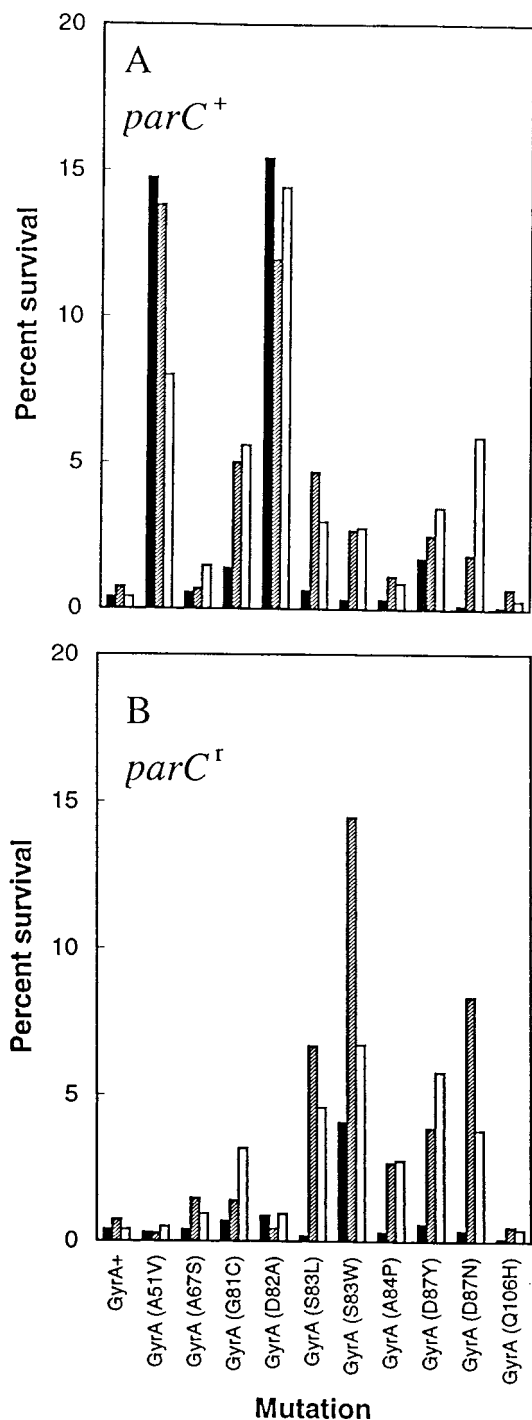


FIG. 5. Bactericidal action of fluoroquinolones against resistant mutants. The survival rates of the indicated mutant strains of *E. coli* were determined following a 2-h incubation in gatifloxacin (filled bars), AM1121 (shaded bars), or ciprofloxacin (open bars) at 10 times the MIC<sub>90</sub> for each compound. (A) *gyrA* mutant strains; (B) *gyrA parC* double mutants. The data shown are the averages of two determinations.

Comparison of lethal action revealed that gatifloxacin was superior to the two C-8-H compounds for most of the *gyrA* mutants examined even when cells were treated with chloramphenicol. An exception was observed when tryptophan was substituted for serine at codon 83: little difference was seen between gatifloxacin and AM1121 (Fig. 6). These observations

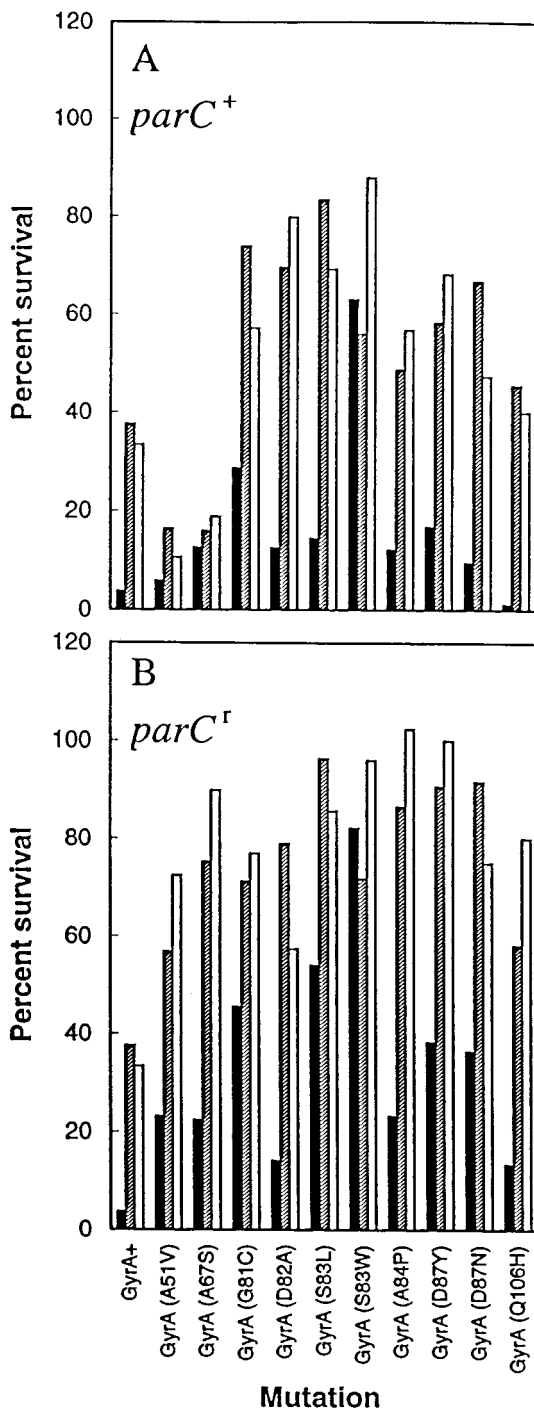


FIG. 6. Bactericidal action of fluoroquinolones against resistant *gyrA* and *gyrA parC* mutants in the presence of chloramphenicol. The survival rates of mutants were determined following a 2-h incubation in chloramphenicol plus gatifloxacin (filled bars), AM1121 (shaded bars), or ciprofloxacin (open bars) at 10 times the respective MIC<sub>90</sub>s (A) *gyrA* mutant strains; (B) *gyrA parC* double mutant strains. The data shown are the averages of two determinations.

should contribute to a better understanding of quinolone-gyrase-DNA complexes when more structural information becomes available.

Resistance associated with mutation of amino acid 82 of GyrA exhibited several unusual features. First, position 82 gyrase mutations arise rarely, if at all, unless topoisomerase IV

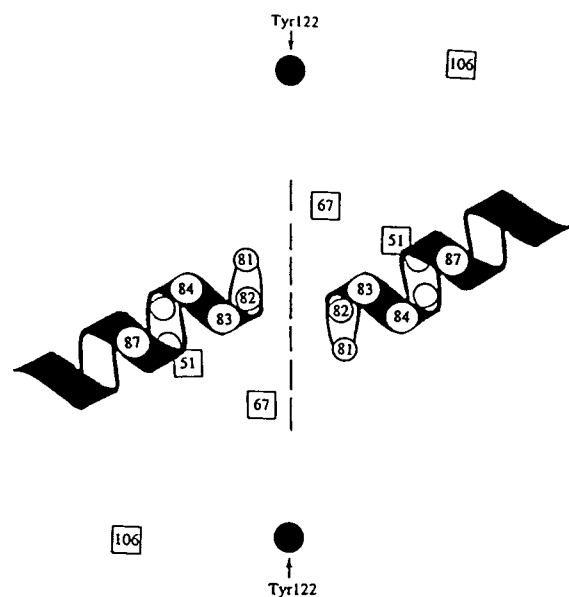


FIG. 7. Relative positions of resistance alleles in GyrA dimer. The numbers indicate amino acid positions in the *E. coli* GyrA protein for resistance mutations and for the active center tyrosine (solid circles). The resistance alleles that reside within  $\alpha$ -helix 4 are shown as open circles; residues outside the helix that confer resistance are shown as open squares. The dashed line approximates the interface of the two GyrA subunits. DNA is predicted to lay across the protein at an angle from upper left to lower right such that the two helices fit in the major groove of DNA (14).

is also resistant (25). Second, introduction of *parC*-mediated resistance into a D82A gyrase mutant rendered the double mutant exceptionally resistant when bacteriostatic action was measured (Fig. 3). Third, when lethal activity was measured, the D82A allele was among the more resistant alleles until a *parC* resistance allele was added (Fig. 5). These observations, which are still unexplained, may reflect special features of this amino acid due to its position at the GyrA dimer interface.

The results described above have several practical implications. First, enhanced ability to block mutant growth and kill mutant cells is expected to restrict the selection of resistant mutants, as observed with fluoroquinolones having structures similar to gatifloxacin (2, 3). Second, the C-8-methoxy group improves activity against *gyrA parC* double mutants, as revealed by comparison of gatifloxacin and AM1121. Consequently, a concentration should exist at which wild-type cells would need to acquire three mutations to express resistance. For *E. coli* that concentration is about 1  $\mu\text{g/ml}$  for the most resistant mutant we examined. This value is within the range of serum concentrations normally achieved by fluoroquinolones (10). If the serum concentration is kept above that level, no mutant would be selected (see reference 3). Finally, the C-8-methoxy group improved the attack of topoisomerase IV (as shown by a comparison of results for gatifloxacin and AM1121 [Fig. 3]), which should make gatifloxacin better able to attack species in which topoisomerase IV is the primary quinolone target. Thus, compounds such as gatifloxacin are likely to have widespread application.

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