

# Fluoroquinolones: Relationships between Structural Variations, Mammalian Cell Cytotoxicity, and Antimicrobial Activity<sup>1</sup>

Mark J. Suto,\* John M. Domagala, Gregory E. Roland, Gail B. Mailloux, and Michael A. Cohen

Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, Michigan 48105

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Fluoroquinolones are potent inhibitors of bacterial topoisomerase II (DNA gyrase). They can also inhibit eukaryotic topoisomerases, which could possibly lead to clastogenicity and/or cellular toxicity. Recent studies have demonstrated a correlation between mammalian cell cytotoxicity of the fluoroquinolones and the potential of these compounds to induce micronuclei, a genetic toxicity endpoint. In an effort to identify potent nontoxic quinolone antibacterials, we have examined the structural features of the fluoroquinolones associated with mammalian cell cytotoxicity. An investigation of a wide variety of substituents at the 1, 5, 7, and 8 positions of a quinolone nucleus was conducted. The results indicate that no one position has a controlling effect on the observed cytotoxicity. Instead, a combination of the various substituents contributes to the effects seen. Certain trends were apparent, such as the fact that compounds with pyrrolidines at the R-7 position were more cytotoxic than those with piperazines, and halogens at R-8 (X-position) were associated with more cytotoxicity relative to hydrogen. A general trend also existed between the cytotoxicity of the compounds and their Gram-positive antibacterial activity. A detailed comparison between the various groups and positional variations as they controlled the cytotoxicity and antibacterial activity is presented.

## Introduction

Topoisomerases are enzymes critical for maintaining and controlling the conformations required for DNA replication and transcription.<sup>2</sup> They are found in both eukaryotic and prokaryotic cells and represent targets for a wide variety of chemotherapeutic agents. For example, inhibitors of eukaryotic topoisomerases have been used as antitumor agents, and inhibitors of bacterial topoisomerase II (DNA gyrase) have been used as highly successful antibacterial agents.<sup>3,4</sup> These compounds elicit their effects by disrupting DNA synthesis and cell division.<sup>4</sup> The fluoroquinolones represent one of the first classes of antibacterials whose mechanism of action is inhibition of DNA gyrase.<sup>5</sup> Studies have shown that quinolone antibacterial agents can also affect eukaryotic topoisomerases, as was recently demonstrated with the nalidixic acid analogue CP-67015.<sup>6</sup> The inhibition of eukaryotic topoisomerase II by this compound resulted in it acting as a direct mutagen in a number of model systems. Studies with a limited series of fluoroquinolones have also shown that their ability to inhibit topoisomerase II was directly correlated with their cytotoxicity on murine cells.<sup>7</sup> Therefore, the use of compounds such as the fluoroquinolones,

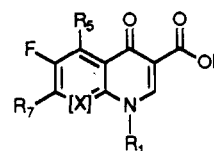


Figure 1.

which function as antibacterials via inhibition of DNA gyrase, could potentially lead to gene mutations and/or toxicities in the host organism.<sup>5</sup> Although the selective antibacterial activity of many of the newer fluoroquinolones is unquestionable, their effects on eukaryotic cells continues to be an area of active research.

Ongoing studies in our laboratories have been directed toward identifying such liabilities as they pertain to the fluoroquinolones as a class. Gracheck et al.<sup>8</sup> demonstrated that mammalian cell cytotoxicity in Chinese hamster V79 cells was predictive of the in vitro genetic toxicity for the fluoroquinolone class of compounds. In this study, a small group of compounds was evaluated in vitro for their ability to inhibit eukaryotic topoisomerase II activity, their cytotoxicity toward mammalian cells, and their induction of micronuclei, a genetic toxicity endpoint.<sup>6,7,9</sup> A strong correlation was seen between the induction of micronuclei in vitro and mammalian cell cytotoxicity ( $r^2 = 0.94$ ).

(1) Portions of this work were presented at the 31st Interscience Conference on Antimicrobial Agents and Chemotherapy, 1991, Chicago, Illinois.

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Table I. Quinolone Standards

| compound number | name          | R <sub>7</sub> | X   | R <sub>1</sub>                    | mammalian cell cytotoxicity IC <sub>50</sub> (μg/mL) | antibacterial activity MIC (Gram -/Gram +, μg/mL) |
|-----------------|---------------|----------------|-----|-----------------------------------|--|---|
| 1               | Norfloxacin   |                | C-H | Et                                | >500   | 0.06/0.61   |
| 2               | Pefloxacin    |                | C-H | Et                                | >500   | 0.07/0.40   |
| 3               | Ofloxacin     |                |     |                                   | >500   | 0.20/0.46   |
| 4               | Ciprofloxacin |                | C-H |                                   | 380  | 0.10/0.91   |
| 5               | Fleroxacin    |                | C-F | CH <sub>2</sub> CH <sub>2</sub> F | >500   | 0.35/1.81   |
| 6               | Tosufloxacin  |                | N   |                                   | 128  | 0.09/0.05   |
| 7               | PD 117558     |                | C-F |                                   | 11   | 0.09/0.02   |

Therefore, we hypothesized that we could use the readily available mammalian cell cytotoxicity data as an indication of a compound's potential to cause genetic toxicity. In an effort to determine those structural features of the fluoroquinolones, which were responsible for the mammalian cell cytotoxicity and ultimately the in vitro genetic toxicity, we examined a series of structurally diverse fluoroquinolones for their cytotoxicity against Chinese hamster V79 cells. The compounds chosen varied in the substituents at the 1 (R-1), 5 (R-5), 7 (R-7), and 8 (X) positions (Figure 1). These data could be used to establish a structure-activity relationship that would be predictive of in vitro genetic toxicity. For comparison, the antibacterial activity of these compounds as represented by the geometric means of the MIC's for Gram-negative and Gram-positive bacteria are also given. The ultimate goal was to identify the structural features which not only provide the potential for reduced genetic toxicity but also potent broad-spectrum antibacterial activity.

### Materials and Methods

**Chemistry.** All of the compounds used in these studies have been previously reported or prepared using standard published procedures.<sup>5b,10,11</sup> In summary, the compounds were prepared by reacting the appropriate quinolone/naphthyridone, substituted at the 7-position with a halogen, with 1.1 equiv of the amine side chain in the presence of excess Et<sub>3</sub>N. Standard workup and depro-

tection of the side chain when necessary provided the target compounds.<sup>11</sup>

For clarity, each of the tables were viewed as separate entities; therefore some of the same structures have different numbers. A complete list is provided.<sup>12</sup>

**Mammalian Cell Cytotoxicity (Clonogenic Cytotoxicity).** Chinese hamster V79 cells were seeded into Costar six-well cluster dishes and incubated overnight at 37 °C in a humidified incubator (95% air, 5% CO<sub>2</sub>). At the time of assay, culture medium (TCM; RPMI 1640, HEPES buffered + 10% fetal bovine serum + gentamicin 50 μg/mL) was removed from wells without disturbing adherent cells and was replaced with 3 mL of fresh medium containing various concentrations of test compounds. All concentrations were tested in duplicate. Cells were incubated with test compounds for 3 h at 37 °C. After incubation, the medium containing compound was aspirated, cells were rinsed twice with Hank's balanced salt solution, and 3 mL of fresh medium was added to each well. Cells were then incubated for an additional 5 days at 37 °C. Any colonies which formed were stained with crystal violet, and cell survival was assessed by counting

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(12) The compounds with the same structure but different numbers are: 7, 12, 26b, and 67; 16, 26a, 36, and 66; 21, 24b, and 43; 24d, 35, and 47; 4, 29a, and 40a; 24c, 33, and 46; 24a and 44; 26d and 68; 26c and 69.

colonies using an Artek Counter Model 880. The  $IC_{50}$  ( $\mu\text{g/mL}$ ) was determined and was defined as the concentration of compound yielding 50% cell survival compared to untreated control cells.

**Antimicrobial Activity.** The compounds were evaluated against five Gram-negative organisms and five Gram-positive organisms using standard microdilution techniques.<sup>13</sup> The data presented represent the geometric mean of the MIC's ( $\mu\text{g/mL}$ ) for the Gram-negative (*Enterobacter cloacae* MA 2646, *Escherichia coli* Vogel, *Klebsiella pneumoniae* MGH-2, *Providencia rettgeri* M 1771, and *Pseudomonas aeruginosa*) and the Gram-positive (*Staphylococcus aureus* H 228, *Staphylococcus aureus* UC-76, *Enterococcus faecalis* MGH-2, *Streptococcus pneumoniae* SV-1, and *Streptococcus pyogenes* C-203) bacteria.

## Results and Discussion

All of the data are listed in Tables I–V. In addition to the various structural features indicated, each table contains the mammalian cell cytotoxicity, expressed as the  $IC_{50}$  in  $\mu\text{g/mL}$ , and the antibacterial activity, expressed as the geometric mean of the MIC's for Gram-negative and Gram-positive organisms ( $\mu\text{g/mL}$ ). Table I contains this data for a series of quinolone standards. Examination of the data in Table I indicates that the mammalian cell cytotoxicity or the antibacterial data alone do not necessarily reflect the utility of the compound. For example, if one compares compound 6 (Tosufloxacin) to compounds 1–5, it would appear that 6 was more cytotoxic (128 versus  $>300 \mu\text{g/mL}$ ) and possibly not a "useful" antibacterial agent. However, if one examines the antibacterial data, compound 6 was also very potent against both Gram-negative and Gram-positive organisms, and its "safety margin" or selectivity index (ratio of  $IC_{50}$  to MIC) was still greater than 1000. Compound 7 was more cytotoxic to mammalian cells, but again due to its increased antibacterial activity the selectivity index was still  $>200$  to 1000. The data in Table I for these quinolone standards clearly indicated that some increase in mammalian cell cytotoxicity could be expected and accepted, particularly if a corresponding increase in antibacterial activity was obtained. Also of note was the fact that although 6 was cytotoxic at  $128 \mu\text{g/mL}$ , no quinolone thus far examined has ever achieved tissue or fluid levels in humans anywhere near those levels.<sup>14</sup> Quinolone concentrations in most tissues usually were found to be in the range of 1–10  $\mu\text{g/mL}$ , meaning at least a 12-fold selectivity with compound 6 would still exist.

**R-1 Substituents.** Table II contains the compounds which varied in the substituent at the R-1 position for a constant R-7 and X-position substituent (Figure 1). Three different series of quinolones were examined. In general, a cyclopropyl group at R-1 resulted in compounds with increased cytotoxicity (lower  $IC_{50}$  values) and increased antibacterial activity (lower geometric means). Other groups at R-1 usually resulted in compounds which were less cytotoxic to mammalian cells, but the effect on antibacterial activity was not as clearly defined. For example, adding a methylene spacer between the N-1 nitrogen and the cyclopropyl group (compound 13) greatly

Table II. R<sub>1</sub> Substituents

| compd no. | R <sub>1</sub> | mammalian cell cytotoxicity $IC_{50}$ , $\mu\text{g/mL}$ | antibacterial activity MIC (Gram -/Gram +, $\mu\text{g/mL}$ ) |
|-----------|----------------|--|---|
| 8         |                | 160  | 0.20/0.10   |
| 9         |                | $>500$   | 0.40/0.15   |
| 10        |                | 58   | 1.82/0.26   |
| 11        |                | $>500$   | 50/9.50   |
| 12        |                | 11   | 0.05/0.01   |
| 13        |                | 240  | 4.17/0.35   |
| 14        |                | $>500$   | 0.26/2.40   |
| 15        |                | 310  | 0.56/0.14   |
| 16        |                | 160  | 0.46/0.10   |
| 17        |                | 190  | 0.80/0.09   |
| 18        |                | $>500$   | 12.5/1.81   |
| 19        |                | 300  | 0.12/0.26   |
| 20        |                | 310  | 0.13/0.70   |
| 21        |                | 30   | 0.04/0.03   |
| 22        |                | 160  | 0.30/0.30   |

diminished the cytotoxicity and the Gram-negative antibacterial activity relative to compound 12, but did not have as great an effect on Gram-positive activity. Another important point was illustrated by compounds 8 versus 12, 15 versus 16, and 19 versus 21. Replacing the R-1 ethyl group with a cyclopropyl group caused an increase in cytotoxicity, but very little change in antibacterial activity was observed. Therefore, the ethyl group at R-1 resulted in compounds which were less cytotoxic to mammalian cells and since there was a minimal loss of antibacterial activity, they had a favorable selectivity index. An ideal target compound might then contain the cytotoxicity profile of the N-1 ethyl series, but the antibacterial activity of the N-1 cyclopropyl group.

**R-7 versus X Substituents.** In general, a wide variety of structural modifications have been carried out at the R-7 and X positions of the fluoroquinolones (Figure 1) in an attempt to improve the antibacterial activity of this class of compounds. Changes in both the R-7 substituent and the X-position have had a dramatic effect on the antibacterial activity. It also appears that these changes are synergistic in that modifications of one of these

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Table III. R<sub>7</sub> versus X Substituents

| compound number | R <sub>7</sub> | mammalian cell cytotoxicity IC <sub>50</sub> (μg/mL), and antibacterial activity MIC (Gram -/Gram +, μg/mL) |                   |                   |                   |                          |                  |
|-----------------|----------------|---|-------------------|-------------------|-------------------|--------------------------|------------------|
|                 |                | a: X = C-H  | b: X = C-F        | c: X = C-Cl       | d: X = C-OMe      | e: X = C-CF <sub>3</sub> | f: X = N         |
| 23              |                | 38<br>0.20/0.05   | 120<br>0.10/0.006 | 150<br>0.10/0.006 | 150<br>0.30/0.02  | 280<br>12.5/6.31         | 58<br>0.26/0.07  |
| 24              |                | 72<br>0.09/0.14   | 30<br>0.04/0.03   | 26<br>0.03/0.04   | 45<br>0.07/0.03   | 300<br>0.20/0.20         | 98<br>0.03/0.13  |
| 25              |                | 81<br>0.20/0.20   | 11<br>0.05/0.01   | 8<br>0.06/≤0.003  | 10<br>0.11/0.03   | 38<br>0.52/0.03          | 51<br>0.14/0.03  |
| 26              |                | 160<br>0.46/0.10  | 11<br>0.09/0.02   | 11<br>0.14/<0.004 | 22<br>0.26/0.02   | 120<br>0.60/0.04         | 160<br>0.21/0.11 |
| 27              |                | 23<br>0.52/0.04   | 8<br>0.13/0.01    | 8<br>0.07/0.007   | 38<br>0.26/0.01   | 110<br>1.0/0.17          | 25<br>0.35/0.03  |
| 28              |                | 205<br>0.40/0.04  | 8<br>0.23/0.03    | 23<br>0.26/0.07   | 120<br>0.52/0.02  | 230<br>>30/0.35          | 58<br>0.60/0.08  |
| 29              |                | 380<br>0.10/0.90  | 47<br>0.07/0.40   | 43<br>0.07/0.06   | 82<br>0.07/0.11   | 160<br>0.15/0.30         | 120<br>0.09/0.21 |
| 30              |                | >500<br>0.08/0.15   | 150<br>0.05/0.20  | 140<br>0.23/0.26  | >500<br>0.34/0.10 | >500<br>0.91/0.80        | 250<br>0.17/1.90 |
| 31              |                | 230<br>0.26/0.17  | 23<br>0.20/0.08   | 18<br>0.08/0.03   | -                 | 20<br>0.26/0.13          | 100<br>0.23/0.23 |
| 32              |                | >500<br>0.91/0.53   | 53<br>0.13/0.06   | 24<br>0.07/0.009  | 69<br>0.23/0.01   | >500<br>0.52/0.11        | 100<br>0.40/1.60 |

positions had an effect on the other. One potential explanation for these observations is that steric interactions, proximity effects, or electronic interactions could be involved. Therefore, we wanted to examine how changes in the R-7 and X-position affected the mammalian cell cytotoxicity data, as well as how these groups interact together. In examination of Table III, moving down one of the rows (a-f) shows the effect of changing the R-7 substituent for a particular X-substituent. If one proceeds across the table, the effect of varying the X-substituent for a particular R-7 substituent is shown. Overall, Table III illustrates the important relationship between the X and R-7 positions with respect to both mammalian cell cytotoxicity and antibacterial activity.

A number of general conclusions could be drawn from this table, in addition to the specific conclusions apparent in a particular series (i.e. a particular X or R-7 substituent). First, increasing the bulk around the distal nitrogen, either through alkylation of the nitrogen or addition of an alkyl group in close proximity, generally resulted in compounds less cytotoxic to mammalian cells (25 versus 26, 27 versus 28, 29 versus 30). The magnitude of the change varied depending upon the X-substituent. Only a very small decrease in antibacterial activity was also observed, so overall the selectivity indices significantly improved (2-8-fold) for the more substituted analogs. Compounds in which the bulk (methyl group) was further removed from the distal nitrogen did not have the same effect (25 versus 27). Second, piperidines were overall more cytotoxic than

piperazines and piperidines. Third, halogens attached to the C-8 carbon (X = C-F and C-Cl, rows b and c) resulted in substantially more cytotoxic compounds as well as some of the most potent antibacterial agents thus far evaluated in our laboratories. However, the selectivity index was also decreased, and the increase in antibacterial activity was not sufficient to compensate for the increased cytotoxicity. Overall, the halogens at the X-position dominated the substituent effects that were seen. This substitution even negated the lack of cytotoxicity seen with those compounds containing a piperazine at R-7. Studies are currently underway to try and determine what the acceptable selectivity index or margin of safety might be. The methoxy group at C-8 (X = OMe, row d) had an effect similar to that seen with the halogens. Of note were the trifluoromethyl derivatives (row e), which did not follow any clear pattern, a trend that was also observed in Table IV.

**R-1 Substituents versus R-7 and X Substituents.** Having established a relationship between R-7 and X, we next wanted to examine this effect for two of the better R-1 substituents, the cyclopropyl group and the difluorophenyl group. Table IV compares these two R-1 substituents over several R-7 and X-substituents. The data demonstrated that the difluorophenyl group was less cytotoxic for all of the compounds except when X was trifluoromethyl (compounds 34 and 39). This trend did not hold when the substituent at R-7 was the 3-methylpiperazine group (compound 42). The difluorophenyl

**Table IV.** R<sub>1</sub>-Substituents: Cyclopropyl versus Difluorophenyl. Mammalian Cell Cytotoxicity IC<sub>50</sub>(μg/mL) and Antibacterial Activity MIC (Gram -/Gram +, μg/mL)

| compound number | R <sub>7</sub> | X                 | data                | R <sub>7</sub> | X                 | data                |
|-----------------|----------------|-------------------|---------------------|----------------|-------------------|---------------------|
| 33              |                | C-Cl              | 26<br>(0.03/0.04)   |                | C-Cl              | 89<br>(0.23/0.20)   |
| 34              |                | C-CF <sub>3</sub> | 300<br>(0.20/0.20)  |                | C-CF <sub>3</sub> | 99<br>(0.52/0.11)   |
| 35              |                | C-OMe             | 45<br>(0.07/0.03)   |                | C-OMe             | 290<br>(0.35/0.35)  |
| 36              |                | C-H               | 160<br>(0.46/0.10)  |                | C-H               | 190<br>(0.80/0.09)  |
| 37              |                | C-Cl              | 11<br>(0.14/<0.004) |                | C-Cl              | 130<br>(1.05/0.17)  |
| 38              |                | C-OMe             | 38<br>(0.26/0.01)   |                | C-OMe             | 250<br>(0.46/0.17)  |
| 39              |                | C-CF <sub>3</sub> | 110<br>(1.00/0.17)  |                | C-CF <sub>3</sub> | 18<br>(2.07/0.10)   |
| 40              |                | C-H               | 380<br>(0.10/0.90)  |                | C-H               | >500<br>(0.11/0.17) |
| 41              |                | C-Cl              | 45<br>(0.08/0.15)   |                | C-Cl              | >500<br>(2.07/0.53) |
| 42              |                | C-CF <sub>3</sub> | 220<br>(0.46/0.53)  |                | C-CF <sub>3</sub> | >500<br>(4.14/0.26) |

derivative 42b, was less cytotoxic than the cyclopropyl analogue, and although the Gram-negative activity was lost, the Gram-positive activity was slightly improved. This case again shows that decreased cytotoxicity may not always be useful, particularly if the antibacterial activity diminishes to the point of inactivity.

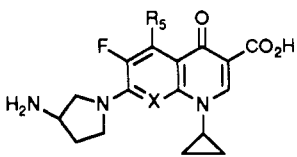
**X and R-5 Substituents.** Table V illustrates the relationship between the R-7 substituent and position X. Another important relationship exists between the R-5 and R-7 positions and the R-5 and the X-position.<sup>11c</sup> The interaction between the latter groups would be electronic, propagated through the aromatic ring. Table V compares a wide variety of compounds for a given R-7 substituent, which vary only in the X-position (43–50 and 66–71) and the R-5 position (51–55 and 72–73), and also included is a series of disubstituted compounds (56–65 and 74–78) for two different R-7 substituents. These data provide an understanding of the interaction between these key positions of the quinolone ring. In summary, substituents at the X-position (R-5 equals H and R-7 constant) had a profound effect on the cytotoxicity, with IC<sub>50</sub> values ranging from 11–290 μg/mL. Substituents at the R-5 position (X-substituent equals C-H, R-7 constant, examples 51–55) had little effect on cytotoxicity relative to their

corresponding parent compounds (R-5 equals hydrogen, 44 and 66). One exception may be compound 52, which seems to be slightly more cytotoxic than the parent 44. Compounds substituted at R-5 were generally more cytotoxic overall than the substituted X-derivatives and in general, the cytotoxicity and the antibacterial activity of the disubstituted analogs were controlled by the substituent at the X-position.

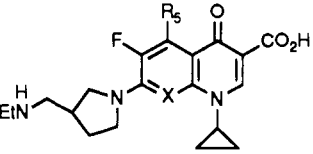
Of note in this series were compounds 53 and 55. These compounds represent examples of quinolones with diminished antibacterial activity, but which were still cytotoxic. This trend was not generally observed since it seemed that a correlation between cytotoxicity and antibacterial activity existed.

### Conclusions and Summary

Herein, we described our initial efforts examining the structural features responsible for the *in vitro* mammalian cell cytotoxicity and antibacterial activity. The data demonstrated that mammalian cell cytotoxicity was not controlled by any one substituent positioned on the quinolone ring. Depending upon the substitution pattern, certain positions may influence the cytotoxicity more than others. Overall, the mammalian cell cytotoxicity was most

Table V. X (C-R<sub>8</sub>) versus R<sub>5</sub> Substituents


| compound number | R <sub>5</sub>  | X                 | mammalian cell cytotoxicity IC <sub>50</sub> (μg/mL) | antibacterial activity MIC (Gram -/Gram +, μg/mL) |
|-----------------|-----------------|-------------------|--|---|
| 43              | H               | C-F               | 30   | 0.04/0.03   |
| 44              | H               | C-H               | 72   | 0.09/0.14   |
| 45              | H               | C-CF <sub>3</sub> | 300  | 0.20/0.20   |
| 46              | H               | C-Cl              | 26   | 0.03/0.04   |
| 47              | H               | C-OMe             | 44   | 0.07/0.03   |
| 48              | H               | C-NO <sub>2</sub> | 69   | 0.46/0.92   |
| 49              | H               | C-NH <sub>2</sub> | 260  | 0.53/2.06   |
| 50              | H               | C-SMe             | 31   | 0.20/0.06   |
| 51              | NH <sub>2</sub> | C-H               | 54   | 0.04/0.05   |
| 52              | Me              | C-H               | 14   | 0.09/0.05   |
| 53              | Et              | C-H               | 46   | 1.81/>2.71  |
| 54              | OH              | C-H               | 72   | 0.04/0.09   |
| 55              | SMe             | C-H               | 92   | >1.58/>3.1  |
| 56              | F               | C-F               | 100  | 0.08/0.37   |
| 57              | F               | C-CF <sub>3</sub> | 190  | 25/25   |
| 58              | NH <sub>2</sub> | C-F               | 15   | 0.01/0.01   |
| 59              | NH <sub>2</sub> | C-Cl              | 59   | 0.08/0.05   |
| 60              | NH <sub>2</sub> | C-OMe             | 100  | 0.11/0.04   |
| 61              | Me              | C-CF <sub>3</sub> | 290  | 0.91/0.79   |
| 62              | Me              | C-F               | 11   | 0.02/0.008  |
| 63              | OH              | C-F               | 43   | 0.03/0.05   |
| 64              | OMe             | C-Cl              | >500   | 2.08/12.48  |
| 65              | Cl              | C-Cl              | >500   | 0.13/0.15   |

|    |                 |                   |      |            |
|----|-----------------|-------------------|------|------------|
| 66 | H               | C-H               | 160  | 0.46/0.10  |
| 67 | H               | C-F               | 11   | 0.09/0.02  |
| 68 | H               | C-OMe             | 22   | 0.26/0.02  |
| 69 | H               | C-Cl              | 11   | 0.14/0.004 |
| 70 | H               | C-NO <sub>2</sub> | 67   | 1.05/0.20  |
| 71 | H               | C-NH <sub>2</sub> | 290  | 0.91/0.23  |
| 72 | NH <sub>2</sub> | C-H               | 61   | 0.35/0.06  |
| 73 | Me              | C-H               | 42   | 0.60/0.05  |
| 74 | F               | C-F               | 46   | 0.56/0.08  |
| 75 | NHMe            | C-F               | 53   | 0.69/0.04  |
| 76 | NHAc            | C-F               | >500 | >25/8.2    |
| 77 | Me              | C-F               | 9    | 0.28/0.01  |
| 78 | Cl              | C-Cl              | 270  | 0.60/0.05  |

dependent upon the groups at R-7 and X. Halogens at X (X = C-Cl or C-F) and 3-substituted pyrrolidines at the R-7 position gave the most cytotoxic derivatives and the most potent antibacterials. Some moderation of the mammalian cell cytotoxicity could be achieved by placing a methyl group on or near the distal nitrogen of the R-7 substituent, with little change in antibacterial activity. Studies are currently in progress directed toward examining the effect chirality at the R-7 position has on cytotoxicity since some differences between various stereoisomers has recently been observed.<sup>15</sup> Additional studies to address the influence of log *P* and p*K*<sub>a</sub>, as well as a number of other Hansch type parameters, are ongoing and will be reported elsewhere.

(15) Domagala, J. M.; Cohen, M.; Kiely, J.; Hagen, S.; Laborde, E.; Schroeder, M.; Sesnie, J.; Suto, M. J. New 7-[3-Aminoethyl]-1-pyrrolidinylquinolones: Outstanding Gram-positive Activity With Reduced Photo- and Cytotoxicity. 31st Interscience Conference on Antimicrobial Agents and Chemotherapy, 1991, Chicago, Illinois; Abstract No. 1439.

In summary, we have presented data illustrating how multiple substituents on the quinolone/naphthyridone nucleus contribute to mammalian cell cytotoxicity and antibacterial activity. The data in general indicate that some of the most cytotoxic compounds were also some of the best antibacterial agents. Therefore, to adequately assess a compound's utility both pieces of information were required (selectivity index). Additional studies (Gracheck et al. and ref 8) have indicated that a window may exist (IC<sub>50</sub> > 100 μg/mL) in which the cytotoxicity seen does not result in additional mammalian cell toxic effects. Studies to further address these issues with the intent of developing screens predictive of potential fluoroquinolone genetic toxicity are in progress.

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