Fluoroquinolones: Relationships between Structural Variations, Mammalian Cell Cytotoxicity, and Antimicrobial Activity[1]

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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.[2] 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.[3,4] These compounds elicit their effects by disrupting DNA synthesis and cell division.[4] The fluoroquinolones represent one of the first classes of antibacterials whose mechanism of action is inhibition of DNA gyrase.[5] Studies have shown that quinolone antibacterial agents can also affect eukaryotic topoisomerases, as was recently demonstrated with the nalidixic acid analogue CP-67015.[6] 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.[7] Therefore, the use of compounds such as the fluoroquinolones,

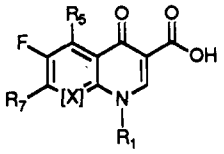


Figure 1.

which function as antibacterials via inhibition of DNA gyrase, could potentially lead to gene mutations and/or toxicities in the host organism.[5] 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.[8] 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.[6,7,9] 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.

(2) Vosberg, H. D. DNA Topoisomerase: Enzymes That Control DNA Conformations. *Curr. Top. Microbiol. Immunol.* **1985**, *114*, 19–102.

(3) Drlica, K. Inhibitors of DNA Topoisomerases. *Biochemistry* **1988**, *27*, 2253–2259.

(4) Liu, L. F. DNA Topoisomerase Poisons as Antitumor Drugs. *Annu. Rev. Biochem.* **1989**, *58*, 351–375.

(5) (a) Fernandes, P. B. Mode of Action, In Vitro and In Vivo Activities of the Fluoroquinolones. *J. Clin. Pharmacol.* **1988**, *28*, 156–168. (b) Domagala, J. M.; Hanna, L. D.; Heifetz, C. M.; Hutt, M. P.; Mich, T. F.; Sanchez, J. P.; Solomon, M. New Structure–Activity Relationships of the Quinolone Antibacterials Using the Target Enzyme. The Development and Application of a DNA Gyrase Assay. *J. Med. Chem.* **1986**, *29*, 394–404 and references therein.

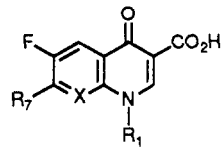
(6) Holden, H. H.; Barret, J. F.; Huntington, C. M.; Muehlbauer, P. A.; Wahrenburg, M. G. Genetic Profile of a Nalidixic Acid Analog: A Model for the Mechanism of Sister Chromatid Exchange. *Environ. Mol. Mutagen.* **1989**, *13*, 238–252.

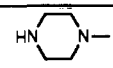
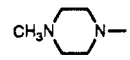
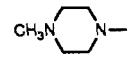
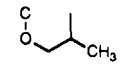
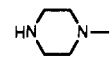
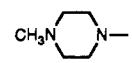
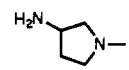
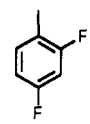
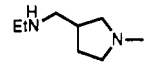
(7) (a) Hoshino, K.; Sato, K.; Akahane, K.; Yoshida, A.; Hayakawa, I.; Sato, M.; Une, T.; Osada, Y. Significance of the Methyl Group on the Oxazine Ring of Ofloxacin Derivatives in the Inhibition of Bacterial and Mammalian Type II Topoisomerases. *Antimicrob. Agents Chemother.* **1991**, *35*, 309–312. (b) Moreau, N. J.; Robaux, N.; Baron, L.; Tabary, X. Inhibitory Effects of Quinolones on Pro- and Eukaryotic DNA Topoisomerases I and II. *Antimicrobial Agents Chemother.* **1990**, *34*, 1955–1960. (c) Kohlbrenner, W. E.; Wideburg, N.; Weigl, D.; Saldivar, A.; Chu, D. T. W. Induction of Calf Thymus Topoisomerase II-Mediated DNA Breakage by the Antibacterial Isothiazoloquinolones A-65281 and A-65282. *Antimicrob. Agents Chemother.* **1992**, *36*, 81–86.

(8) Gracheck, S. J.; Mychalonka, M.; Gambino, L.; Cohen, M.; Roland, G.; Ciaravino, V.; Worth, D.; Theiss, J. C.; Heifetz, C. Correlations of Quinolone Inhibition Endpoints vs Bacterial DNA Gyrase, Mammalian Topoisomerase I and II, Cell Clonogenic Survival and In Vitro Micronuclei Induction. 31st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, Illinois, 1991; Abstract No. 1488.

0022-2623/92/1835-4745$03.00/0

Table I. Quinolone Standards



compound number	name	R_7	X	R_1	mammalian cell cytotoxicity IC_{50}(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_2CH_2F	>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.[5b,10,11] 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_3N. Standard workup and deprotection of the side chain when necessary provided the target compounds.[11]

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.[12]

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_2). 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

(9) Ciaravino, V; Suto, M. J.; Theiss, J. C. High Capacity In Vitro Micronucleus Assay for Assessment of Chromosome Damage: Results with Quinolone/Naphthyridone Antibacterials. *Mutation Res.* **1992**, in press.

(10) (a) Wentland, P. Structure-Activity of Fluoroquinolones. In *The New Generation of Quinolones*; Siporin, C., Heifetz, C., Domagala, J., (Eds.; Marcel Dekker: New York, 1990; pp 1–43. (b) Vergin, H; Metz, R; Review of Developments in Fluoroquinolones. *Drugs Today* **1991**, *27*, 177–192. (c) Chu, D. T. W.; Fernandes, P. B. Recent Developments in the Field of Quinolone Antibacterial Agents. *Adv. Drug Res.* **1991**, *21*, 39–144.

(11) (a) Domagala, J. M.; Heifetz, C. L.; Hutt, M. P.; Mich, T. F.; Nichols, J. B.; Solomon, M.; Worth, D. F. 1-Substituted 7-[3-[(Ethylamino)methyl]-1-pyrrolidinyl]-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acids. New Quantitative Structure–Activity Relationships at N_1 for the Quinolone Antibacterials. *J. Med. Chem.* **1988**, *31*, 991–1001. (b) Sanchez, J. P.; Domagala, J. M.; Hagen, S. E.; Heifetz, C. L.; Hutt, M. P.; Nichols, J. B.; Trehan, A. K. Quinolone Antibacterial Agents. Synthesis and Structure–Activity Relationships of 8-Substituted Quinolone-3-carboxylic Acids and 1,8-Naphthyridine-3-carboxylic Acids. *J. Med. Chem.* **1988**, *31*, 983–991. (c) Domagala, J. M.; Bridges, A. J.; Culbertson, T. C.; Gambino, L.; Hagen, S. E.; Karrick, G.; Porter, K.; Sanchez, J. P.; Sesnie, J. A.; Spense, F. G.; Szotek, D.; Wemple, J. Synthesis and Biological Activity of 5-Amino- and 5-Hydroxyquinolones, and the Overwhelming Influence of the Remote N_1-Substituent in Determining the Structure–Activity Relationship. *J. Med. Chem.* **1991**, *34*, 1142–1154.

(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} (μg/mL) was determined and was defined as the concentration of compound yielding 50% cell survival compared to untreated control cells.

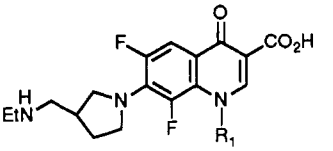
Antimicrobial Activity. The compounds were evaluated against five Gram-negative organisms and five Gram-positive organisms using standard microdilution techniques.[13] The data presented represent the geometric mean of the MIC's (μg/mL) for the Gram-negative (Enterobacter cloacae MA 2646, Escherichia coli Vogel, Klebsiella pneumonia MGH-2, Providencia rettgeri M 1771, and Pseudomonas aeruginosa) and the Gram-positive (Staphylococcus aureus H 228, Staphylococcus aureus UC-76, Enterococus faecalis MGH-2, Streptococcus pneumonia SV-1, and Streptococcus pyogenes C-203) bacteria.

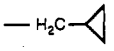
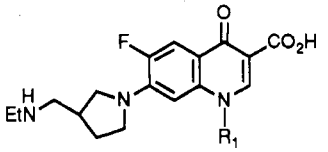
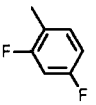
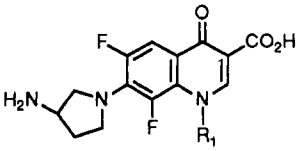
Results and Discussion

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 μg/mL, and the antibacterial activity, expressed as the geometric mean of the MIC's for Gram-negative and Gram-positive organisms (μg/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 μg/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 μg/mL, no quinolone thus far examined has ever achieved tissue or fluid levels in humans anywhere near those levels.[14] Quinolone concentrations in most tissues usually were found to be in the range of 1–10 μg/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 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.

Table II. R_1 Substituents



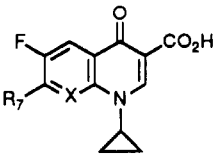
compd no.	R_1	mammalian cell cytotoxicity IC_{50}, μg/mL	antibacterial activity MIC (Gram −/Gram +, μg/mL)
8	$-CH_2CH_3$	160	0.20/0.10
9	$-CH_2CH_2F$	>500	0.40/0.15
10	$-CH_2CF_3$	58	1.82/0.26
11	$-CH_2CH_2OH$	>500	50/9.50
12		11	0.05/0.01
13		240	4.17/0.35
14	–NHMe	>500	0.26/2.40
			
15	$-CH_2CH_3$	310	0.56/0.14
16		160	0.46/0.10
17		190	0.80/0.09
18	–NHMe	>500	12.5/1.81
			
19	$-CH_2CH_3$	300	0.12/0.26
20		310	0.13/0.70
21		30	0.04/0.03
22		160	0.30/0.30

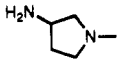
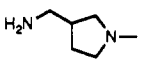
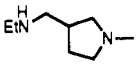
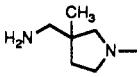
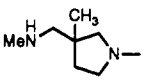
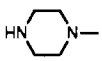
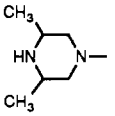
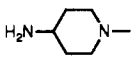
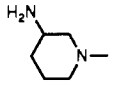
R-7 versus X Substituents. In general, a wide variety of structural modifications have been carried out at the R-7 and X positions of the fluoroquinolones (Figure 1) in an attempt to improve the antibacterial activity of this class of compounds. Changes in both the R-7 substituent and the X-position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

(13) Cohen, M. A.; Griffen, T. J.; Bien, P. A.; Heifetz, C. L.; Domagala, J. M. In Vitro Activity of Cl-934, a Quinolone Carboxylic Acid Active Against Gram-Positive and -Negative Bacteria. *Antimicrob. Agents Chemother.* **1985**, *28*, 766–772.

(14) Gerding, D. N.; Hitt, J. A. Tissue Penetration of the New Quinolones in Humans. *Rev. Infect. Dis.* **1989**, *11*, S1046.

Table III. R_7 versus X Substituents



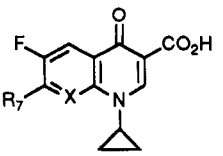
compound number	R_7	mammalian cell cytotoxicity IC_{50} (μg/mL), and antibacterial activity MIC (Gram −/Gram +, μg/mL)					
		a: X = C–H	b: X = C–F	c: X = C–Cl	d: X = C–OMe	e: X = C–CF_3	f: X = N
23		38 0.20/0.05	120 0.10/0.006	150 0.10/0.006	150 0.30/0.02	280 12.5/6.31	58 0.26/0.07
24		72 0.09/0.14	30 0.04/0.03	26 0.03/0.04	45 0.07/0.03	300 0.20/0.20	98 0.03/0.13
25		81 0.20/0.20	11 0.05/0.01	8 0.06/≤0.003	10 0.11/0.03	38 0.52/0.03	51 0.14/0.03
26		160 0.46/0.10	11 0.09/0.02	11 0.14/<0.004	22 0.26/0.02	120 0.60/0.04	160 0.21/0.11
27		23 0.52/0.04	8 0.13/0.01	8 0.07/0.007	38 0.26/0.01	110 1.0/0.17	25 0.35/0.03
28		205 0.40/0.04	8 0.23/0.03	23 0.26/0.07	120 0.52/0.02	230 >30/0.35	58 0.60/0.08
29		380 0.10/0.90	47 0.07/0.40	43 0.07/0.06	82 0.07/0.11	160 0.15/0.30	120 0.09/1.21
30		>500 0.08/0.15	150 0.05/0.20	140 0.23/0.26	>500 0.34/0.10	>500 0.91/0.80	250 0.17/1.90
31		230 0.26/0.17	23 0.20/0.08	18 0.08/0.03	–	20 0.26/0.13	100 0.23/0.23
32		>500 0.91/0.53	53 0.13/0.06	24 0.07/0.009	69 0.23/0.01	>500 0.52/0.11	100 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.

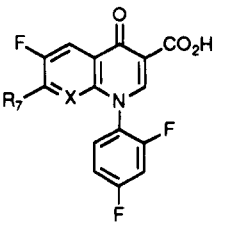
A number of general conclusions could be drawn from this table, in addition to the specific conclusions apparent in a particular series (i.e. a particular X or R-7 substituent). First, increasing the bulk around the distal nitrogen, either through alkylation of the nitrogen or addition of an alkyl group 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, pyrrolidines 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

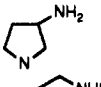
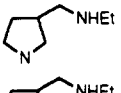
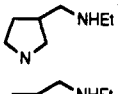
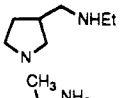
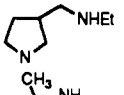
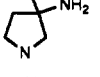
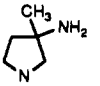
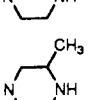
Table IV. R_1-Substituents: Cyclopropyl versus Difluorophenyl. Mammalian Cell Cytotoxicity IC_{50}(μg/mL) and Antibacterial Activity MIC (Gram −/Gram +, μg/mL)



a



b

compound number	R_7	X	data	R_7	X	data
33		C–Cl	26 (0.03/0.04)		C–Cl	89 (0.23/0.20)
34		C–CF_3	300 (0.20/0.20)		C–CF_3	99 (0.52/0.11)
35		C–OMe	45 (0.07/0.03)		C–OMe	290 (0.35/0.35)
36		C–H	160 (0.46/0.10)		C–H	190 (0.80/0.09)
37		C–Cl	11 (0.14/<0.004)		C–Cl	130 (1.05/0.17)
38		C–OMe	38 (0.26/0.01)		C–OMe	250 (0.46/0.17)
39		C–CF_3	110 (1.00/0.17)		C–CF_3	18 (2.07/0.10)
40		C–H	380 (0.10/0.90)		C–H	>500 (0.11/0.17)
41		C–Cl	45 (0.08/0.15)		C–Cl	>500 (2.07/0.53)
42		C–CF_3	220 (0.46/0.53)		C–CF_3	>500 (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.

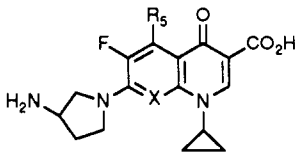
X and R-5 Substituents. Table V illustrates the relationship between the R-7 substituent and position X. Another important relationship exists between the R-5 and R-7 positions and the R-5 and the X-position.[11c] The interaction between the latter groups would be electronic, propagated through the aromatic 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_{50} 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.

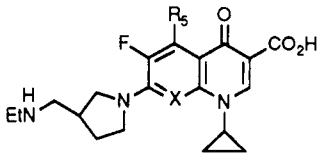
Of note in this series were compounds **53** and **55**. These compounds represent examples of quinolones with diminished antibacterial activity, but which were still cytotoxic. This trend was not generally observed since it seemed that a correlation between cytotoxicity and antibacterial activity existed.

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-R8) versus R5 Substituents



compound number	R_5	X	mammalian cell cytotoxicity IC_{50} (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_3	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_2	69	0.46/0.92
49	H	C–NH_2	260	0.53/2.06
50	H	C–SMe	31	0.20/0.06
51	NH_2	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_3	190	25/25
58	NH_2	C–F	15	0.01/0.01
59	NH_2	C–Cl	59	0.08/0.05
60	NH_2	C–OMe	100	0.11/0.04
61	Me	C–CF_3	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_2	67	1.05/0.20
71	H	C–NH_2	290	0.91/0.23
72	NH_2	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.[15] Additional studies to address the influence of log P and pK_a, 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-pyrrolidinyl]quinolones: 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 substitutents 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_{50} > 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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