

Drug Features That Contribute to the Activity of Quinolones against Mammalian Topoisomerase II and Cultured Cells: Correlation between Enhancement of Enzyme-Mediated DNA Cleavage In Vitro and Cytotoxic Potential

SARAH H. ELSEA,¹ PAUL R. MCGUIRK,² THOMAS D. GOOTZ,²
MELINDA MOYNIHAN,² AND NEIL OSHEROFF^{1*}

*Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-0146,¹
and Department of Immunology and Infectious Diseases, Pfizer Central Research,
Pfizer, Inc., Groton, Connecticut 06340²*

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CP-115,953 [6,8-difluoro-7-(4'-hydroxyphenyl)-1-cyclopropyl-4-quinolone-3-carboxylic acid] is a novel quinolone that is highly active against topoisomerase II in vitro and in mammalian cells in culture (M. J. Robinson, B. A. Martin, T. D. Gootz, P. R. McGuirk, M. Moynihan, J. A. Sutcliffe, and N. Osheroff, *J. Biol. Chem.* 266:14585–14592, 1991). However, the features of the drug that contribute to its activity towards mammalian systems have not been characterized. Therefore, CP-115,953 and a series of related quinolones were examined for their activity against calf thymus topoisomerase II and cultured mammalian cells. CP-115,953 stimulated DNA cleavage mediated by the type II enzyme with a potency that was ~600-fold greater than that of the antimicrobial quinolone ciprofloxacin and ~50-fold greater than that of the antineoplastic drug etoposide. As determined by the ability to enhance enzyme-mediated DNA cleavage, quinolone activity towards calf thymus topoisomerase II was enhanced by the presence of a cyclopropyl group at the N-1 ring position and by the presence of a fluorine at C-8. Furthermore, the 4'-hydroxyphenyl substituent at the C-7 position was critical for the potency of CP-115,953 towards the mammalian type II enzyme. In this regard, the aromatic nature of the C-7 ring as well as the presence and the position of the 4'-hydroxyl group contributed greatly to drug activity. Finally, the cytotoxicity of quinolones in the CP-115,953 series towards mammalian cells paralleled the in vitro stimulation of DNA cleavage by topoisomerase II rather than the inhibition of enzyme-catalyzed DNA relaxation. This correlation strongly suggests that these quinolones promote cell death by converting topoisomerase II to a cellular poison.

Topoisomerase II is the target for a wide variety of clinically relevant antineoplastic drugs (21, 34). A number of these compounds, including etoposide, amsacrine, doxorubicin, and mitoxantrone, currently are being used for the treatment of human cancers (21, 34). Despite the structural diversity of topoisomerase II-targeted drugs, these agents share a common basis for their antineoplastic action. All of these drugs stabilize covalent enzyme-cleaved DNA complexes that are normal reaction intermediates in the catalytic cycle of topoisomerase II (21, 29, 34). As a consequence of their action on enzyme-mediated DNA cleavage-religation, these drugs convert topoisomerase II into a cellular poison (20) and promote the formation of protein-associated breaks in the genomes of treated cells (21, 34).

DNA gyrase, a prokaryotic type II topoisomerase (30, 36), is also the target for a number of clinically important drugs (14, 22, 30, 43). The most potent drug class targeted towards gyrase, the quinolones, contains some of the most active antimicrobial agents in use for the treatment of human infections (14, 43). Like their antineoplastic counterparts, quinolone-based antimicrobial agents convert gyrase into a cellular poison by enhancing enzyme-mediated DNA breakage (14, 20, 22, 30, 36, 43).

Quinolones have long been known to inhibit the overall catalytic activity of topoisomerase II, albeit at concentrations well above their therapeutic range (3, 8, 13, 15–17,

23–25, 27). Recently, some of the more potent quinolone-based drugs, such as ciprofloxacin (Fig. 1) and CP-67,015, also were reported to stimulate DNA cleavage mediated by the eukaryotic type II enzyme (3). Since that initial report, a number of novel quinolones with activity against topoisomerase II, eukaryotic cells, or mammalian tumors have been described (8, 9, 18, 19, 31, 32, 37, 39–41). In contrast to clinically relevant quinolone antimicrobial agents, many of these latter compounds contain an aromatic substituent at the C-7 position (3, 9, 18, 31, 32, 39, 40).

The quinolone that displays the highest activity towards the eukaryotic type II enzyme is CP-115,953 [6,8-difluoro-7-(4'-hydroxyphenyl)-1-cyclopropyl-4-quinolone-3-carboxylic acid] (Fig. 1) (8, 31, 32). This compound is cytotoxic towards mammalian cells and is the first quinolone-based drug reported to enhance topoisomerase II-mediated DNA breakage with a potency greater than that of a commonly used antineoplastic agent, such as etoposide (31). Unlike etoposide (26, 33), CP-115,953 and related compounds increase levels of DNA breakage without impairing the ability of the eukaryotic enzyme to religate cleaved nucleic acids (31, 32). Thus, quinolones in the CP-115,953 series constitute a novel mechanistic class of topoisomerase II-targeted drugs.

Before the clinical value of quinolones as potential antineoplastic agents can be fully evaluated, features of the drugs that contribute to activity towards mammalian systems must be established. Therefore, CP-115,953 and several related derivatives were examined for their ability to en-

* Corresponding author.

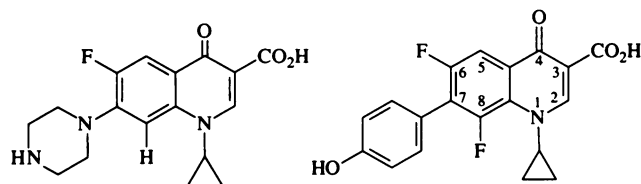
**Ciprofloxacin****CP-115,953**

FIG. 1. Structures of ciprofloxacin and CP-115,953. The ring numbering system shown for CP-115,953 is applicable to all quinolone-based compounds used in the present study.

hance DNA cleavage or to inhibit DNA relaxation mediated by calf thymus topoisomerase II. While substituents at the N-1 and C-8 positions contributed to drug action, both the aromatic nature and the 4'-hydroxyl moiety of the C-7 substituent were critical for the potent stimulation of enzyme-mediated DNA cleavage. Finally, a strong relationship was observed between the ability of quinolones in the CP-115,953 series to enhance topoisomerase II-mediated DNA breakage in vitro and to promote cell death in mammalian cultures.

MATERIALS AND METHODS

Chemicals. DNA topoisomerase II was purified from calf thymus by the procedure of Andersen et al. (1) and was the generous gift of P. S. Jensen, J. Alsner, and O. Westergaard (University of Aarhus). Negatively supercoiled bacterial plasmid pBR322 DNA was prepared as previously described (31). Ciprofloxacin and norfloxacin were obtained from Sigma Chemical Co. (St. Louis, Mo.). Difluorociprofloxacin was kindly provided by J. M. Domagala (Warner Lambert/Parke-Davis Pharmaceuticals). All other quinolones were synthesized at Pfizer Central Research by the procedure of Gilligan et al. (11). Quinolone derivatives CP-80,080, CP-115,953, CP-115,954, CP-115,955, and difluorociprofloxacin were dissolved as 25 mM solutions in 0.1 N NaOH and diluted to 5 mM stock solutions with 10 mM Tris-HCl (pH 8.0). CP-67,804 was dissolved as a 15 mM solution in 0.1 N NaOH and diluted to a 1 mM stock solution with 10 mM Tris-HCl (pH 8.0). Quinolone derivatives CP-67,803, CP-68,807, CP-151,774, CP-162,600, CP-171,472, CP-171,473, ciprofloxacin, and norfloxacin were dissolved as 5 mM solutions in dimethyl sulfoxide. Quinolone stock solutions were stored in the dark at -85°C . Etoposide (VePesid) was obtained from Bristol Laboratories; Tris and ethidium bromide were obtained from Sigma; sodium dodecyl sulfate (SDS) and proteinase K were obtained from E. Merck Biochemicals (West Point, Pa.); ATP was obtained from Pharmacia LKB Biotechnology (Piscataway, N.J.); and cell culture media, phosphate-buffered saline, and fetal bovine serum were obtained from GIBCO Biologicals (Grand Island, N.Y.). All other chemicals were analytical reagent grade.

Topoisomerase II-mediated DNA cleavage. DNA cleavage assays were carried out as described by Osheroff and Zechiedrich (28). Reaction mixtures contained 100 nM topoisomerase II and 5 nM negatively supercoiled pBR322 DNA in a total volume of 20 μl of assay buffer (10 mM Tris-HCl [pH 7.9], 50 mM NaCl, 50 mM KCl, 5 mM MgCl_2 , 0.1 mM EDTA, 2.5% glycerol). DNA cleavage-religation equilibria were established by incubating samples at 37°C for 6 min.

Cleavage products were trapped (2, 10) by the addition of 2 μl of 10% SDS, which was followed by the addition of 1.5 μl of 250 mM EDTA and 2 μl of a 0.8-mg/ml solution of proteinase K. Samples were incubated at 45°C for 30 min to allow digestion of topoisomerase II by proteinase K. Final products were mixed with 2 μl of loading buffer (60% sucrose, 0.05% bromophenol blue, 0.05% xylene cyanol FF, 10 mM Tris-HCl [pH 7.9]), heated at 70°C for 2 min, and subjected to electrophoresis in 1% agarose (EM Science, Gibbstown, N.J.) gels in 40 mM Tris-acetate (pH 8.3)–2 mM EDTA–1 μg of ethidium bromide per ml. DNA bands were visualized by transillumination with UV light (300 nm) and were photographed through Kodak 23A and 12 filters with Polaroid type 665 positive-negative film. The amount of DNA was quantitated by scanning negatives with an E-C Apparatus model EC910 scanning densitometer using Hoefer GS-370 Data System software. Under the conditions used, the intensity of the bands in the negative was directly proportional to the amount of DNA present. The effects of drugs were examined over a concentration range of 0 to 500 μM . An amount of diluent equal to that in drug-containing samples was added to all control samples. No drug-induced DNA cleavage was observed in the absence of topoisomerase II.

It should be noted that topoisomerase II establishes DNA cleavage-religation equilibria both before and after its DNA strand passage event (29). Since the enzyme requires ATP binding for strand passage and the assays described above were carried out in the absence of a high-energy cofactor, all of the data generated represent the pre-strand passage DNA cleavage reaction of topoisomerase II.

The term EC_2 (effective concentration of drug required to enhance double-stranded DNA cleavage twofold) was used as a relative measure of quinolone stimulation of topoisomerase II-mediated DNA cleavage. First, as expected for drug-enzyme interactions, dose-response curves for quinolones were saturable. However, even for compounds with low activity against topoisomerase II, the dose-response curves in DNA cleavage assays generally were linear up to a twofold enhancement of enzyme-mediated DNA breakage. Thus, EC_2 values were believed to reflect the initial slopes of drug concentration curves and hence drug potency. Second, compounds that were unable to double levels of DNA breakage when used at 500 μM were believed to have marginal (at best) activity.

Topoisomerase II-mediated DNA relaxation. DNA relaxation assays were carried out as described by Osheroff et al. (27). Reaction mixtures contained 0.7 nM calf thymus topoisomerase II and 5 nM supercoiled pBR322 in a total volume of 20 μl of assay buffer that contained 1 mM ATP. DNA relaxation was carried out at 37°C for 15 min. Reactions were stopped by the addition of 3 μl of 0.77% SDS–77 mM EDTA. Samples were mixed with 2 μl of loading buffer, heated for 2 min at 70°C , and subjected to electrophoresis on 1% agarose gels in Tris-borate (pH 8.3)–2 mM EDTA. DNA in the gels was visualized and quantitated as described above. The effects of drugs were examined over a concentration range of 0 to 500 μM . An amount of diluent equal to that in drug-containing samples was added to all control samples.

Cytotoxicity of quinolone derivatives towards mammalian cells. Wild-type Chinese hamster ovary (CHO) cells and A-431 human cells were cultured as monolayers at 37°C under 5% CO_2 in F-12 nutrient mixture (CHO) or Dulbecco modified Eagle medium (A-431) supplemented with 10 μg of gentamicin per ml and 10% fetal bovine serum. Drug cytotoxicity was determined by a colony-forming assay (12,

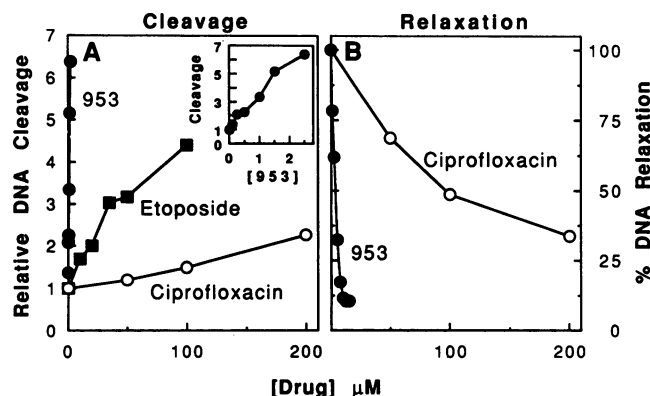


FIG. 2. Effects of CP-115,953 (953) (●) on the DNA cleavage (A) and DNA relaxation (B) activities of calf thymus topoisomerase II. Effects of ciprofloxacin (○) and etoposide (■) are shown for comparison. Double-stranded DNA cleavage was monitored by the conversion of negatively supercoiled plasmid molecules to linear molecules. In the absence of drug, levels of DNA cleavage were set arbitrarily to 1.0 and levels of DNA relaxation were set arbitrarily to 100%. The inset in panel A shows an expanded scale for the enhancement of DNA cleavage by CP-115,953. Micromolar quinolone concentrations are given. Data are the averages of two to seven independent experiments.

31). Assays employed log-phase, trypsinized cells that were seeded at ~ 250 cells per well in 35-mm plates for 18 h prior to drug treatment. Both CHO and A-431 cells were treated with drugs (dissolved as 10 mM solutions in dimethyl sulfoxide) for 1 h at 37°C. Cells were rinsed twice with phosphate-buffered saline and incubated in fresh medium for 5 (CHO) or 6 (A-431) days. The culture medium was removed, cells were rinsed, and colonies were stained with 2% crystal violet in methanol and counted. Plating efficiencies were approximately 95% for CHO cells and 85% for A-431 cells.

RESULTS

Activity of quinolone CP-115,953 against mammalian topoisomerase II. While quinolones are highly efficacious against DNA gyrase, they generally display minimal activity towards the eukaryotic type II enzyme (13). Even ciprofloxacin, the most active oral antimicrobial agent in clinical use (14, 43), shows only low activity against calf thymus topoisomerase II. As shown in Fig. 2A, ciprofloxacin had little effect on enzyme-mediated DNA cleavage. The EC_{50} for this compound was 171 μM (Table 1). Furthermore, the ability of ciprofloxacin to inhibit the overall catalytic activity of the calf thymus enzyme (as monitored by a DNA relaxation assay) was considerably lower than that typically observed for most topoisomerase II-targeted antineoplastic drugs (21, 34). The drug concentration required to inhibit DNA relaxation 50% (IC_{50}) for ciprofloxacin was 106 μM (Fig. 2B and Table 1).

In marked contrast to ciprofloxacin, quinolone CP-115,953 was a potent effector of mammalian topoisomerase II. As shown in Fig. 2A, CP-115,953 greatly stimulated DNA cleavage mediated by the calf thymus enzyme. The EC_{50} for this quinolone was 0.3 μM (Fig. 2A and Table 1); that for etoposide was 17 μM (Fig. 2A). In addition to its effects on DNA cleavage, CP-115,953 inhibited the overall catalytic activity of mammalian topoisomerase II with an IC_{50} of 3.2 μM (Fig. 2B and Table 1).

Features of CP-115,953 that promote activity against mam-

malian topoisomerase II. CP-115,953 differs from ciprofloxacin by substitutions at ring positions C-7 and C-8 and from the widely used quinolone antimicrobial agent norfloxacin by substitutions at N-1, C-7, and C-8 (5, 31, 38). However, CP-115,953 displayed a potency against calf thymus topoisomerase II that was ~ 600 - or $\sim 1,600$ -fold greater, respectively, than that of ciprofloxacin or norfloxacin (Table 1). Considering the antineoplastic potential of CP-115,953 (8, 31, 32), the above-mentioned ring positions were examined for their contributions to quinolone potency against the mammalian type II enzyme. This was accomplished by characterizing a series of quinolone derivatives that differed only at single ring positions for their ability to enhance DNA cleavage or to inhibit DNA relaxation mediated by calf thymus topoisomerase II. A summary of results is given in Table 1 and detailed below.


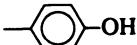

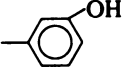

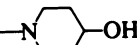





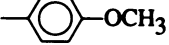



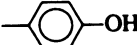

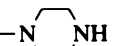

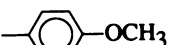
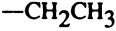
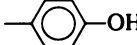
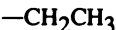

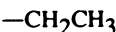
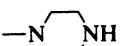
(i) **Ring position N-1.** Considerable evidence indicates that the presence of a cyclopropyl as opposed to an ethyl group at position N-1 increases quinolone potency against DNA gyrase (5, 38) and *Drosophila* topoisomerase II (31). A similar result was found for the mammalian type II enzyme (Table 1). By comparing the DNA cleavage-enhancing activity of ciprofloxacin with that of norfloxacin or the activity of CP-115,953 with that of CP-67,804, it was determined that substitution of an ethyl group at N-1 decreased quinolone potency against calf thymus topoisomerase II ~ 3 - or ~ 40 -fold, respectively. A similar effect was observed when compounds were examined for their ability to inhibit enzyme-catalyzed DNA relaxation.

(ii) **Ring position C-8.** Previous studies suggest that the inclusion of a fluorine group at the C-8 position has little positive effect and in some cases has a detrimental effect on quinolone potency against DNA gyrase (6, 7, 32). In contrast, the C-8 fluorine enhances activity against *Drosophila* topoisomerase II ~ 2.5 -fold (32). The presence of this fluorine group also led to a modest increase in potency against the mammalian enzyme (Table 1). The substitution of a hydrogen for the fluorine at C-8 decreased the ability of difluorociprofloxacin (which is converted to ciprofloxacin by the removal of the fluorine) to enhance enzyme-mediated DNA cleavage ~ 2.7 -fold. Moreover, the potency of CP-115,953 (which is converted to CP-115,955 by the substitution) was reduced ~ 13 -fold. While a similar decrease in the ability to inhibit DNA relaxation was observed for CP-115,955, removal of the C-8 fluorine had little effect on the inhibitory properties of difluorociprofloxacin.

(iii) **Ring position C-7.** Although the N-1 cyclopropyl group and the C-8 fluorine group contribute to quinolone activity against mammalian topoisomerase II, they cannot account for the dramatic difference in potency found between CP-115,953 and the quinolone antimicrobial agents. However, substitution of a piperazine ring (difluorociprofloxacin) or a bromine (CP-151,774) for the 4'-hydroxyphenyl ring of CP-115,953 decreased the DNA cleavage-enhancing properties of the quinolone ~ 200 - or $\geq 1,900$ -fold, respectively. This finding strongly suggests that the C-7 ring substituent is critical for the activity of CP-115,953 against the mammalian enzyme. Since the C-7 substituent of CP-115,953 differs from the piperazine ring of difluorociprofloxacin primarily by its aromatic nature and by the presence of a 4'-hydroxyl group, these two features were examined for their contributions to drug activity.

To characterize the importance of aromaticity to quinolone function, the 4'-hydroxyphenyl ring was substituted for by a 4'-hydroxypiperidine group (converting CP-115,953 to CP-171,472). As shown in Table 1, this substitution

TABLE 1. Activities of quinolones in the CP-115,953 series towards topoisomerase II

Compound	Ring Substituent			DNA Cleavage		DNA Relaxation	
	N-1	C-7	C-8	EC ₂ ^a ± SE (μM)	Relative Potency ^b	IC ₅₀ ^c ± SE (μM)	Relative Potency ^b
CP-115,953			F	0.3 ± 0.1	570	3.2 ± 0.2	33
CP-171,473			F	20 ± 2	8.6	203 ± 8	0.5
CP-171,472			F	53 ± 6	3.2	140 ± 18	0.8
Difluorociprofloxacin			F	63 ± 8	2.7	132 ± 27	0.8
CP-162,600			F	105 ± 6	1.6	198 ± 1	0.5
CP-115,954			F	>500 ^d	<0.3	>500 ^e	<0.2
CP-151,774			F	>500 ^f	<0.3	>500 ^e	<0.2
CP-115,955			H	4 ± 1	43	33 ± 3	3.2
Ciprofloxacin			H	171 ± 11	1.0	106 ± 10	1.0
CP-80,080			H	>500 ^g	<0.3	343 ± 8	0.3
CP-67,804			F	12 ± 4	14	110 ± 5	1.0
CP-68,807			F	>500 ^h	<0.3	>500 ^e	<0.2
Norfloxacin			H	492 ± 4	0.3	>500 ^e	<0.2

^a Values represent the averages of two to seven independent experiments.^b The relative potency of ciprofloxacin was set arbitrarily to 1.0.^c Values represent the averages of two to four independent experiments.^d The maximal stimulation of DNA cleavage observed was ~1.2-fold.^e Topoisomerase II had ~65% residual activity remaining at 500 μM drug.^f The maximal stimulation of DNA cleavage observed was ~1.3-fold.^g CP-80,080 was inhibitory rather than stimulatory. DNA cleavage decreased 50% at 100 μM drug.^h The maximal stimulation of DNA cleavage observed was ~1.7-fold.

decreased the relative potency for enhancement of DNA cleavage ~180-fold. Therefore, the aromatic nature of the C-7 ring appears to be important for the activity of CP-115,953 against calf thymus topoisomerase II. It should be noted that the C-7 substituent in CP-171,472 contains a nitrogen rather than a carbon bridge atom. It is unlikely that this difference contributes to the reduced activity of CP-171,472, since the geometry of a nitrogen bridge is essentially identical to that of a carbon bridge.

Several ring substitutions were characterized to determine

the contribution of the 4'-hydroxyl group to quinolone potency (Table 1). First, removal of this substituent (converting CP-115,953 to CP-162,600) decreased drug activity in enzyme-mediated DNA cleavage assays ~350-fold. Furthermore, the comparable derivative in the less potent CP-67,804 series (i.e., CP-68,807) was unable to double levels of DNA cleavage even at concentrations up to 500 μM. Second, conversion of the 4'-hydroxyl group to a methoxy group greatly diminished the ability of the quinolone to stimulate DNA cleavage by topoisomerase II. For all purposes, CP-

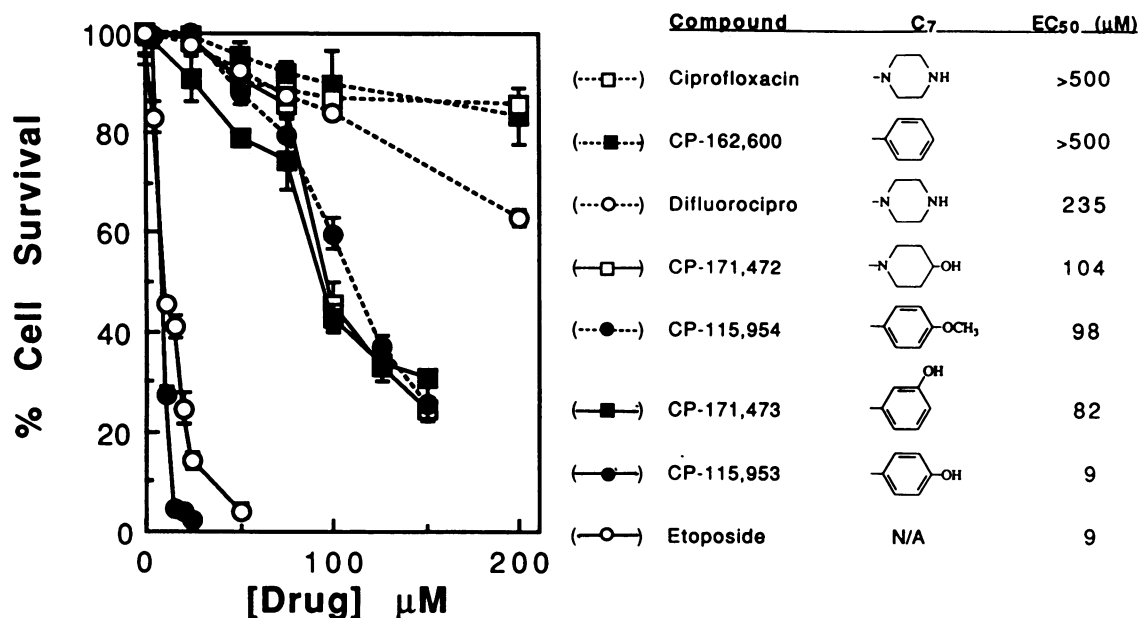


FIG. 3. Cytotoxicity of quinolones in the CP-115,953 series towards CHO cells. Assays were carried out as described in Materials and Methods. Data represent the averages of two or three independent experiments. Standard errors are indicated by vertical bars. Etoposide and ciprofloxacin are shown as controls. EC₅₀ values and the substituent at the C-7 position for each of the quinolones used are shown to the right of the cytotoxicity plot. Difluorocipro, difluorociprofloxacin. N/A, not applicable.

115,954 (which is in the CP-115,953 series) and CP-80,080 (which is the comparable derivative in the CP-115,955 series) lost their DNA cleavage-enhancing properties. Finally, even moving the hydroxyl group one ring carbon from the 4' to the 3' position (converting CP-115,953 to CP-171,473) diminished drug activity in DNA cleavage assays ~70-fold.

Taken together, these results provide strong evidence that the aromatic nature of the C-7 ring substituent as well as the presence and the position of the 4'-hydroxyl group are critical for the potent enhancement of topoisomerase II-mediated DNA cleavage by CP-115,953. While the ring substituents discussed above also appear to contribute to the inhibition of enzyme-catalyzed DNA relaxation by quinolones (Table 1), two important points should be noted. First, in contrast to results obtained in DNA cleavage experiments, all of the above-mentioned derivatives of CP-115,953 inhibited DNA relaxation with a potency that was less than that of ciprofloxacin. Second, the range of IC₅₀ values generated for these substituted quinolones was considerably narrower than the corresponding range of EC₂ values (Table 1). The lack of correlation ($r \approx 0.36$) between quinolone enhancement of DNA cleavage and inhibition of DNA relaxation was observed previously for the *Drosophila* type II topoisomerase (32). It is clear that the structure-activity relationships that govern the potency of quinolone action against these two enzyme activities are separate.

Cytotoxicity of quinolones towards mammalian cells. To characterize the features of quinolones that contribute to their in vivo activity, the cytotoxicity of several compounds towards CHO cells was assessed. Previous studies (31, 32) demonstrated that the substitution of an ethyl group for a cyclopropyl group at N-1 or the deletion of the C-8 fluorine decreased the in vivo potency of CP-115,953 ~8- or ~2-fold, respectively. Therefore, the present study was confined to examining the influence of the C-7 ring substituent. Cytotox-

icity data for ciprofloxacin and etoposide were included, however, as controls.

The activity of quinolones in the CP-115,953 series against CHO cells is shown in Fig. 3. As previously reported (31), the parent quinolone was equipotent to etoposide at killing this mammalian cell line. The drug concentration required to kill 50% of the cells (EC₅₀) for CP-115,953 was ~9 μM. In contrast, ciprofloxacin displayed little cytotoxicity towards CHO cells. At 500 μM ciprofloxacin (the highest drug concentration used), >80% of the cells remained viable.

As demonstrated for quinolone potency against mammalian topoisomerase II, the aromatic nature of the C-7 ring substituent and the presence and the position of the 4'-hydroxyl group on the C-7 substituent were important for the activity of CP-115,953 towards CHO cells. Substitution of the aromatic C-7 4'-hydroxyphenyl ring by either an aliphatic 4'-hydroxypiperidine group (CP-171,472) or a piperazine ring (difluorociprofloxacin) increased EC₅₀ values ~12- or ~25-fold. Removal of the 4'-hydroxyl group from the C-7 substituent (CP-162,600) resulted in a quinolone that was approximately equipotent to ciprofloxacin. Even moving the 4'-hydroxyl group to the 3'-position (CP-171,473) decreased the cytotoxicity of the drug nearly 10-fold.

The only potential anomaly observed for any member of the CP-115,953 series involved the 4'-methoxy-substituted compound (CP-115,954). Although this quinolone derivative showed almost no activity against calf thymus topoisomerase II, some cytotoxicity for CHO cells was observed (EC₅₀ ≈ 98 μM). The physiological basis for the in vivo activity of CP-115,954 is not known. However, it should be noted that the conversion of methoxy groups to hydroxyl groups is a common step in phase I drug metabolism (4). Thus, it is possible that some CP-115,954 may be converted to CP-115,953 in the cell.

The cytotoxic potential of CP-115,953 and related quino-

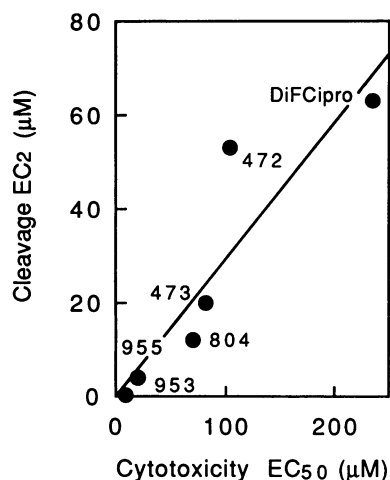


FIG. 4. Correlation between enhancement of DNA cleavage and cytotoxicity for CP-115,953 and related quinolones. The EC₂ and EC₅₀ values are from Table 1 and Fig. 3, respectively. The EC₅₀ values for CP-115,955 and CP-67,804 are from Robinson et al. (31, 32). Only quinolones that displayed EC₂ and EC₅₀ values of ≤500 μM were used. The *r* value calculated from the plot is ~0.90. Data for CP-115,953 (953), CP-115,955 (955), CP-67,804 (804), CP-171,473 (473), CP-171,472 (472), and difluorociprofloxacin (DiFCipro) are shown.

lones paralleled their ability to stimulate DNA cleavage mediated by mammalian topoisomerase II (Fig. 4). For compounds that displayed EC₂ and EC₅₀ values of ≤500 μM, the correlation coefficient (i.e., the *r* value) for these two quinolone activities was ~0.90. In contrast, the ability of quinolones to promote cell death did not correlate (*r* ≈ 0.35) with their ability to inhibit enzyme-catalyzed DNA relaxation. For example, while difluorociprofloxacin, CP-171,472, CP-115,954, and CP-117,473 all were less potent than ciprofloxacin at inhibiting DNA relaxation, these compounds all were considerably more potent (minimally two- to sixfold) than ciprofloxacin at promoting cell death.

To confirm the results described with CHO cells, the cytotoxicity of quinolones in the CP-115,953 series towards human A-431 carcinoma cells was assessed (data not shown). Once again, CP-115,953 was a potent cytotoxin (EC₅₀ ≈ 14 μM; that for etoposide was ~11 μM). Furthermore, any of the above alterations at the C-7 position decreased quinolone potency towards this squamous cell line by at least 1 order of magnitude.

DISCUSSION

Because of the importance of quinolones as antimicrobial agents, relationships between drug structure and activity toward DNA gyrase have been studied extensively (5, 14, 38, 43). In contrast, drug features that promote the activity of quinolones towards the mammalian type II enzyme have not been well characterized. The results of the present study demonstrate that the substituent at the C-7 position of CP-115,953 (the most potent topoisomerase II-targeted quinolone reported to date [8, 31, 32]) is critical for drug action against calf thymus topoisomerase II. In addition, the potency of CP-115,953 is enhanced by the presence of a cyclopropyl group at position N-1 and by the presence of a fluorine at position C-8.

It is not clear why the 4'-hydroxyl group of the C-7 ring is

so important for the activity of CP-115,953. One possibility is that this substituent interacts with topoisomerase II through the formation of a hydrogen bond. This suggestion is consistent with previous hypotheses concerning quinolone-gyrase interactions (35, 42) and is supported by the fact that most quinolones with activity against the eukaryotic type II enzyme contain a C-7 ring with a hydrogen-bonding group either at the 4'-position (3, 19, 39-41) or as a substituent off the 4'-position (18, 31, 32, 41). A second and intriguing possibility is that the C-7 4'-hydroxyphenyl ring mimics the active-site tyrosine of topoisomerase II (26, 29) and acts as a nucleophile when in the enzyme-DNA complex. Following attack on the nucleic acid backbone by the 4'-hydroxyl group, cleaved DNA could then be transferred rapidly to topoisomerase II. This could allow for both the stimulation of DNA breakage and the lack of inhibition of enzyme-mediated religation previously ascribed to CP-115,953 (31). Ultimately, further mechanistic studies will be required to determine whether either of these possibilities has credence.

While all clinically relevant quinolone antimicrobial agents contain an aliphatic group at C-7, many quinolones that display potency towards the eukaryotic enzyme contain an aromatic substituent at this position (3, 18, 31, 32, 39, 40). Thus, it is possible that a planar ring structure is necessary to properly position the 4'-hydroxyl group for a productive interaction within the topoisomerase II-DNA complex.

The cytotoxicity of quinolones in the CP-115,953 series towards mammalian cells correlates with the ability to enhance topoisomerase II-mediated DNA cleavage. In contrast, the cytotoxic potential of many of the compounds examined was significantly greater than that predicted on the basis of the inhibition of enzyme-catalyzed DNA relaxation. Thus, as demonstrated for other drug classes (21, 34), the ability to enhance DNA cleavage (i.e., convert topoisomerase II to a cellular poison) rather than the ability to inhibit DNA strand passage appears to be the quinolone function that predicts *in vivo* potency.

CP-115,953 is equipotent to etoposide in promoting cell death but is considerably more potent in enhancing topoisomerase II-mediated DNA cleavage. The underlying reasons for the relative difference in the *in vivo* and *in vitro* activities of the quinolone are unclear. Potentially, CP-115,953 may be taken up by cells more slowly or metabolized or extruded more rapidly than etoposide. Alternatively, the conversion of transient enzyme-mediated DNA breaks to permanent cellular lesions may be less efficient in the presence of a drug, such as CP-115,953, that acts primarily by stimulating the rate of DNA cleavage (31) rather than in the presence of a drug, such as etoposide, that acts primarily by inhibiting the rate of DNA religation (26, 33).

The quinolone CP-115,953 is highly active against topoisomerase II *in vitro* and is cytotoxic towards mammalian cells in culture (31, 32). Moreover, a recent report indicates that topoisomerase II is the primary physiological target of CP-115,953 in eukaryotic cells (8). Taken together, these findings strongly suggest that quinolone-based drugs, which currently are used exclusively as antimicrobial agents, may in the future play a role in the treatment of human cancers. The features that contribute to quinolone potency towards mammalian systems described in the present study provide a basis for the potential development of members of this drug class as topoisomerase II-targeted antineoplastic drugs.

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