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Induction of Calf Thymus Topoisomerase II-Mediated DNA Breakage by the Antibacterial Isothiazoloquinolones A-65281 and A-65282

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A number of quinolones and related antibacterial compounds were screened for activity against calf thymus topoisomerase II by using the P4 unknotting and DNA breakage assays. Several compounds from different structural classes which inhibited DNA unknotting with 50% inhibitory concentrations ranging from 8 to 25 μg/ml were identified. Two experimental isothiazoloquinolones from this group, designated A-65281 and A-65282, were also found to induce considerable DNA breakage mediated by calf thymus topoisomerase II, with ^{32}P-end-labeled pBR322 as the substrate. These compounds were nearly as potent as teniposide, with DNA breakage activity evident at concentrations as low as 4 μg/ml. However, some differences in DNA cleavage patterns from those with teniposide were evident. These studies have thus identified a new class of agents which have activity against both bacterial and eukaryotic type II topoisomerases. The implications of these data for the selectivity of topoisomerase-directed compounds and the potential toxicity of such compounds developed as antibacterial agents are discussed.

Several classes of antibacterial and antitumor agents which are believed to exert their cytotoxic effects by interacting with DNA topoisomerases have been identified (for a recent review, see reference 20). The bacterial type II topoisomerase, DNA gyrase, has long been known to be the site of action of nalidixic acid (33). This enzyme is also the target of a closely related class of potent antibacterial agents, the 4-quinolones (37). A large body of evidence indicates that the eukaryotic homolog of DNA gyrase, topoisomerase II, is involved in mediating the cytotoxic effects of a variety of antitumor agents such as ellipticine, teniposide, etoposide, adriamycin, and 4′-(9-acridinylamino)-methansulfon-*m*-anisidide (m-AMSA) (20). Other, although fewer, cytotoxic agents are directed against type I topoisomerases. Camptothecin and its congeners presently constitute the only well-characterized class of cytotoxic agents known to interact with eukaryotic topoisomerase I (16), although other classes of compounds which inhibit the catalytic activity of this enzyme (18) or induce topoisomerase I-mediated DNA breakage (38) have recently been identified.

DNA damage appears to be essential to the mode of action of all classes of topoisomerase-directed cytotoxic agents, and it is believed to initiate a cascade of events that lead to cell death (20). Early mode-of-action studies, using elegant analytical techniques to quantitate DNA breakage in vitro (see reference 19 for a recent review), demonstrated that many DNA-intercalating antitumor agents shared the ability to cause severe DNA damage (26–28, 39, 40). Suggestions that topoisomerases were involved in mediating the effects of these and other drugs (22, 26, 28, 39) have been confirmed by more recent work with reconstituted systems in which significant drug-induced DNA fragmentation was demonstrated for a variety of agents by using highly purified topoisomerase II preparations (3, 23, 25, 34, 35). A similar pattern of effects has been demonstrated for the quinolone antibacterial agents. Treatment of bacteria with the quinolone prototype, nalidixic acid, results in extensive chromosomal damage (7, 11). Drug-induced DNA breakage can also be catalyzed by purified DNA gyrase (8, 10, 36). In related studies it was recently demonstrated that additional quinolone-binding sites appear upon the formation of gyrase-DNA complexes and that occupancy of these sites correlates with the occurrence of DNA breakage (30).

In general, the agents directed against type II topoisomerases are specific for either bacterial or eukaryotic enzymes. However, in view of the mechanistic similarities and the sequence homologies shared by the bacterial and eukaryotic enzymes, the possibility exists that some agents interact with both classes. Indeed, several antibacterial quinolones which inhibit the P4 DNA-unknotting activity of various eukaryotic type II topoisomerases have been identified (1, 15, 17). Also, ciprofloxacin and several experimental fluroroquinolones induce topoisomerase II-mediated DNA breakage (1, 12, 24). These findings raised the possibility that additional classes of compounds that interact with both prokaryotic and eukaryotic enzymes will be identified. Additionally, they underscored the need to carefully assess the cross-reactivity of any topoisomerase-directed antibacterial agent against eukaryotic topoisomerases. Studies to address this important question and their implications for potential quinolone toxicity have been discussed in a recent review (13).

We report here the identification of two closely related antibacterial isothiazoloquinolones, designated as A-65281 and A-65282, which are potent inhibitors of the P4-unknotting reaction catalyzed by calf thymus topoisomerase II. Moreover, these compounds are potent inducers of DNA breakage by calf thymus topoisomerase II. These data thus

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establish a new class of topoisomerase II-directed agents that interact with both bacterial and eukaryotic enzymes.

MATERIALS AND METHODS

Topoisomerase II purification. The steps used to purify calf thymus topoisomerase II were adapted from several previously published procedures (9, 14, 32). Briefly, nuclei were isolated from 0.5 to 1 kg of frozen calf thymus as described previously (14). The nuclear pellet was resuspended without any washing steps in 300 ml of lysis buffer (5 mM KPO_4 [pH 7.0], 0.35 M NaCl, 5 mM dithiothreitol, 10 μg each of leupeptin, chymostatin, bestatin, and trypsin inhibitor per ml, 1 mM phenylmethylsulfonyl fluoride). The nuclei were lysed by frequent hand stirring with a glass rod and held on ice for 30 min. The resulting thick gelatinous suspension was centrifuged at 16,000 × *g* for 10 min. The supernatant was recovered, and the pellets were washed with another 100 ml of lysis buffer and centrifuged again. The supernatant fractions were combined and applied by gravity to a 250-ml column of HA-Ultrogel (IBF Biotechnics) equilibrated with a mixture of 0.2 M KPO_4 (pH 7), 10% glycerol, and 1 mM dithiothreitol. The column was then washed with 100 to 200 ml of equilibration buffer. The topoisomerase II activity was eluted in batch mode with 400 ml of 1 M KPO_4–10% glycerol–1 mM dithiothreitol, diluted fivefold with cold 10% glycerol containing 1 mM dithiothreitol, and applied to a column of S-Sepharose (4.5 by 12 cm) equilibrated with 50 mM Tris-HCl (pH 7.5)–0.1 M KCl–10% glycerol–1 mM dithiothreitol. After being washed with 1 column volume of this equilibration buffer, the column was developed at 10 to 15 ml/min with a 2-liter gradient of 0.1 to 1 M KCl in the same buffer. Fractions containing topoisomerase II activity were routinely frozen at −80°C. Final purification of diluted S-Sepharose fractions was done on a 20-ml MonoQ column (Pharmacia) with a 0.1 to 0.75 M KPO_4 gradient (pH 7.0) containing 10% glycerol and 1 mM dithiothreitol. Topoisomerase II fractions were concentrated by using HA-Ultrogel. This procedure yields highly purified preparations of topoisomerase II as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE); however, the activity of the highly purified enzyme was unstable after MonoQ chromatography. For this reason S-Sepharose fractions were routinely used in DNA breakage assays. The specific activity of pooled fractions at this level of purity was typically 25 to 50 P4-unknotting units per μg of total protein. Maximum stability was achieved by adding bovine serum albumin (molecular biology grade; Bethesda Research Laboratories) to a concentration of 0.1 mg/ml, concentrating the mixture 10-fold by ultrafiltration (Amicon YM-30), and storing it at −80°C. Working stocks were kept at −20°C.

P4-unknotting assay. Knotted P4 phage DNA was prepared as described previously (21). The P4 DNA-unknotting assay was done essentially as described previously (14) in 40-μl reaction volumes containing 50 mM Tris-Cl (pH 7.6), 100 mM KCl, 10 mM $MgCl_2$, 0.5 mM EDTA, 0.5 mM dithiothreitol, 30 μg of bovine serum albumin per ml, 1 mM ATP, drug at the concentrations indicated, 100 to 200 ng of knotted P4 DNA, and 1 U of topoisomerase II. Reactions were run at 37°C for 30 min. Aliquots (25 μl) were then electrophoresed in a 1% agarose gel, and the DNA was visualized by transillumination with UV light after staining with ethidium bromide. One unit of topoisomerase activity is defined as the amount of enzyme necessary to maximally unknot 200 ng of P4 knotted DNA in 30 min at 37°C under standard reaction conditions.

The 50% inhibitory concentrations (IC_{50}s) for the inhibition of P4 unknotting were estimated from the levels of inhibition seen in samples containing serial dilutions of inhibitor. The concentration of drug which inhibited the formation of unknotted product DNA by approximately 50% was defined as the IC_{50}.

DNA breakage assay. The DNA breakage assay was based on that described by Tewey et al. (34). The substrate used for breakage assays was pBR322 that had been linearized by *Eco*RI treatment and end labeled with Klenow fragment and [α-^{32}P]ATP. The assays were done in 20-μl reaction mixtures containing 50 mM Tris-HCl (pH 7.6), 50 mM KCl, 10 mM $MgCl_2$, 0.5 mM EDTA, 0.5 mM dithiothreitol, 50 μg of bovine serum albumin per ml, 50 ng of end-labeled DNA (approximately 5,000 cpm determined by Cerenkov light), 200 U of topoisomerase II, and drug at the concentrations indicated in the figures. The reaction was started by the addition of enzyme, and incubation continued at 37°C for 15 min. Reactions were stopped by the addition of 2 μl of quench solution that contained 5% SDS and 1.5 mg of predigested proteinase K (Bethesda Research Laboratories) per ml, and the mixtures were heated at 50°C for 45 min. Electrophoresis buffer (10 μl; 3% SDS, 15% Ficoll, 0.15 mg of bromophenol blue per ml) was added, and 25-μl aliquots were electrophoresed overnight in a 1% agarose gel. The bands were visualized by autoradiography after the gel had been dried by standard procedures. The amount of added enzyme was adjusted to minimize the background breakage observed in the absence of drug in order to facilitate the comparison of DNA cleavage patterns induced by different drugs. The assay was sensitive enough to detect topoisomerase-mediated DNA breakage induced by a variety of well-known agents (see below); however, at this enzyme-to-DNA ratio, high concentrations (>128 μg/ml) of test compounds (e.g., teniposide) were required to induce the cleavage of all of the labeled DNA substrate (results not shown).

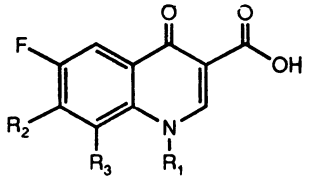
Compounds were initially screened at a final assay concentration of 128 μg/ml. Any active compounds were assayed again at 128, 64, 32, 16, and 4 μg/ml to determine whether they induced strong, moderate, or weak breakage. Strong breakage indicates that the level of breakage at ≤32 μg of test drug per ml was roughly comparable to the breakage seen with 16 to 32 μg of teniposide. Moderate breakage indicates that levels of DNA cleavage similar to that seen with the teniposide controls could be approached at the highest concentrations of test compound used. Weak but detectable cleavage at high drug concentrations is defined as weak breakage.

Other materials and reagents. Etoposide and teniposide were obtained from Bristol Myers-Squibb, Wallingford, Conn. The synthesis of A-65281 and A-65282 has been described elsewhere (4). All other compounds tested for calf thymus topoisomerase II activity were synthesized as part of the DNA gyrase inhibitors program at Abbott Laboratories.

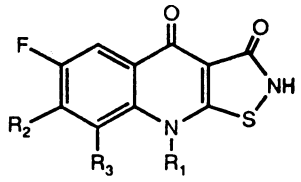
RESULTS

A number of quinolones and related compounds were screened for activity in assays designed specifically for eukaryotic topoisomerase II in order to identify chemical structure types that would interact with both the bacterial and eukaryotic enzymes. The ability of type II topoisomerases to catalyze DNA strand passage reactions provides the basis for the P4 DNA-unknotting assay (21), which is commonly used to measure the catalytic activity of eukaryotic topoisomerase II. However, as noted above, the

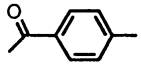
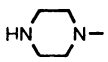
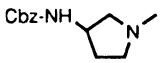
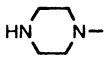
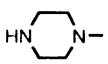
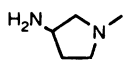
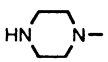
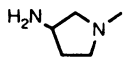
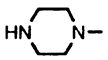
Table 1. Summary of P4 Unknotting and DNA Breakage Results



A



B

	structure class	R_1	R_2	R_3	unknotting IC_{50}(ug/ml)	DNA breakage[2]
A-61057	A			H	12	none
A-61526	A			H	25-50	none
norfloxacin	A	ethyl		H	n.d.[1]	none
ciprofloxacin	A			H	n.d.[1]	none
A-64927	B			H	16-25	none
A-65281	B			F	8	strong
A-65282	B			F	8	strong
A-65364	B	ethyl		F	25-50	weak

[1]No detectable inhibition at 50 μg/ml. [2]See Materials and Methods for description of weak, moderate, and strong breakage.

ability to induce topoisomerase-mediated breakage appears to correlate better with the cytotoxic activity of such compounds (20). This breakage activity can be readily detected by agarose gel electrophoresis after addition of SDS to drug-enzyme-DNA mixtures (see reference 34 for an example). Such assays have been widely used to study drug-topoisomerase interactions and have helped to prove the involvement of topoisomerases in the mode of action of such agents.

Inhibition of P4 DNA unknotting. Table 1 summarizes the effects of a number of quinolones and related compounds on the P4-unknotting and DNA breakage activity catalyzed by calf thymus topoisomerase II. Compounds that were active at ≤128 μg/ml fell into two major structural classes, those which had the standard quinolone structure (class A) and those from a new structural class in which the C-3 quinolone carboxylate group was replaced by an isothiazolone ring (class B) (5, 6). Potent unknotting inhibitors were found from both structural classes, with the most active compounds having IC_{50}s in the range of 8 to 25 μg/ml. Thus these compounds are considerably more potent than those reported previously (15, 17). Figure 1 illustrates the DNA unknotting catalyzed by calf thymus topoisomerase II and the dose dependence for inhibition typically seen with these compounds. The results obtained with A-65281 and A-65282 are shown. The knotted DNA substrate runs as a highly diffuse smear on agarose gels (Fig. 1A, lane 1), which reflects substantial heterogeneity in the extent of knotting of the closed-circular P4 DNA molecules (21). Unknotting by topoisomerase II resolves the DNA into topologically relaxed, closed-circular molecules which migrate as a single band (Fig. 1A, lane 2). The inhibition of DNA unknotting occurred in a normal, dose-dependent manner with all active compounds (data not shown). It should be noted that although A-65281 and A-65282 induce DNA breakage (see below), no breakage would be detectable in the unknotting assay because of the low levels of enzyme used.

Figure 1B shows the results obtained with etoposide and teniposide, two well-known inhibitors of topoisomerase II that also induce considerable DNA damage. Careful comparison of the dose responses seen with these compounds indicates that A-65281 is equipotent to teniposide and etoposide. A-65282 appears slightly more potent at inhibiting the P4 DNA unknotting; however, the levels of drug required for unknotting inhibition are higher than the antibacterial MICs and IC_{50}s for inhibition of DNA gyrase typically seen with

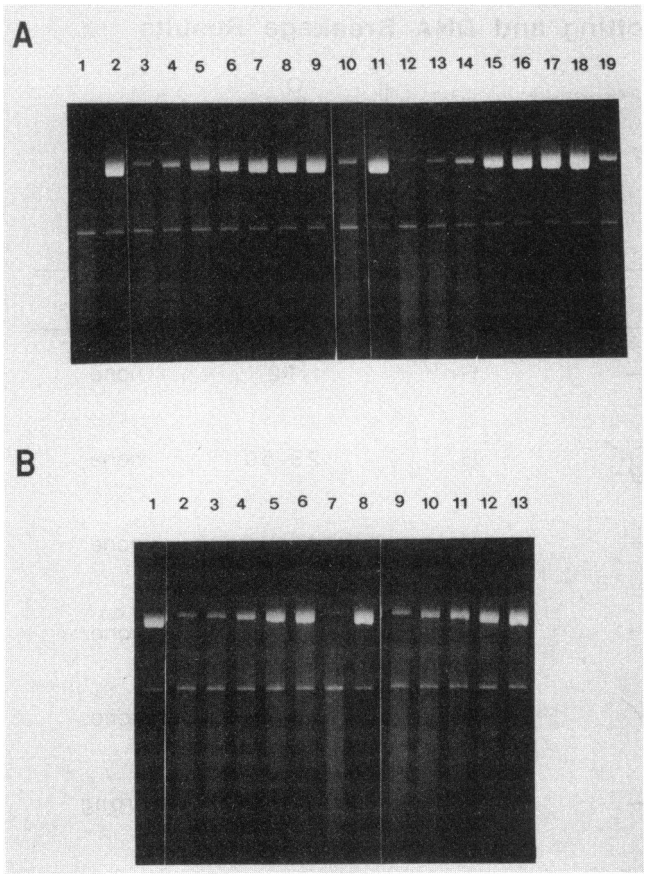


FIG. 1. Inhibition of P4 DNA unknotting by A-65281 and A-65282. The figure shows ethidium bromide-stained agarose gels that illustrate the extent of P4 DNA unknotting by calf thymus topoisomerase II in the presence of various drug concentrations. (A) The knotted P4 DNA substrate is shown in lane 1 as a diffuse smear. After resolution of the knots by calf topoisomerase II the DNA runs as a distinct band (lanes 2 and 11). Lanes 3 through 9 contain 50, 25, 16, 8, 4, 2, and 1 μg, respectively, of A-65281 per ml. Lanes 12 through 18 contain 50, 25, 16, 8, 4, 2, and 1 μg, respectively of A-65282 per ml. Lanes 10 and 19 contain 100 μg of etoposide per ml. (B) Lanes 1 and 8 contain just topoisomerase II. Lane 7 is a minus-enzyme control. Lanes 2 through 6 contain 50, 25, 12.5, 6.25, and 3.1 μg, respectively, of teniposide per ml. Lanes 9 through 13 contain 100, 50, 25, 12.5, 6.25 μg, respectively, of etoposide per ml.

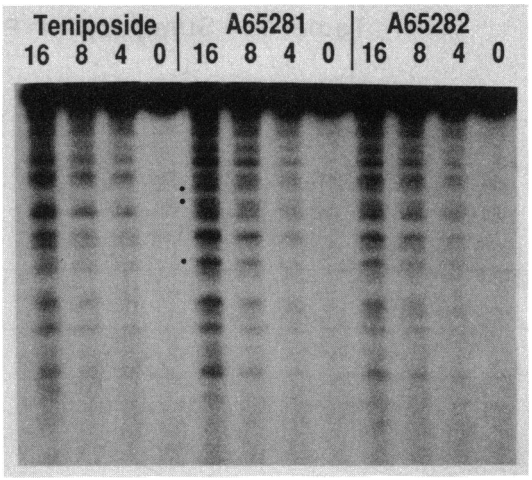


FIG. 2. Drug-induced DNA breakage by calf thymus topoisomerase II. This autoradiogram shows the extent and pattern of breakage of end-labeled pBR322 induced by various levels of A-65281, A-65282, and teniposide. Lanes are designated according to the level of drug present. The units used are micrograms per milliliter. Although similar, there are some differences in cleavage patterns induced by the two classes of drugs. The dots adjacent to the lane containing 16 μg of A65281 per ml indicate the DNA fragments induced by the isothiazoloquinolones which are less prominent or absent when teniposide is used.

isothiazoloquinolones (5, 6), indicating that there is still a high degree of specificity for the bacterial enzyme.

The possible effects of DNA binding should be considered when interpreting these results. A number of quinolones have been reported to bind to DNA in a nonintercalative mode (29, 31). Although the role of these drug-DNA interactions in the biological effects of these compounds is not clear, they could account for the weak inhibition of P4 DNA unknotting seen previously (15, 17). For such effects to explain the results reported here, significant drug-DNA interactions must occur at relatively low drug concentrations. Further studies are needed to investigate the effects of drug-DNA interactions on the inhibition of DNA unknotting by topoisomerase II.

Inhibitor-induced DNA breakage mediated by calf thymus topoisomerase II. It has been proposed that the biological activity of topoisomerase-directed cytotoxic agents results from the ability of the drug to stabilize a complex in which the DNA is covalently linked to the enzyme (commonly referred to as a cleavable complex) (20). This complex is easily disrupted by SDS, resulting in double-stranded DNA breakage which is readily detectable in vitro after electrophoresis when high levels of topoisomerase II are used with ^{32}P-end-labeled DNA substrate (34). Interestingly, of the compounds listed in Table 1, only two isothiazoloquinolones, A-65281 and A-65282, induced substantial topoisomerase II-mediated DNA breakage in this assay. The DNA cleavage patterns obtained with A-65281, A-65282, and teniposide are shown in the autoradiograph in Fig. 2. DNA breakage is indicated by the generation of DNA fragments with higher mobility than the end-labeled linear pBR322 substrate. The level of topoisomerase used in this assay is much greater than that used in the unknotting assay; however, the amount added was adjusted so that very little breakage of DNA is evident in the absence of drug. Separate controls showed that no drug-induced DNA breakage was detectable in the absence of enzyme at drug concentrations exceeding 100 μg/ml (data not shown); however, all three compounds induced substantial DNA cleavage when enzyme was present. Breakage was evident at concentrations of both A-65281 and A-65282 as low as 4 μg/ml. Comparisons with the data obtained with teniposide indicate that all three compounds have similar potencies. Although direct comparisons with other published studies in which this assay was used are difficult, these results suggest that A-65281 and A-65282 are at least as potent as CP-67,015 at inducing topoisomerase II-mediated DNA breakage (1).

The banding patterns of the cleavage products shown in Fig. 2 are consistent with an enzyme-mediated process at preferred sites along the DNA. The sequence specificity observed in the presence of the isothiazoloquinolones is similar, but not identical, to the sequence specificity observed in the presence of teniposide. The fragments induced by the isothiazoloquinolones which are less prominent or

absent when teniposide is used are indicated in the figure. More sensitive techniques are necessary to identify the exact sites of cleavage induced by the different drugs and to establish whether the patterns observed reflect real differences in cleavage site specificity or changes in the relative frequency of DNA breakage at common cleavage sites, or both. Differences in DNA cleavage patterns induced by different drugs have been observed previously and probably result from the ability of different drug classes to stabilize the drug-DNA-enzyme complex at different sites along the DNA substrate (see reference 20 and citations therein). The extent to which direct drug-DNA interactions contribute to these specificities and how they correlate with the biological effects of different classes of compounds are not known. The DNA cleavage pattern obtained with A-65281 was also distinctly different from that obtained with m-AMSA (results not shown).

These results are in accord with previous observations that DNA-unknotting inhibitors do not necessarily have the ability to induce DNA breakage (15, 17). It should also be noted that the isothiazolone moiety is not sufficient by itself to confer this activity, since several isothiazoloquinolones did not induce any detectable DNA breakage (Table 1; data not shown). However, one interesting structural feature shared by A-65281, A-65282, and CP-67015 (see reference 1 for structure) is the 6,8-difluoro substitution on the quinolone ring system. The importance of this difluoro structure is also suggested by the lack of DNA breakage activity of A-64927, which differs from A-65282 only by the removal of the fluorine at C-8 (Table 1). The weak DNA breakage seen with A-65364 suggests that the cyclopropyl group also contributes significantly to the observed effects. These results are in accord with the results reported very recently (24) for two quinolone derivatives, CP-67,804 and CP-115,953. A cyclopropyl group at the N position (CP-115,953) results in more potent DNA breakage activity than is observed with an ethyl substitution (CP-67,804). Further work is under way to identify which structural aspects of the isothiazoloquinolones contribute to their DNA breakage activity and to further evaluate the biological activity of this class of topoisomerase II-directed agents.

Neither norfloxacin nor ciprofloxacin showed any detectable inhibition of P4 DNA-unknotting activity when tested at 50 μg/ml. Moreover, no DNA breakage was evident with either compound at concentrations up to 128 μg/ml. This is in contrast to previous reports of ciprofloxacin-induced DNA breakage by calf thymus topoisomerase II (1, 12). The reason for this discrepancy is not known; however, in view of the dose response reported previously (1, 12), it is quite possible that the highest concentration of ciprofloxacin tested here (128 μg/ml) was not sufficient to give detectable cleavage, particularly at the moderate enzyme-to-DNA ratios we chose to use for better cleavage pattern comparisons. Under the assay conditions described in Materials and Methods, DNA breakage by more potent agents such as ellipticine, m-AMSA, or adriamycin was detectable at concentrations of ≤ 1 μg/ml, which is in accord with the results seen previously (23, 24, 25) for these drugs. Each class of drug induced different cleavage patterns (data not shown).

DISCUSSION

These studies have identified several isothiazoloquinolones which have significant activity against both bacterial and eukaryotic type II topoisomerases. As a class, the isothiazoloquinolones have quite potent antibacterial activity (5, 6) against a wide range of organisms and low IC_{50}s for the inhibition of bacterial DNA gyrase (6). In general, an isothiazoloquinolone is significantly more active than its corresponding parent quinolone (6). A-65281 is a potent DNA gyrase inhibitor (IC_{50} = 0.1 μg/ml against *Escherichia coli* DNA gyrase [6a]), with MICs against different strains of *E. coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Pseudomonas aeruginosa* all well below 1.0 μg/ml (6). A-65282 has a similar profile (3a). Although the biological activity of these two compounds reflects a high degree of selectivity for DNA gyrase, their potency against topoisomerase II provides additional evidence that common structural themes may emerge for inhibitors that induce DNA breakage by both classes of enzymes. As pointed out previously (1, 2, 12, 13), such data also suggest the need to investigate the potential toxicity of compounds developed as antibacterial agents against bacterial DNA gyrase which might result from their interaction with human topoisomerase II. This issue has been raised regarding ciprofloxacin (1, 2, 12, 13), which has DNA breakage activity when tested at high concentrations with purified topoisomerase II (1, 12) and was recently found to induce double-strand breaks in human lymphoblastoid cells (2). It is not clear how these in vitro results might correlate with potential toxicity (see reference 13 and citations therein). Nor is it clear which structural features of topoisomerase II-directed inhibitors correlate with toxicity. Thus, even within closely related structure types, compounds should be evaluated on a case-by-case basis.

In addition to further defining structure-function relationships, consideration will have to be given to issues such as how different compounds interact with topoisomerases in situ and how preferred in vitro cleavage sites correlate with in vivo activity. Such information would better establish how well in vitro activities serve as predictors of toxicity; in addition, the elucidation of the structural features essential to topoisomerase-directed activity offers the prospect of designing more potent and/or specific agents that might find utility in the anti-infective or antitumor areas.

ACKNOWLEDGMENTS

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REFERENCES

1. **Barrett, J. F., T. D. Gootz, P. R. McGuirk, C. A. Farrell, and S. A. Sokolowski.** 1989. Use of in vitro topoisomerase II assays for studying quinolone antibacterial agents. Antimicrob. Agents Chemother. **33**:1697–1703.
2. **Bredberg, A., M. Brant, and M. Jaszyk.** 1991. Ciprofloxacin-induced inhibition of topoisomerase II in human lymphoblastoid cells. Antimicrob. Agents Chemother. **35**:448–450.
3. **Chen, G. L., L. Yang, T. C. Rowe, B. D. Halligan, K. M. Tewey, and L. F. Liu.** 1984. Nonintercalative antitumor drugs interfere with the breakage-reunion reaction of mammalian DNA topoisomerase II. J. Biol. Chem. **259**:13560–13566.

3a. **Chu, D. T. W.** Unpublished data.

4. **Chu, D. T. W., and A. K. Claiborne.** 1990. Practical synthesis of iminochlorothioformates: application of iminochlorothioformates in the synthesis of novel 2,3,4,9-tetrahydroisothiazolo [5,4-b][1,8]naphthyridine-3,4-diones and 2,3,4,9-tetrahydroisothiazolo[5,4-b]quinoline-3,4-dione derivatives. J. Heterocycl. Chem. **27**:1191–1195.
5. **Chu, D. T. W., P. B. Fernandes, A. K. Claiborne, L. Shen, and A. G. Pernet.** 1988. Structure-activity relationships in quinolone antibacterials: design, synthesis and biological activities of novel isothiazoloquinolones. Drugs Exp. Clin. Res. **14**:379–383.
6. **Chu, D. T. W., P. B. Fernandes, A. K. Claiborne, L. L. Shen,**

and A. G. Pernet. 1989. Structure-activity relationships in quinolone antibacterials: replacement of the 3-carboxylic acid group, p. 37–45. *In* P. B. Fernandes (ed.), Quinolones. J. R. Prous, Barcelona, Spain.

6a. **Chu, D. T. W., and L. Shen.** Unpublished data.

7. **Cozzarelli, N. R.** 1980. DNA gyrase and the supercoiling of DNA. Science **207**:953–960.

8. **Domagala, J. M., L. D. Hanna, C. L. Heifetz, M. P. Hutt, T. F. Mich, J. P. Sanchez, and M. Solomon.** 1986. New structure-activity relationships of the quinolone antibacterial using the target enzyme. The development and application of a DNA gyrase assay. J. Med. Chem. **29**:394–404.

9. **Drake, F. H., J. P. Zimmerman, F. L. McCabe, H. F. Bartus, S. R. Per, D. M. Sullivan, W. E. Ross, M. R. Mattern, R. K. Johnson, S. T. Crooke, and C. K. Mirabelli.** 1987. Purification of topoisomerase II from amsacrine-resistant P388 leukemia cells: evidence for two forms of the enzyme. J. Biol. Chem. **262**: 16739–16747.

10. **Elwell, L. P., L. M. Walton, J. M. Besterman, and A. Hudson.** 1990. Use of an in vitro DNA strand-breakage assay to monitor compound interactions with DNA gyrase, p. 87–102. *In* G. C. Crumplin (ed.), The 4-quinolones: antibacterial agents in vitro. Springer-Verlag, London.

11. **Gellert, M.** 1981. DNA topoisomerases. Annu. Rev. Biochem. **50**:879–910.

12. **Gootz, T. D., J. F. Barrett, H. E. Holden, V. A. Ray, and P. R. McGuirk.** 1990. Selective toxicity: the activities of 4-quinolones against eukaryotic DNA topoisomerases, p. 159–171. *In* G. C. Crumplin (ed.), The 4-quinolones: antibacterial agents in vitro. Springer-Verlag, London.

13. **Gootz, T. D., J. F. Barrett, and J. A. Sutcliffe.** 1990. Inhibitory effects of quinolone agents on eukaryotic topoisomerases and related test systems. Antimicrob. Agents Chemother. **34**:8–12.

14. **Halligan, B. D., K. A. Edwards, and L. F. Liu.** 1985. Purification and characterization of a type II DNA topoisomerase from bovine calf thymus. J. Biol. Chem. **260**:2475–2482.

15. **Hoshino, K., K. Sato, T. Une, and Y. Osada.** 1989. Inhibitory effects of quinolones on DNA gyrase of *Escherichia coli* and topoisomerase II of fetal calf thymus. Antimicrob. Agents Chemother. **33**:1816–1818.

16. **Hsiang, Y.-H., R. Hertzberg, S. Hecht, and L. F. Liu.** 1985. Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. J. Biol. Chem. **260**:14873–14878.

17. **Hussy, P., G. Maass, B. Tummler, F. Grosse, and U. Schomburg.** 1986. Effect of 4-quinolones and novobiocin on calf thymus DNA polymerase, a primase complex, topoisomerases I and II, and growth of mammaliam lymphocytes. Antimicrob. Agents Chemother. **29**:1073–1078.

18. **Ikegami, Y., N. Takeuchi, M. Hanada, Y. Hasegawa, K. Ishii, T. Andoh, T. Sato, K. Suzuki, H. Yamaguchi, S. Miyazaki, K. Nagai, S.-I. Watanabe, and T. Saito.** 1990. Topostin, a novel inhibitor of mammalian DNA topoisomerase I from *Flexibacter topostinus* sp. nov. II. Purification and some properties of topostin. J. Antibiot. **43**:158–162.

19. **Kohn, K. W.** 1991. Principles and practice of DNA filter elution. Pharmacol. Ther. **49**:55–77.

20. **Liu, L. F.** 1989. DNA topoisomerase poisons as antitumor drugs. Annu. Rev. Biochem. **58**:351–375.

21. **Liu, L. F., and J. L. Davis.** 1981. Novel topologically knotted DNA from bacteriophage P4 capsids: studies with DNA topoisomerases. Nucleic Acids Res. **9**:3979–3989.

22. **Minocha, A., and B. H. Long.** 1984. Inhibition of the DNA catenation activity of type II topoisomerase by VP16-213 and VM26. Biochem. Biophys. Res. Commun. **122**:165–170.

23. **Nelson, E. M., K. M. Tewey, and L. F. Liu.** 1984. Mechanism of antitumor drug action: poisoning of mammalian DNA topoisomerase II on DNA by 4′-(9-acridinylamino)-methanesulfon-m-anisidide. Proc. Natl. Acad. Sci. USA **81**:1361–1365.

24. **Robinson, M. J., B. A. Martin, T. D. Gootz, P. R. McGuirk, M. Moynihan, J. A. Sutcliffe, and N. Osheroff.** 1991. Effects of quinolone derivatives on eukaryotic topoisomerase II: a novel mechanism for enhancement of enzyme-mediated DNA cleavage. J. Biol. Chem. **266**:14585–14592.

25. **Ross, W., T. Rowe, B. Glisson, J. Yalowich, and L. Liu.** 1984. Role of topoisomerase II in mediating epipodophyllotoxin-induced DNA cleavage. Cancer Res. **44**:5857–5860.

26. **Ross, W. E., and M. O. Bradley.** 1981. DNA double-strand breaks in mammalian cells after exposure to intercalating agents. Biochim. Biophys. Acta **654**:129–134.

27. **Ross, W. E., D. L. Glaubiger, and K. W. Kohn.** 1978. Protein-associated DNA breaks in cells treated with adriamycin or ellipticine. Biochim. Biophys. Acta **519**:23–30.

28. **Ross, W. E., D. L. Glaubiger, and K. W. Kohn.** 1979. Qualitative and quantitative aspects of intercalator-induced DNA strand breaks. Biochim. Biophys. Acta **562**:41–50.

29. **Shen, L. L., J. Baranowski, T. Wai, and D. T. W. Chu.** 1989. The binding of quinolones to DNA: should we worry about it? p. 159–170. *In* P. B. Fernandes (ed.), Quinolones. J. R. Prous, Barcelona, Spain.

30. **Shen, L. L., W. E. Kohlbrenner, D. Weigl, and J. Baranowski.** 1989. Mechanism of quinolone inhibition of DNA gyrase. J. Biol. Chem. **264**:2973–2978.

31. **Shen, L. L., and A. G. Pernet.** 1985. Mechanism of inhibition of DNA gyrase by analogues of nalidixic acid: the target of the drugs is DNA. Proc. Natl. Acad. Sci. USA **82**:307–311.

32. **Strausfeld, U., and A. Richter.** 1989. Simultaneous purification of DNA topoisomerase I and II from eukaryotic cells. Prep. Biochem. **19**:37–48.

33. **Sugino, A., C. L. Peebles, K. N. Kreuzer, and N. R. Cozzarelli.** 1977. Mechanism of action of nalidixic acid: purification of *E. coli* nalA gene product and its relationship to DNA gyrase and a novel nicking-closing enzyme. Proc. Natl. Acad. Sci. USA **74**:4767–4771.

34. **Tewey, K. M., G. L. Chen, E. M. Nelson, and L. F. Liu.** 1984. Intercalative antitumor drugs interfere with the breakage-reunion reaction of mammalian DNA topoisomerase II. J. Biol. Chem. **259**:9182–9187.

35. **Tewey, K. M., T. C. Rowe, L. Yang, B. D. Halligan, and L. F. Liu.** 1984. Adriamycin-induced DNA damage mediated by mammalian DNA topoisomerase II. Science **226**:466–468.

36. **Walton, L., and L. P. Elwell.** 1988. In vitro cleavable-complex assay to monitor antimicrobial potency of quinolones. Antimicrob. Agents Chemother. **32**:1086–1089.

37. **Wolfson, J. S., and D. C. Hooper.** 1989. Fluoroquinolone antimicrobial agents. Clin. Microbiol. Rev. **2**:378–424.

38. **Yamashita, Y., S.-Z. Kawada, N. Fujii, and H. Nakano.** 1991. Induction of mammalian DNA topoisomerase I and II mediated DNA cleavage by saintopin, a new antitumor agent from fungus. Biochemistry **30**:5838–5845.

39. **Zwelling, L. A., S. Michaels, L. C. Erickson, R. S. Ungerleider, M. Nichols, and K. W. Kohn.** 1981. Protein-associated DNA strand breaks in L1210 cells treated with the DNA intercalating agents m-AMSA and adriamycin. Biochemistry **20**:6553–6563.

40. **Zwelling, L. A., S. Michaels, D. Kerrigan, Y. Pommier, and K. W. Kohn.** 1982. Protein-associated deoxyribonucleic acid strand breaks produced in mouse leukemia L1210 cells by ellipticine and 2-methyl-9-hydroxyellipticinium. Biochem. Pharmacol. **31**:3261–3267.