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Stimulation of Topoisomerase II-Mediated DNA Cleavage by Ellipticine Derivatives: Structure-Activity Relationship

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SUMMARY

Ellipticines are aromatic compounds that intercalate between DNA base pairs and display significant antitumor activity. The cytotoxicity of these compounds is mediated by DNA topoisomerase II, and the presence of a hydroxy group at position 9 of the pyridocarbazole ring system of ellipticines has been found to be essential for high levels of cytotoxicity. The ability of 13 ellipticine derivatives to stabilize the topoisomerase II-DNA covalent complex *in vitro* was studied, and the data obtained with five pairs of hydroxylated and nonhydroxylated analogues indicate that the hydroxy group at position 9 plays a crucial role in the stabilization of the complex. The influence, upon the complex stabilization, of various substituents at positions 1, 2, 5, and 6 of the pyridocarbazole ring system was investigated. The interaction with DNA of four ellipticine derivatives was studied in the topoisomerase II standard medium. Results suggest that the degree of unwinding might be a determinant of topoisomerase II-DNA-drug complex stability. In addition, the 5-ethyl derivative was observed to induce covalent complex stabilization by a cooperative mechanism.

Ellipticine and its derivatives belong to a group of antitumor drugs that bind to DNA by intercalation between adjacent base pairs (1). One of these derivatives, elliptinium (NMHE), has shown significant activity against advanced breast cancer (2). Induction of protein-associated DNA strand breaks was observed by the alkaline elution technique in cultured mammalian cells treated with ellipticine (3, 4), and the protein covalently linked to the DNA was later suggested to be a topoisomerase II subunit (5, 6). These observations led to the hypothesis that the cytotoxicity of ellipticine derivatives was mediated by DNA topoisomerase II, an enzyme that controls DNA topology by introducing transient protein-bridged DNA double-strand breaks to allow the passage of an intact DNA segment through the break (7). This hypothesis was strengthened by the analysis of ellipticine-resistant cell lines, showing that DNA topoisomerase II activity is altered in these cells, compared with the drug-sensitive cells (8–10).

In vitro studies with purified DNA topoisomerase II showed that ellipticine and NMHE were able to stabilize the enzyme-DNA covalent complex in a fashion similar to that of 4′-(9-acridinylamino)methanesulfon-*m*-anisidide (*m*-AMSA) or epipodophyllotoxin derivatives (11, 12). Furthermore, NMHE, which is a more potent antitumor drug than ellipticine, was found to stabilize the enzyme-DNA covalent complex more efficiently than ellipticine (11). To generalize this observation, we studied the enzyme-DNA complex stabilization by a series of ellipticine derivatives substituted at different positions of the pyridocarbazole ring system (Table 1). The results presented here demonstrate the crucial role played by a hydroxy group at position 9 of ellipticine in the stabilization of the enzyme-DNA complex. Furthermore, the properties of four ellipticine analogues with respect to complex stabilization are reported.

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Materials and Methods

Drugs, enzymes, DNA, and supplies. Of the ellipticine series (Table 1), compounds **1**–**10** and **13** were synthesized by Dr. E. Lescot and Dr. B. Psaume (IGR, Villejuif, France) and **11** and **12** were provided by the SANOFI Co. (Sisteron, France). These drugs were dissolved at a concentration of 2 mM just before use (compounds **1**, **2**, and **3** in 50% dimethylsulfoxide/10 mM HCl, compound **4** in 10 mM HCl, compound **5** in 50% dimethylsulfoxide, and the remaining drugs in distilled water).

Restriction endonucleases were purchased from New England Biolabs (Beverly, MA). *Escherichia coli* DNA polymerase I (Klenow fragment) and proteinase K were purchased from Boehringer Mannheim (Hamburg, Germany). DNA topoisomerase I and II were purified from calf thymus, using previously published procedures (13–16).

Circular pBR322 DNA and circular pSP65 DNA were purified from *E. coli* (*hsd*R, *hsd*M, *rec*A13) according to published methods (17). [α-^{32}P]dATP was purchased from Amersham (Buckinghamshire, UK). Agarose and polyacrylamide were purchased from Seakem (FMC Bio-

ABBREVIATIONS: SDS, sodium dodecyl sulfate; compounds **1**–**13**, abbreviations given in Table 1.

products, Rockland, ME) and Boehringer Mannheim, respectively. Autoradiography was performed with R-X film (Fuji-Photo Film Co., Minami-Ashigara-shi, Japan).

Labeling procedure for pBR322 DNA. For preparation of the 3′-end-labeled pBR322 fragment, circular pBR322 DNA was cut with *Eco*RI restriction endonuclease and then labeled with [α-^{32}P]dATP, using the large fragment of *E. coli* DNA polymerase I (Klenow fragment), at 20° for 20 min. The labeled DNA was purified by two cycles of ethanol precipitation and was further cut with *Hind*III restriction endonuclease. This procedure generated two fragments, one large and one small, which were both labeled at one end. These two DNA fragments were present in the reaction of topoisomerase II-mediated DNA cleavage.

Topoisomerase II-mediated DNA cleavage reactions. pBR322 DNA (3′-end-labeled; 6×10^4 cpm) was incubated with topoisomerase II (17 μg/ml), in the presence or absence of drug, in cleavage buffer (40 mM Tris·HCl, pH 7.5, 100 mM KCl, 10 mM $MgCl_2$, 0.5 mM EDTA, 1 mM ATP) for 15 min at 37°. The cleavage reaction was terminated by the addition of SDS and proteinase K to final concentrations of 0.4% and 0.1 mg/ml, respectively, and the mixture was incubated for an additional 30 min at 50°. Cleavage reactions were performed in 15 μl and, after proteinase K digestion, 5 μl of loading buffer (0.05% bromophenol blue, 50 mM EDTA, 50% sucrose) were added to samples. The products of cleavage reactions were separated on 1% agarose-0.1% SDS gels for about 15 hr (2.5 V/cm) in TBE (89 mM Tris base, 89 mM boric acid, 2.5 mM EDTA, pH 8.3) containing 0.1% SDS, in order to remove DNA-bound drug molecules, which otherwise retard the electrophoretic migration of DNA fragments. The gels were then dried on a 3MM paper sheet and autoradiographed with Fuji X-ray films.

When negatively supercoiled pBR322 plasmid DNA was used as a substrate for topoisomerase II-mediated cleavage, reaction mixtures contained 110 ng of plasmid DNA and topoisomerase II (1.1 μg/ml) in 15 μl of cleavage buffer. Samples were otherwise treated as described above for samples loaded on agarose gels, except that a known amount of the pBR322 2694-base pair *Pvu*I-*Pvu*II fragment was added as an internal standard after SDS-proteinase K treatment, in order to allow precise quantification of pBR322 DNA form III. The reaction products were then mixed with loading buffer (0.05% bromophenol blue, 50 mM EDTA. 50% sucrose) and analyzed by polyacrylamide gel electrophoresis. The products of topoisomerase II-mediated cleavage of circular pBR322 DNA were assessed by electrophoresis on 4.2% polyacrylamide gels [29:1 acrylamide to bis(acrylamide) ratio] in TBE at 3 V/cm for about 18 hr, at 11°. In these polyacrylamide gels, forms I and II remain at the top of the gel and only form III (linearized DNA) migrates in the gel (18, 19). The gels were stained for 1 hr in an aqueous solution of ethidium bromide (0.5 μg/ml). DNA bands were visualized by transillumination with UV light (300 nm) and were photographed using Polaroid type 665 positive/negative film.

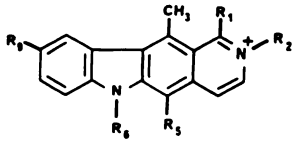
Quantification of DNA cleavage stimulated by drugs. DNA cleavage stimulated by ellipticine derivatives was quantified by means of densitometer scanning (Chromoscan 3; Joyce-Loebl, Gateshead, England) of autoradiograms. The densitometer was connected to a computer, which stored and analyzed the data.

To quantify the circular pBR322 DNA double-strand cleavage, negative films were scanned with the densitometer. The peak areas of sharp bands corresponding to form III permit an effective quantification. The gel was calibrated with a known amount of linearized pBR322 DNA; 100% of form III corresponds to complete linearization of 110 ng of supercoiled pBR322.

Interaction with DNA. The DNA-ellipticine derivative binding constants were determined at 25° by a method of competition with ethidium bromide, as described (20), using calf thymus DNA (Boehringer Mannheim) in medium containing 40 mM Tris·HCl, pH 7.5, 100 mM KCl, 10 mM $MgCl_2$, and 0.5 mM EDTA. The binding of ethidium

TABLE 1

Chemical structures of the ellipticine derivatives and effect of various substitutions on stimulation of topoisomerase II-mediated DNA cleavage



Compound	R_1	R_2	R_5	R_6	R_9	Abbreviation	Concentration[a]	Relative Frequency[b]
							μM	
1 Ellipticine	H		CH_3	H	H		4	1.6 ± 0.1
2 9-Hydroxyellipticine	H		CH_3	H	OH	9-OH-Ellipticine	4	4.0 ± 0.3
3 9-Methoxyellipticine	H		CH_3	H	OCH_3	9-OCH_3-Ellipticine	4	0.8 ± 0.1
4 9-Aminoellipticine	H		CH_3	H	NH_2	9-NH_2-Ellipticine	4	2.4 ± 0.5
5 2-Methylellipticinium	H	CH_3	CH_3	H	H	NME	2	1.9 ± 0.1
6 2-Methyl-9-hydroxyellipticinium	H	CH_3	CH_3	H	OH	NMHE	2	4.7 ± 0.2
7 1,2-Dimethylellipticinium	CH_3	CH_3	CH_3	H	H	1-Me-NME	2	1.3 ± 0.2
8 1,2-Dimethyl-9-hydroxyellipticinium	CH_3	CH_3	CH_3	H	OH	1-Me-NMHE	2	6.2 ± 1.1
9 2,6-Dimethylellipticinium	H	CH_3	CH_3	CH_3	H	6-Me-NME	1	1.8 ± 0.5
10 2,6-Dimethyl-9-hydroxyellipticinium	H	CH_3	CH_3	CH_3	OH	6-Me-NMHE	1	3.3 ± 0.3
11 2-Diethylaminoethylellipticinium	H	$(CH_2)_2N^+(C_2H_5)_2$	CH_3	H	H	DE	2	1.6 ± 0.1
12 2-Diethylaminoethyl-9-hydroxyellipticinium	H	$(CH_2)_2N^+(C_2H_5)_2$	CH_3	H	OH	DHE	2	3.6 ± 0.1
13 2,11-Dimethyl-5-ethyl-9-hydroxy-6*H*-pyrido[4,3-*b*]carbazolium	H	CH_3	C_2H_5	H	OH	EPC	2	8.2 ± 1.6

[a] The drug concentrations indicated correspond to the optimal concentrations, for cleavage, for the 9-OH derivatives. Nonhydroxylated derivatives were assayed at the same concentrations as their 9-OH counterparts.

[b] Cumulative frequencies of cleavage in regions 1–3 of pBR322 (as defined in the text) were expressed for each drug relative to the cumulative frequency in the absence of drug. Each value, with variation range, is the mean of two independent determinations.

to DNA was studied by fluorimetry using a SFM 23/B spectrofluorimeter (Kontron, Zurich, Switzerland). Fluorescence excitation and emission wavelengths were 540 and 610 nm, respectively. The binding constants determined by this procedure allowed calculation, as described previously (21), of the bound drug/nucleotide ratio from the DNA and total drug concentrations, assuming a value of 0.2 molecules of bound drug/nucleotide at saturation.

Unwinding of the DNA helix was measured by the method of Keller (22), with some modifications. The 3005-base pair supercoiled pSP65 DNA (170 ng) was incubated with calf topoisomerase I in the presence of increasing concentrations of drugs (2×10^{-7} to 2.5×10^{-6} M) in 40 mM Tris·HCl, pH 7.5, 100 mM KCl, 10 mM $MgCl_2$, 0.5 mM EDTA, for 15 min at 37°. Reaction mixtures (30 μl) were stopped by extraction with phenol/chloroform/isoamyl alcohol (25:24:1, v/v/v), vortexed, and centrifuged for 30 sec at room temperature. To 15 μl of supernatant were added 4 μl of loading buffer (0.05% bromophenol blue, 50 mM EDTA, 30% glycerol, 1% SDS). Samples were electrophoresed (2.8 V/cm) in 1% agarose gels containing 0.4 μg/ml chloroquine, at 20°. The gels were stained and photographed, and the negatives were scanned as described above. The median of the topoisomer distribution was determined as described (23), and the change in the linking number was calculated for each drug concentration by comparison with the sample relaxed in the absence of drug.

Results

Structure-activity relationship for stimulation of DNA cleavage. The effect of the 13 ellipticine derivatives shown in Table 1 on the topoisomerase II-mediated double-strand DNA cleavage was studied by agarose gel electrophoresis, using as substrate a pBR322 fragment (*Eco*RI-*Hind*III) labeled with ^{32}P at its *Eco*RI end (Fig. 1). Stimulation of cleavage varied as a function of the drug concentration (see below), and the data presented in Fig. 1 and Table 1 were obtained in the vicinity of the optimal drug concentration for DNA cleavage. The cleavage patterns shown in Fig. 1 indicate that ellipticine derivatives stimulated DNA cleavage in several regions, whose positions on the pBR322 map were previously determined using size markers (24). A quantitative analysis of cleavage frequency was carried out for three regions of pBR322 (Fig. 1, *arrows*) where the DNA cleavage was strongly stimulated by ellipticine. Region 1 spans approximately nucleotides 1035 to 1125, region 2 nucleotides 2385 to 2475, and region 3 nucleotides 2730 to 2820. These three regions correspond to previously characterized strong ellipticine-stimulated cleavage sites (sites 1059 and 1083, region 1; site 2431, region 2; and site 2750, region 3) (24). Cleavage in other regions was weak or not stimulated by ellipticine derivatives.

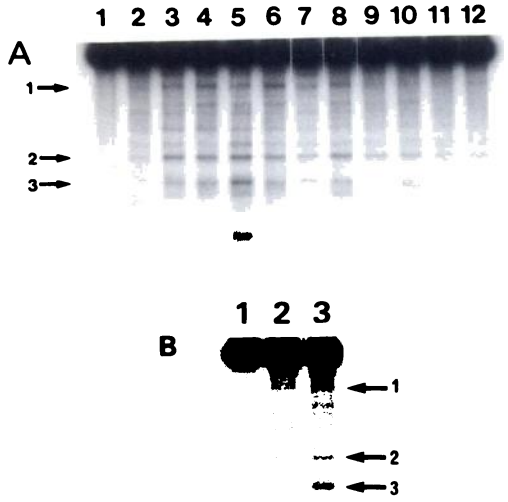


Fig. 1. DNA double-strand break cleavage patterns induced by calf thymus topoisomerase II in the presence of different ellipticine derivatives. pBR322 DNA labeled with ^{32}P at its *Eco*RI ends was incubated with topoisomerase II and each drug at the same concentration as in Table 1 (except 9-NH_2-ellipticine at 8 μM). *Arrows*, three particular regions of cleavage defined in the text. Controls without enzyme and drug (*lane 1*) or without drug (*lane 2*) are shown in both panels. A, *Lane 3*, 9-OH-ellipticine; *lane 4*, NMHE; *lane 5*, EPC; *lane 6*, 1-Me-NMHE; *lane 7*, 6-Me-NMHE; *lane 8*, DHE; *lane 9*, DE; *lane 10*, NME; *lane 11*, 1-Me-NME; *lane 12*, 6-Me-NME. B, *Lane 3*, 9-NH_2-ellipticine.

Under our experimental conditions, the average number of cleavages per fragment is <1, and multiple cleavage of one fragment is very rare. Therefore, band intensity is proportional to the cleavage frequency in the corresponding region and is not affected by the distance of the region from the labeled end.

The cleavage frequency was determined for each ellipticine derivative in the three regions defined above, relative to the cleavage in the absence of drug. The cumulative relative frequencies for the three regions are presented in Table 1. For a given ellipticine derivative, cleavage stimulation was approximately equivalent in the three regions analyzed. The patterns of DNA cleavage induced by the different ellipticine derivatives were similar (Fig. 1A). However, 9-NH_2-ellipticine strongly stimulated cleavage in region 3 but not in regions 1 and 2, yielding a different DNA cleavage pattern for this compound (Fig. 1B). From the data of Table 1, two classes of ellipticine derivatives may be distinguished, i.e., the 9-OH derivatives, which stimulate cleavage by a factor of 3.3–8.2, and derivatives that are neither hydroxylated nor 9-NH_2-substituted, for which the relative frequency of DNA cleavage is between 0.8 and 1.9. The effect of different substitutions at position 9 was further investigated by determining the cleavage frequency as a function of the drug concentration (Fig. 2). Ellipticine and 9-OCH_3-ellipticine slightly stimulated DNA cleavage at concentrations of 4 μM or 2 μM, whereas a more marked stimulation was observed with 9-OH-ellipticine and 9-NH_2-ellipticine, with a

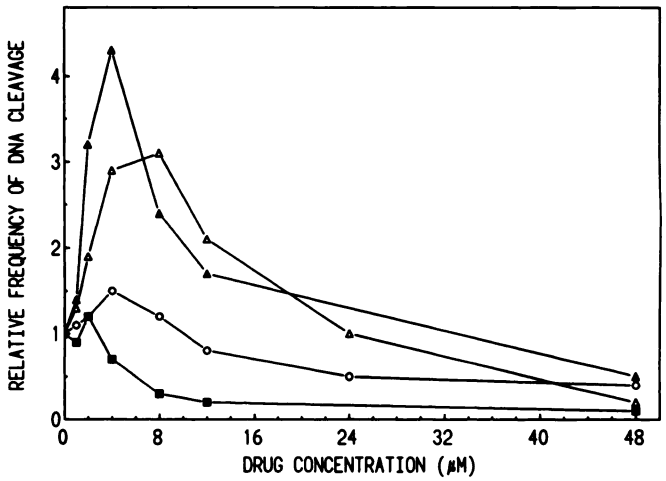


Fig. 2. DNA topoisomerase II-induced DNA cleavage in the presence of 9-substituted ellipticine derivatives. Cumulative frequencies of cleavage in regions 1–3 of pBR322 (as defined in the text) are calculated relative to cumulative frequencies in the absence of drug. ▲, 9-OH-ellipticine; △, 9-NH_2-ellipticine; ○, ellipticine; ■, 9-OCH_3-ellipticine.

bell-shaped curve characteristic of DNA-intercalating compounds (11, 25).

The data shown in Table 1 indicate that the quaternarization of the nitrogen atom at position 2 by a methyl group or a diethylaminoethyl chain does not significantly change the cleavage potency (compare ellipticine with NME and DE and compare 9-OH-ellipticine with NMHE and DHE). For the NME derivatives, substitutions at the 1- or 6-positions do not significantly change the strength of topoisomerase II-mediated DNA cleavage (compare NME with 1-Me-NME and 6-Me-NME). For the NMHE derivatives, (i) addition of a methyl group at position 6 slightly decreases the cleavage potency (compare NMHE with 6-Me-NMHE), (ii) in contrast, addition of a methyl group at position 1 does not decrease the cleavage potency (compare NMHE with 1-Me-NMHE), and (iii) the replacement of a methyl group at position 5 by an ethyl group significantly increases the cleavage potency (compare NMHE and EPC). Furthermore, in contrast to the other ellipticine derivatives (for example, NMHE and 1-Me-NMHE), which stabilize the enzyme-DNA complex over a narrow concentration range, EPC is able to stabilize this complex over a much wider concentration range (data not shown). These observations prompted us to analyze the DNA interaction of three typical NMHE analogues, compared with NMHE.

Interaction of EPC, 1-Me-NMHE, NMHE, and 6-Me-NMHE with DNA. The interaction of these drugs with DNA was studied, in the topoisomerase II standard medium, by competition with the fluorescent DNA-intercalating compound ethidium bromide. The binding constants (K_{app}) and the DNA unwinding angles induced upon drug binding, determined by measuring the change in the linking number of a circular DNA molecule relaxed by topoisomerase I in the presence of the drug at various concentrations (Fig. 3), are presented in Table 2. These data indicate that the replacement of a methyl group at position 5 by an ethyl group increases the DNA cleavage frequency and decreases the DNA binding constant and the unwinding angle.

Relation between DNA cleavage and DNA-bound drug. The previous results led us to study in more detail, for EPC and NMHE, the drug concentration dependence of the cleavage reaction. The relevant parameter in this experiment is the number of drug molecules bound per nucleotide, which can be determined using the value of the binding constant and the drug and DNA concentrations. Unlabeled circular pBR322 DNA was used as substrate because its concentration could be determined accurately. The results shown in Fig. 4 indicate that EPC stimulates cleavage more efficiently and in a wider range of bound drug than does NMHE. Cleavage is maximum for $0.08 \leq r \leq 0.12$ with NMHE and for $0.09 \leq r \leq 0.16$ with EPC (r is the number of molecules of bound drug/nucleotide ratio). Furthermore, the nonlinear variation of cleavage as a function of r in the initial part of the curve, more clearly apparent with EPC than with NMHE, suggests a cooperative effect of the drug for DNA cleavage.

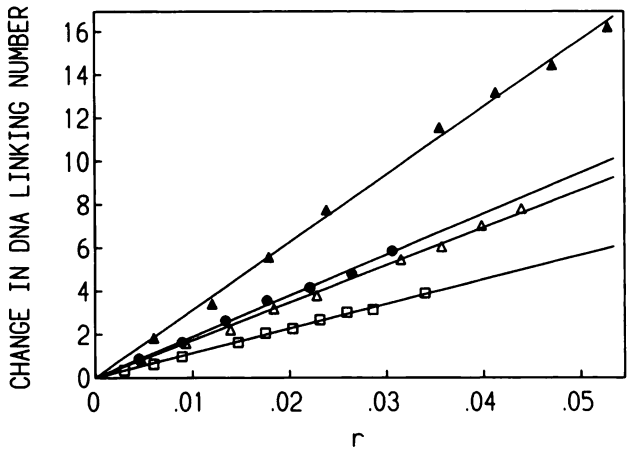


Fig. 3. Unwinding of pSP65 DNA by NMHE (●), 1-Me-NMHE (△), 6-Me-NMHE (▲), and EPC (□) in the topoisomerase II medium. The change in DNA linking number produced by topoisomerase I in the presence of different concentrations of the drugs was determined as described in Materials and Methods. The bound drug/nucleotide molar ratios (r) were calculated using the binding constant values determined for each drug (21).

TABLE 2

Relation between interaction of drugs with DNA and topoisomerase II-mediated DNA cleavage

Compound	K_{app}[a]	Unwinding angle[b]	Relative Frequency of cleavage[c]
	$\times 10^6\ M^{-1}$		
EPC	2.3	7.0°	8.2 ± 1.6
1-Me-NMHE	2.9	10.4°	6.2 ± 1.1
NMHE	5.3	11.5°	4.7 ± 0.2
6-Me-NMHE	14.3	18.7°	3.3 ± 0.3

[a] DNA binding constant.
[b] Unwinding angle of pSP65 DNA per intercalated drug molecule.
[c] Cumulative frequencies of cleavage taken from Table 1.

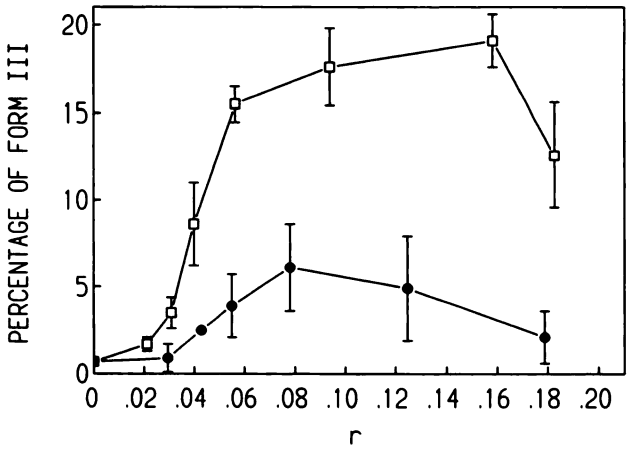


Fig. 4. Extent of DNA topoisomerase II-mediated double-strand cleavage of circular pBR322 DNA as a function of the amount of NMHE (●) or EPC (□) bound to DNA. The bound drug/nucleotide molar ratios (r) were calculated as in Fig. 3. *Error bars*, standard deviations of at least three independent determinations.

Discussion

Previous observations indicate that the presence of an OH group at position 9 of ellipticine derivatives greatly enhances their cytotoxicities and antitumor properties (1). Accordingly, the bioactivation of these derivatives by hydroxylation (26) and of 9-methoxy derivatives by a demethylation reaction (27) is probably involved in the cytotoxicity of these compounds. Concurrently, preliminary data obtained by Tewey *et al.* (11) with two ellipticine derivatives bearing two different substitutions (ellipticine and NMHE) suggested that an OH group in position 9 might be a major factor in the stimulation of topoisomerase II-mediated DNA cleavage. Our data obtained with

five pairs of hydroxylated and nonhydroxylated analogues indicate that the average relative frequency of DNA cleavage increase from 1.6 to 4.4 upon hydroxylation at position 9, supporting the notion that topoisomerase II is the cellular target of ellipticine derivatives. Previous data obtained in our laboratory (25) with γ-carboline derivatives, which are structurally related to ellipticines, show that an 8-OH substitution (which is equivalent to substitution of the 9-position in the ellipticine series), is essential for topoisomerase II-mediated DNA cleavage. Among isoflavone derivatives, an OH substitution is also essential for topoisomerase II-mediated DNA cleavage; genistein efficiently stimulates cleavage, whereas its methoxylated counterpart, biochanin A, does not (28).

Hydroxylation at position 9 of ellipticine derivatives does not affect their ability to intercalate into DNA (29) but does significantly increase their affinity for DNA. Identical ratios of bound drug/nucleotide can be obtained with hydroxylated and nonhydroxylated derivatives by raising the concentration of the latter. However, the relative frequency of DNA cleavage is always lower with the nonhydroxylated derivatives (Fig. 2 and data not shown). We conclude that intercalation is not the only factor to play a role, but stereospecific interactions of the OH group with the DNA-enzyme complex should also be considered in the mechanism of DNA cleavage stimulation. Monnot *et al.* (18) have suggested that the OH group of ellipticine derivatives protrudes into the DNA major groove. This OH group might be hydrogen bonded with the enzyme, contributing to the ternary complex stabilization. By analogy with 9-OH-substituted derivatives, it might be assumed that the NH_2 group of 9-NH_2-ellipticine protrudes into the DNA major groove and forms a hydrogen bond with the enzyme. However, the ternary complex formed probably has a slightly different structure, because the DNA cleavage pattern induced by 9-NH_2-ellipticine is different from that induced by the other ellipticine derivatives. Therefore, the specificity of DNA cleavage, which is mainly determined by the structure of the chromophore, can also be modulated to some extent by certain substitutions on the chromophore. Our data indicate that substitutions at positions 1 and 2 of ellipticine do not significantly affect cleavage frequency. However, for the NMHE derivatives, methylation at position 6 decreases slightly the stimulation of topoisomerase II-mediated DNA cleavage. The replacement of a methyl group at position 5 by an ethyl group increases the cleavage frequency, but cleavage occurs in the same regions, indicating that the specificity of cleavage is unchanged.

The determination of the EPC and NMHE binding constants, in the topoisomerase II standard medium, allowed determination of the number of molecules of bound drug/nucleotide, indicating that the cleavage stimulation is maximum for two to three drug molecules intercalated per turn of the DNA helix. Furthermore, a cooperative effect is clearly apparent in the complex stabilization by EPC (Fig. 4), suggesting an interaction of two drug molecules with the complex, probably one with each enzyme subunit in a symmetrical fashion. Upon addition of a methyl group on the N-6 atom of NMHE, the nitrogen atom becomes positively charged, and the DNA binding constant increases by a factor of about 2.5. The 6-Me-NMHE is also characterized by an increased unwinding angle value and a lower cleavage frequency. More generally, for the four drugs shown in Table 2, there appears to be a decrease of the unwinding angle when the drug-DNA binding constant decreases, as well as a higher cleavage stimulation. This observation suggests that steric factors, contributing to alter the drug-DNA interaction, have a stabilizing effect on the covalent complex, and this leads to the conclusion that the DNA unwinding angle might be a relevant parameter in the analysis of the formation and stability of the enzyme-DNA-drug ternary complex.

Because an ethyl substitution at position 5 of ellipticine derivatives influences their interaction with DNA and DNA-topoisomerase II, it might be interesting to study the effect of other substituents at this position on the DNA binding constant, DNA unwinding angle, and topoisomerase II-mediated DNA cleavage, as well as the pharmacological properties of these derivatives.

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