BIOCHEMISTRY
including biophysical chemistry & molecular biology

ARTICLE

pubs.acs.org/biochemistry

Interactions between the Etoposide Derivative F14512 and Human Type II Topoisomerases: Implications for the C4 Spermine Moiety in Promoting Enzyme-Mediated DNA Cleavage

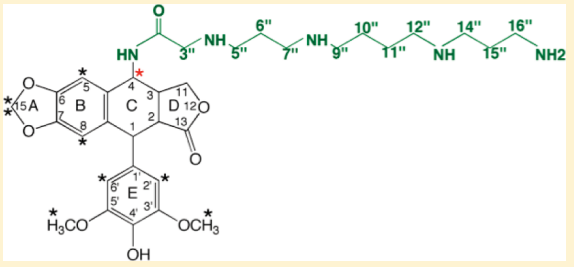
Amanda C. Gentry,[†] Steven L. Pitts,[†] Michael J. Jablonsky,[§] Christian Bailly,[∥] David E. Graves,[§] and Neil Osheroff*[,†,‡]

[†]Department of Biochemistry and [‡]Department of Medicine (Hematology/Oncology), Vanderbilt University School of Medicine, Nashville, Tennessee 37232-0146, United States

[§]Department of Chemistry, University of Alabama at Birmingham, Birmingham, Alabama 35294, United States

[∥]Institut de Recherche Pierre Fabre, 31035 Toulouse cedex 1, France

ABSTRACT: F14512 is a novel etoposide derivative that contains a spermine in place of the C4 glycosidic moiety. The drug was designed to exploit the polyamine transport system that is upregulated in some cancers. However, a preliminary study suggests that it is also a more efficacious topoisomerase II poison than etoposide [Barret et al. (2008) Cancer Res. 68, 9845−9853]. Therefore, we undertook a more complete study of the actions of F14512 against human type II topoisomerases. As determined by saturation transfer difference ^{1}H NMR spectroscopy, contacts between F14512 and human topoisomerase IIα in the binary enzyme−drug complex are similar to those of etoposide. Although the spermine of F14512 does not interact with the enzyme, it converts the drug to a DNA binder [Barret et al. (2008)]. Consequently, the influence of the C4 spermine on drug activity was assessed. F14512 is a highly active topoisomerase II poison and stimulates DNA cleavage mediated by human topoisomerase IIα or topoisomerase IIβ. The drug is more potent and efficacious than etoposide or TOP-53, an etoposide derivative that contains a C4 aminoalkyl group that strengthens drug−enzyme binding. Unlike the other drugs, F14512 maintains robust activity in the absence of ATP. The enhanced activity of F14512 correlates with a tighter binding and an increased stability of the ternary topoisomerase II−drug−DNA complex. The spermine−drug core linkage is critical for these attributes. These findings demonstrate the utility of a C4 DNA binding group and provide a rational basis for the development of novel and more active etoposide-based topoisomerase II poisons.



Etoposide is a widely prescribed anticancer agent that is used as front line therapy to treat a variety of human malignancies.[1−4] The drug is currently in its fourth decade of clinical use and kills cells by stabilizing covalent topoisomerase II-cleaved DNA complexes that are requisite intermediates in the catalytic cycle of the enzyme.[1−8] These transient "cleavage complexes" are converted to permanent DNA strand breaks by collisions with polymerases and other DNA tracking systems, which in turn leads to the accumulation of chromosomal aberrations and triggers cell death pathways.[4,6−11] Because etoposide converts type II topoisomerases to cellular toxins, it is referred to as a topoisomerase II poison.[4−8]

Although regimens that include etoposide generate successful outcomes against a range of cancers, the cytotoxic nature of the drug induces significant side effects, including cardiotoxicity, myelosuppression, and gastrointestinal toxicity.[1,2,12,13] Furthermore, ∼2−3% of patients who are treated with etoposide go on to develop specific secondary leukemias that involve the mixed lineage leukemia (MLL) gene at chromosomal band 11q23.[4,14−16] Since the toxic side effects of etoposide are predominantly mechanism-based (i.e., result from the actions of the drug against topoisomerase II), they are difficult to eliminate. Etoposide and other anticancer drugs affect both isoforms of human topoisomerase II, topoisomerase IIα, and topoisomerase IIβ.[5−8,17] Evidence suggests that topoisomerase IIβ may play a more important role than topoisomerase IIα in mediating off-target toxicities and generating leukemic chromosomal translocations.[18−20]

One way to decrease the off-target toxicity of etoposide and related drugs is to increase their specificity for malignant tissues. To this point, the polyamine transport system is more active in proliferating cells than resting cells, and the uptake of polyamines by some cancer cells is even greater.[21−24] Therefore, in an effort to enhance the uptake of etoposide by cancer cells, F14512 was developed (Figure 1).[25] This derivative replaces the carbohydrate moiety at the C4 position of etoposide, which does not interact with topoisomerase II in the binary enzyme−drug complex,[26,27] with the polyamine spermine. The spermine moiety is conjugated to the etoposide core through a glycyl spacer and was selected for optimal activity among a large series of conjugates.[25]

F14512 is taken up by mammalian cells through the polyamine transport system and, compared to etoposide, displays increased

Received: January 20, 2011
Revised: March 16, 2011
Published: March 17, 2011

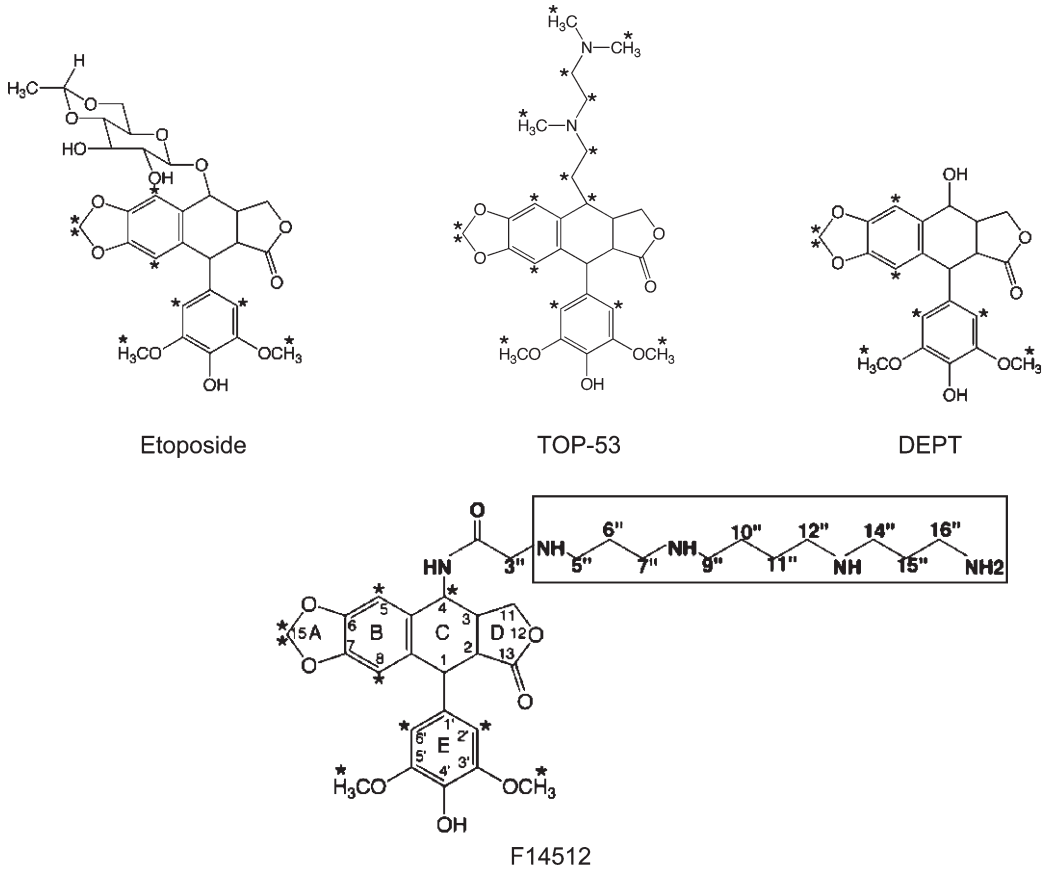


Figure 1. Structure of etoposide and derivatives. The spermine moiety at the C4 position of F14512 is indicated by the box. Asterisks mark protons that interact with topoisomerase II in the binary enzyme−drug complex as determined by STD ^{1}H NMR spectroscopy[26,27] (see Figure 2 and Table 1).

potency against most cancer cell lines.[25] Furthermore, F14512 is more active than the parent compound against a mouse MX-1 human breast tumor xenograft model and displays a higher therapeutic index and lower systemic toxicity.[25] The drug also displays high activity against a variety of other xenograph models, including solid tumors[28] and leukemia,[29] and currently is in phase I clinical trials for the treatment of acute myeloid leukemia.

Although the spermine moiety originally was attached to F14512 in order to change the cellular uptake properties of etoposide, the inclusion of the polyamine had two additional effects.[25] First, it altered the DNA binding properties of the compound. While etoposide displays little interaction with DNA (in the absence of topoisomerase II),[30,31] F14512 is a DNA binder, most likely interacting with the double helix through the minor groove.[25] Second, as determined by preliminary *in vitro* studies, F14512 appears to be a more efficacious topoisomerase II poison than etoposide.[25]

Given the high activity of F14512 in preclinical cancer models, it is important to understand how the drug interacts with potential cellular targets. Therefore, the effects of the C4 spermine moiety on drug function against human type II topoisomerases were analyzed. Results indicate that the C4 polyamine increases the potency and efficacy of the drug against both isoforms of human topoisomerase II but does not change the fundamental mechanism of etoposide action (i.e., inhibition of DNA ligation). Rather, the linkage between the spermine and the drug core appears to enhance drug binding and the stability of etoposide in the ternary enzyme−drug−DNA complex.

EXPERIMENTAL PROCEDURES

Enzymes and Materials. Human topoisomerase IIα and topoisomerase IIβ were expressed in *Saccharomyces cerevisiae*[32] and purified as described previously.[33] Human topoisomerase I was purchased from Topogen. Negatively supercoiled pBR322 DNA was prepared from *Escherichia coli* using a Plasmid Mega Kit (Qiagen) as described by the manufacturer. Positively supercoiled pBR322 DNA was prepared by incubating negatively supercoiled plasmid with *Archaeoglobus fulgidus* reverse gyrase as described by McClendon et al.[34] Positively and negatively supercoiled plasmids contained an equivalent number of superhelical twists but were of opposite handedness. [γ-^{32}P]ATP (~5000 Ci/mmol) was obtained from NEN. Etoposide and ciprofloxacin were from Sigma. TOP-53 was a gift from Taiho Pharmaceuticals. F14512 (patent WO 2005/100363) was synthesized as described previously.[25] 4′-Demethylepipodophyllotoxin (DEPT) was the gift of Dr. Norma Dunlap (Middle Tennessee State University). Etoposide, TOP-53, DEPT, and F14512 were stored at 4 °C as 20 mM stock solutions in 100% DMSO. Ciprofloxacin was stored at −20 °C as a 40 mM stock solution in 0.1 N NaOH. All other chemicals were analytical reagent grade.

STD ^{1}H NMR Spectroscopy. NMR spectra were generated using conditions similar to those described previously.[26,27] All NMR experiments were performed at 283 K using a Bruker Avance 700 MHz spectrometer equipped with a 5 mm cryoprobe with *z* gradients. NMR buffers contained 10 mM sodium phosphate (pH 7.7), 250 mM KCl, 0.1 mM Na_2EDTA, and

5 mM $MgCl_2$. Samples (400 μL) contained 5 μM human topoisomerase IIα and 500 μM F14512 and were maintained at 4 °C until data were collected. STD ^{1}H NMR experiments employed a pulse scheme similar to that reported by Mayer and Meyer.[35] A 2 s saturation pulse was used for the saturation, with on- and off-resonance irradiation frequencies of 0.5 and −71 ppm, respectively. The water signal was suppressed using excitation sculpting with gradients. For each experiment (on- and off-resonance irradiation), 256 scans were collected with a 2 s recycle time. Difference spectra were prepared by subtracting the on-resonance spectrum from the off-resonance spectrum. Signals resulting in the difference spectrum represent the NOE difference signals generated by the transfer of irradiation energy from the enzyme to the bound ligand. Ligand protons in close spatial proximity with the enzyme displayed larger NOE signals. Mapping of the NOE signals with their proton assignments on the ligand revealed the ligand-binding epitope to human topoisomerase IIα. Spectra were processed using Bruker Topspin software.

Plasmid DNA Cleavage. DNA cleavage reactions were carried out using the procedure of Fortune and Osheroff.[36] Topoisomerase II DNA cleavage assays contained 110 nM human topoisomerase IIα or 220 nM human topoisomerase IIβ and 10 nM negatively or positively supercoiled pBR322 in a total of 20 μL of DNA cleavage buffer [10 mM Tris-HCl (pH 7.9), 5 mM $MgCl_2$, 100 mM KCl, 0.1 mM EDTA, and 2.5% (v/v) glycerol]. Assays were carried out in the absence of compound or in the presence of 0−100 μM etoposide, TOP-53, DEPT, spermine, or F14512, or in the presence of a 1:1 mixture of etoposide + spermine or DEPT + spermine. Some reactions were carried out in the presence of 1 mM ATP or APP(NH)P. Competition experiments also contained 0−1000 μM ciprofloxacin. Reaction mixtures were incubated at 37 °C for 6 min, and enzyme−DNA cleavage complexes were trapped by the addition of 2 μL of 5% SDS followed by 2 μL of 250 mM EDTA (pH 8.0). Proteinase K (2 μL of a 0.8 mg/mL solution) was added, and samples were incubated at 45 °C for 30 min to digest the enzyme. Samples were mixed with 2 μL of agarose gel loading buffer [60% sucrose in 10 mM Tris−HCl (pH 7.9)], heated at 45 °C for 5 min, and subjected to electrophoresis in 1% agarose gels in 40 mM Tris-acetate (pH 8.3) and 2 mM EDTA containing 0.5 μg/mL ethidium bromide. DNA bands were visualized with long-range ultraviolet light and quantified using an Alpha Innotech digital imaging system. DNA cleavage was monitored by the conversion of supercoiled plasmid DNA to linear molecules.

DNA Cleavage Site Utilization. DNA cleavage sites were mapped using a modification[37] of the procedure of O'Reilly and Kreuzer.[38] The pBR322 DNA substrate was linearized by treatment with *Hin*dIII. Terminal 5′-phosphates were removed by treatment with calf intestinal alkaline phosphatase and replaced with [^{32}P]phosphate using T4 polynucleotide kinase and [γ-^{32}P]ATP. The DNA was treated with *Eco*RI, and the 4332 bp singly end-labeled fragment was purified from the small *Eco*RI-*Hin*dIII fragment by passage through a CHROMA SPIN+TE-100 column (Clontech). Reaction mixtures contained 4 nM labeled pBR322 DNA substrate and 110 nM human topoisomerase IIα or 220 nM topoisomerase IIβ in 20 μL of DNA cleavage buffer supplemented with 0.5 mM ATP in the absence or presence of 5−10 μM etoposide, 5−10 μM TOP-53, or 1−5 μM F14512. Reaction mixtures were incubated at 37 °C for 6 min, and enzyme−DNA cleavage complexes were trapped by the addition of 2 μL of 5% SDS followed by 2 μL of 250 mM EDTA (pH 8.0). Proteinase K (2 μL of a 0.8 mg/mL solution) was added, and samples were incubated at 45 °C for 30 min to digest the enzyme. DNA products were ethanol precipitated and resuspended in 6 μL of 40% formamide, 10 mM NaOH, 0.02% xylene cyanol FF, and 0.02% bromophenol blue. Samples were subjected to electrophoresis in denaturing 6% polyacrylamide sequencing gels. Gels were dried in vacuo, and DNA cleavage products were visualized with a Bio-Rad Molecular Imager FX.

DNA Ligation. DNA ligation mediated by topoisomerase IIα was monitored according to the procedure of Byl et al.[39] DNA cleavage/ligation equilibria were established for 6 min at 37 °C in the absence or presence of 50 μM etoposide, 50 μM TOP-53, or 10 μM F14512. Ligation was initiated by shifting samples from 37 to 0 °C. Reactions were stopped at time points ranging from 0 to 30 s by the addition of 2 μL of 5% SDS followed by 2 μL of 250 mM EDTA (pH 8.0). Samples were mixed with 2 μL of agarose gel loading buffer and processed and analyzed as described under plasmid DNA cleavage. Linear DNA cleavage product at time zero was set to 100%, and DNA ligation was monitored by the loss of linear DNA.

DNA Intercalation. DNA intercalation was monitored as described previously.[40] Intercalation reaction mixtures contained 20 nM topoisomerase I, 5 nM pBR322 DNA, and 0−100 μM F14512, 20 μM ethidium bromide, or 50 μM etoposide, in a total of 20 μL of 50 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 50 mM KCl, 10 mM $MgCl_2$, and 0.5 mM DTT. Mixtures were incubated at 37 °C for 10 min, extracted with a phenol/chloroform/isoamyl alcohol mixture (25:24:1), and added to 3 μL of 0.77% SDS and 77 mM EDTA (pH 8.0). Samples were mixed with 2 μL of agarose gel loading buffer, heated at 45 °C for 5 min, and subjected to electrophoresis in a 1% agarose gel in 100 mM Tris-borate (pH 8.3) and 2 mM EDTA. Gels were stained with 1 μg/mL ethidium bromide, and DNA bands were visualized as described for plasmid DNA cleavage.

Persistence of Ternary Topoisomerase IIα−Drug−DNA Complexes. The persistence of topoisomerase IIα−drug−DNA complexes was determined using a modification of the procedure of Bandele et al.[41] Initial reactions contained 50 nM DNA, 550 nM topoisomerase IIα, and 100 μM etoposide or 10 μM F14512 in a total of 20 μL of DNA cleavage buffer. Reactions were incubated at 37 °C for 6 min and then diluted 20-fold with DNA cleavage buffer at 37 °C. Samples (20 μL) were removed at times ranging from 0 to 150 min, and DNA cleavage was stopped with 2 μL of 5% SDS. Samples were processed as described above for plasmid cleavage assays. Levels of DNA cleavage were set to 100% at time zero, and the persistence of cleavage complexes was determined by the decay of linear reaction product over time.

RESULTS AND DISCUSSION

Interactions between F14512 and Human Topoisomerase IIα. Previous studies indicate that interactions between topoisomerase II and etoposide are critical for drug activity and mediate the entry of etoposide into the ternary enzyme−drug−DNA complex.[26,27,42,43] Furthermore, drug contacts in the binary topoisomerase II−drug complex, including the C15 geminal protons of the A-ring, the C5 and C8 protons of the B-ring, and the C2′ and C6′ protons and the 3′- and 5′-methoxyl protons of the pendant E-ring (as determined by STD ^{1}H NMR spectroscopy) have predictive value for the actions of etoposide within the ternary drug−enzyme−DNA complex.[26,27,44,45] Indeed, alterations of A-ring and E-ring substituents dramatically

Table 1. Drug Substituents That Interact with Human Topoisomerase IIα in the Binary Enzyme−Drug Complex As Determined by STD ^{1}H NMR Spectroscopy[a]

etoposide		F14512	
substituent proton	resonance	substituent proton	resonance
1	4.39	1	4.52
2	3.33	2	3.18
3	2.83	3	2.99
4	4.82	**4**	**5.00**
5	**6.70**	**5**	**6.61**
8	**6.32**	**8**	**6.32**
11R, 11S	4.15, 4.20	11R, 11S	3.90, 4.30
15R, 15S	**5.70**	**15R, 15S**	**5.70**
2′, 6′	**6.18**	**2′, 6′**	**6.17**
3′, 5′-OCHH$_3$	**3.49**	**3′, 5′-OCHH$_3$**	**3.49**
1″	4.43	3″	3.78
2″	3.07	5″	2.97
3″	3.33	6″	2.05
4″	3.17	7″	3.04
5″	3.24	9″, 12″	2.90
6″, 6″	3.42, 4.01	10″, 11″	1.72
7″	4.70	14″, 16″	3.07
-CH$_3$	1.10	15″	1.97

[a] Resonances that display nuclear Overhauser effects in STD ^{1}H NMR spectroscopy experiments along with the substituent protons that they represent are indicated in bold.

decrease the efficacy of etoposide against topoisomerase II. In contrast, removal of the C4 glycosidic group (which generates DEPT, Figure 1) has relatively little effect on the ability of the drug to poison topoisomerase II.

F14512 shares an identical core (rings A−E) with etoposide but contains a spermine group in place of the glycosidic moiety at the C4 position (Figure 1). Therefore, as a first step in characterizing the activity of the compound against human type II topoisomerases, we used STD ^{1}H NMR spectroscopy to define the substituents on F14512 that contact topoisomerase IIα in the binary enzyme−drug complex. With the exception of one additional contact with the enzyme (the C4 proton of the C-ring) the substituents on the drug core that contact topoisomerase IIα in the binary complex were identical to those previously described for etoposide (Table 1 and Figure 2).[26,27] It is notable that no significant nuclear Overhauser enhancement (NOE) signals were observed for any of the protons of the spermine moiety of F14512. This result suggests that there is limited interaction, if any, between this portion of F14512 and the enzyme.

Although the C4 substituents of etoposide and F14512 do not interact with topoisomerase IIα, it is possible to substitute this position with a protein-binding group. To this point, TOP-53 is a derivative of etoposide that contains a C4 aminoalkyl side chain (Figure 1).[46] In contrast to results with etoposide or F14512, every proton associated with this side chain of TOP-53 contacts topoisomerase IIα in the binary complex.[26] Furthermore, the presence of the aminoalkyl group increases the binding affinity of TOP-53 for topoisomerase II and significantly enhances the potency and efficacy of the drug against the type II enzyme (Figure 3).[26,47]

In contrast to the C4 aminoalkyl side chain of TOP-53, the spermine moiety of F14512 interacts with DNA and converts the drug to a DNA binder.[25] Taken together, these results suggest that the C4 substituents of compounds in the etoposide family have the potential to influence drug activity by two distinct mechanisms: enhanced binding to the enzyme or enhanced binding to DNA. Consequently, we wanted to more fully investigate the influence of the C4 spermine on F14512 activity.

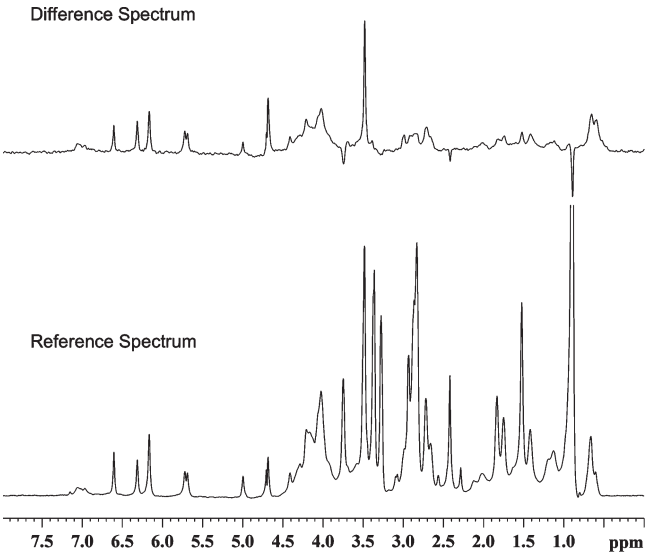


Figure 2. Interaction of F14512 with human topoisomerase IIα as determined by STD ^{1}H NMR spectroscopy. Difference (top) and reference (off-resonance, bottom) spectra are shown. Spectra are representative of at least two independent experiments.

F14512 Poisons Human Type II Topoisomerases. Preliminary results using human topoisomerase IIα suggest that F14512 is a more efficacious poison than etoposide.[25] Therefore, to examine the activity of F14512 in greater detail, the effects of the drug on DNA cleavage mediated by human type II topoisomerases were compared to those of etoposide and TOP-53. As seen in Figure 3, F14512 was severalfold more potent and more efficacious than either drug. Similar results were observed with topoisomerase IIα (left panel) and topoisomerase IIβ (right panel). In all cases, F14512 generated the highest levels of double-stranded, single-stranded, and total topoisomerase II-mediated DNA strand breaks. These results demonstrate that the inclusion of the DNA-binding C4 spermine in F14512 has a marked effect on drug activity. Furthermore, this effect is even greater than that generated by the enzyme-binding aminoalkyl group in TOP-53.

It is notable that F14512 is considerably more active against topoisomerase IIα than topoisomerase IIβ. Comparable or higher levels of DNA scission were observed with the α isoform, despite the fact that the enzyme concentration employed was only one-half that used in topoisomerase IIβ assays (Figure 3). Furthermore, at concentrations of F14512 that exceeded 15 μM, multiple cleavage events per plasmid were observed with topoisomerase IIα, such that it became impossible to accurately quantify levels of double-stranded DNA breaks.

Eukaryotic type II topoisomerases are homodimeric proteins.[48−50] Each protomer active site of the enzyme cuts one strand of the DNA to generate a double-stranded break.[51] The two enzyme protomers are not fully coordinated and, as a result, also create single-stranded breaks.[52−54] The double-stranded:single-stranded cleavage ratio differs between drug classes, with etoposide yielding

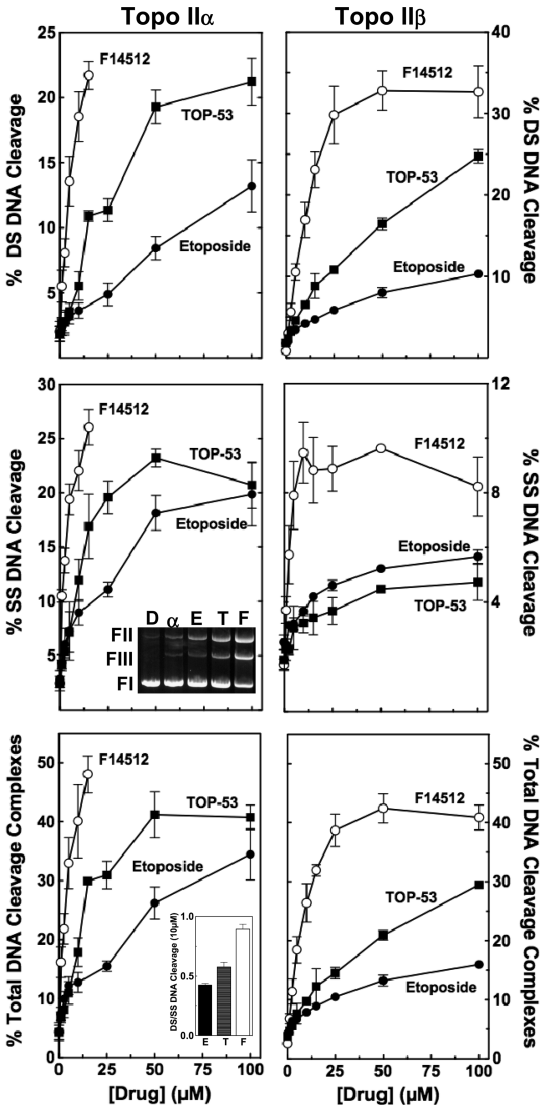


Figure 3. F14512 is a potent topoisomerase II poison. The effects of etoposide (closed circles), TOP-53 (closed squares), and F14512 (open circles) on the cleavage of negatively supercoiled pBR322 plasmid DNA by human topoisomerase IIα (left panels) and topoisomerase IIβ (right panels) were determined. The percentage of double-stranded (top panels), single-stranded (middle panels), and total DNA cleavage complexes (bottom panels) is shown. Error bars represent the standard deviation of three independent experiments. The inset in the left middle panel shows an agarose gel of topoisomerase IIα-mediated cleavage of negatively supercoiled pBR322 plasmid DNA in the absence of drug (α) or in the presence of 15 μM etoposide (E), TOP-53 (T), or F14512 (F). A DNA standard (D) also is shown. The positions of supercoiled (form I, FI), nicked circular (form II, FII), and linear (form III, FIII) molecules are indicated. The inset in the bottom left panel shows the ratio of double-stranded:single-stranded (DS/SS) breaks generated by topoisomerase IIα in the presence of 10 μM etoposide, TOP-53, or F14512.

a relatively low ratio (~0.5:1 at 10 μM drug) with topoisomerase IIα (Figure 3, lower left panel inset).[52] Of the three drugs, F14512 induced the highest ratio of double-stranded:single-stranded breaks (~1:1 at 10 μM drug with topoisomerase IIα) (Figure 3, lower left panel inset).

Type II topoisomerases require ATP binding and hydrolysis for overall catalytic activity.[55−57] Several studies have demonstrated that etoposide displays maximal activity only in the presence of

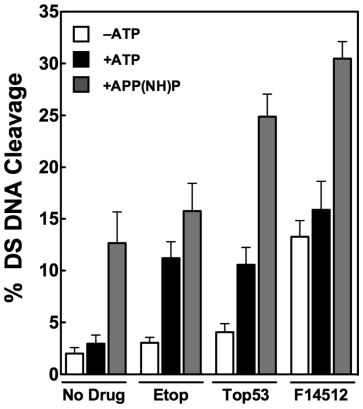


Figure 4. Effects of ATP on drug-stimulated DNA cleavage mediated by human topoisomerase IIα. Assays were carried out in the absence of ATP (closed bars) or in the presence of 1 mM ATP (open bars) or the nonhydrolyzable ATP analogue APP(NH)P (gray bars). Reactions contained no drug (ND) or 5 μM etoposide, TOP-53, or F14512. Error bars represent the standard deviation of three independent experiments.

a high-energy cofactor,[58] although the mechanistic basis for this property is not understood. As seen in Figure 4, levels of topoisomerase IIα-mediated DNA cleavage induced by etoposide and TOP-53 drop ~3-fold and ~2-fold, respectively, in the absence of ATP. In contrast, F14512 was far better at maintaining activity in the absence of ATP, with cleavage levels declining only 15% in assays that lacked the cofactor.

Drug-induced DNA cleavage also was assessed in the presence of the nonhydrolyzable ATP analogue APP(NH)P. This analogue allows type II topoisomerases to go through one round of DNA strand passage but does not support enzyme turnover.[56] Once again, the highest levels of DNA cleavage were observed in the presence of F14512 as compared to etoposide or TOP-53. Furthermore, the spermine-linked drug displayed the least dependence (~2-fold) on APP(NH)P for its enhanced activity as compared to the other drugs (~5−6-fold higher levels of cleavage in the presence of the nonhydrolyzable ATP analogue) (Figure 4).

Because the C4 spermine moiety of F14512 interacts with DNA, it is possible that it alters the cleavage site specificity of topoisomerase IIα and topoisomerase IIβ. In order to assess this issue, we determined the DNA sites that are cleaved by the human enzymes in the presence of etoposide, TOP-53, and F14512 (Figure 5). A similar (but not identical) array of sites was cleaved with all three drugs. This finding suggests that the addition of the spermine moiety to the etoposide core does not promote site-specific DNA interactions and is consistent with the general DNA properties of the polyamine. A similar conclusion was reached in an earlier study that examined a more limited sampling of DNA sites cleaved by topoisomerase IIα.[25]

Etoposide increases levels of covalent topoisomerase II−DNA cleavage complexes by inhibiting the ability of the enzyme to ligate DNA breaks.[59,60] Therefore, a ligation assay was employed to determine whether the inclusion of the spermine moiety alters the mechanistic basis for drug action. As seen in Figure 6, etoposide, TOP-53, and F14512 are all potent inhibitors of DNA ligation mediated by human topoisomerase IIα. Given the strong inhibition, it is not possible to determine whether the higher levels of DNA cleavage generated by F14512 reflect an enhanced inhibition of the ligation reaction.

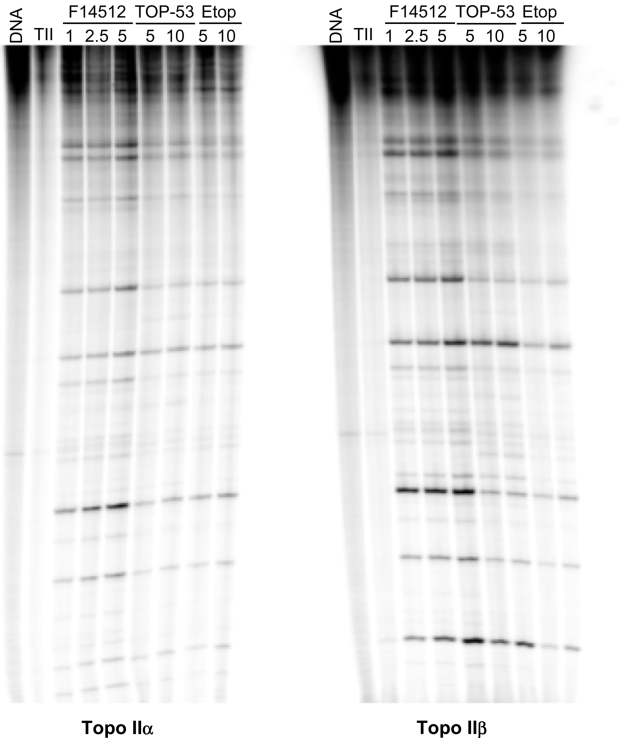


Figure 5. Effects of etoposide derivatives on sites of DNA cleavage mediated by human topoisomerase IIα (left) and topoisomerase IIβ (right). An autoradiogram of a polyacrylamide gel is shown. DNA cleavage reactions were carried out in the absence of drug (TII) or in the presence of the indicated concentrations of F14512, TOP-53, or etoposide. A DNA standard (DNA) also is shown. Results are representative of three independent experiments.

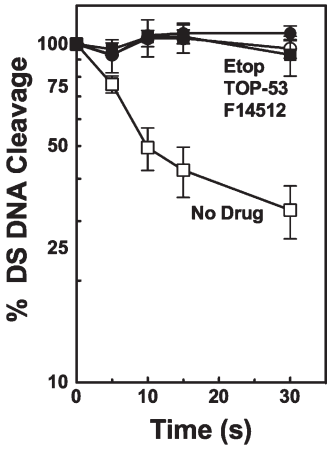


Figure 6. Effects of etoposide derivatives on DNA ligation mediated by human topoisomerase IIα. Reactions were carried out in the absence of drug (open squares) or in the presence of 50 μM etoposide (closed circles), 50 μM TOP-53 (closed squares), or 10 μM F14512 (open circles). Ligation is expressed as the percent loss of linear DNA, which was set to 100% at time zero. Error bars represent the standard deviation of three independent experiments.

F14512−DNA Interactions Are Nonintercalative. It has long been known that etoposide binds weakly if at all to DNA in the absence of topoisomerase II.[30,31] In contrast, a previous study that assessed changes in DNA melting temperatures, as

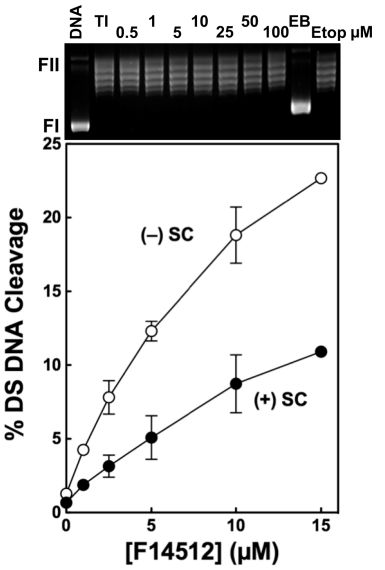


Figure 7. F14512 is not a DNA intercalator. The ability of F14512 to intercalate into negatively supercoiled pBR322 plasmid DNA was determined using a topoisomerase I relaxation assay (top). An agarose gel is shown. DNA relaxation was carried out in the absence of drugs (TI) or in the presence of 0−100 μM F14512, 20 μM ethidium bromide (EB), or 20 μM etoposide (Etop). A DNA standard (DNA) also is shown. The gel is representative of three independent experiments. The bottom panel shows the effects of F14512 on the ability of human topoisomerase IIα to cleave negatively (open circles) and positively (closed circles) supercoiled pBR322 plasmid molecules. Error bars represent the standard deviation of three independent experiments.

well as the ultraviolet and circular dichroism spectra of DNA, concluded that F14512 is a DNA binding drug.[25] While changes in circular dichroism suggest that F14512 binds to the double helix through the minor groove, the study did not determine the DNA binding mode of the drug.[25]

Another study found that attachment of spermine to anthracene (which is a poor DNA intercalator) generated an intercalative drug.[61] Therefore, two experiments were carried out to determine whether F14512 intercalates into DNA. The first experiment utilized a topoisomerase I-DNA relaxation assay to monitor intercalation. This assay is based on the fact that intercalative agents induce constrained negative supercoils and compensatory unconstrained positive superhelical twists in covalently closed circular DNA.[17,40,62] Therefore, as the concentration of an intercalative compound increases, a plasmid that is negatively supercoiled or relaxed (i.e., contains no superhelical twists) appears to become positively supercoiled. Treatment of an intercalated plasmid with topoisomerase I removes the unconstrained positive DNA superhelical twists. Subsequent extraction of the compound allows the local drug-induced unwinding to redistribute in a global manner and manifest itself as a net negative supercoiling of the plasmid. Thus, in the presence of an intercalative agent, topoisomerase treatment converts relaxed plasmids to negatively supercoiled molecules. As seen in Figure 7 (top), F14512 displayed no ability to intercalate over the concentration range that promoted topoisomerase II-mediated DNA cleavage (0−100 μM).

The second experiment takes advantage of the finding that topoisomerase IIα maintains 2−3-fold higher levels of DNA cleavage complexes with negatively supercoiled DNA as compared to

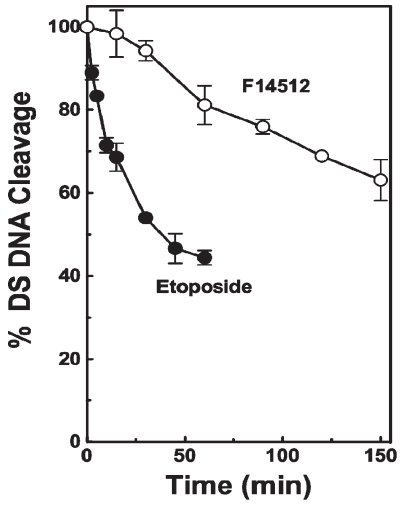


Figure 8. Effects of etoposide derivatives on the persistence of ternary topoisomerase IIα−drug−DNA cleavage complexes. Assays were carried out in the presence of 100 μM etoposide (closed circles) or 10 μM F14512 (open circles) and negatively supercoiled pBR322 plasmid DNA. After initial reaction mixtures attained DNA cleavage−ligation equilibria, they were diluted 20-fold with DNA cleavage buffer. The persistence of cleavage complexes was assessed by monitoring the loss of double-stranded DNA breaks (linear product) over time. Cleavage at time zero was set to 100%. Error bars represent the standard error of two independent assays.

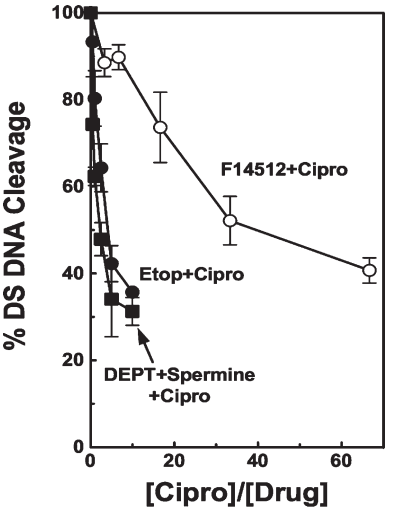


Figure 9. F14512 binds more tightly than etoposide in the ternary topoisomerase IIα−drug−DNA complex. A ciprofloxacin competition assay was employed. DNA cleavage complexes were established in the presence of etoposide (Etop, closed circles), F14512 (open circles), or a 1:1 mixture of DEPT + spermine (closed squares) and 0−1000 μM ciprofloxacin (Cipro). Levels of DNA cleavage generated by human topoisomerase IIα in the presence of ciprofloxacin alone were subtracted from those seen in the presence of etoposide derivatives + ciprofloxacin. Cleavage in the absence of ciprofloxacin was set to 100%, and results are presented as a ratio of ciprofloxacin concentration to etoposide derivative. Error bars represent the standard deviation of three independent experiments.

positively supercoiled molecules.[34,63] While this relationship is maintained over a broad concentration range in the presence of noncintercalative topoisomerase II poisons, it does not hold when intercalative drugs are utilized.[63] In this latter case, cleavage levels of negatively supercoiled plasmids relative to positively supercoiled substrates drop as the concentration of intercalator increases. Over the concentration range of F14512 examined, the percent cleavage of negatively supercoiled plasmids remained 2−3-fold higher than that seen with the corresponding positively supercoiled substrate (Figure 7, bottom). These findings provide strong evidence that F14512 does not utilize an intercalative mode to bind DNA.

Enhanced Stability and Tighter Drug Binding in the Ternary Topoisomerase IIα−F14512−DNA Complex. F14512 displays similar contacts as etoposide with human topoisomerase IIα in the binary enzyme−drug complex (see Table 1 and Figure 2) but has additional interactions with the double helix in the binary DNA−drug complex.[25] Since F14512 is a more potent topoisomerase II poison than etoposide, we wanted to see if these DNA interactions contribute to an enhanced stability or a tighter drug binding of the spermine-linked drug in the ternary topoisomerase IIα−drug−DNA complex. Two approaches were used to address these issues. In the first, the persistence of DNA cleavage complexes[41] established in the presence of F14512 or etoposide was determined. This was accomplished by establishing DNA cleavage−ligation equilibria in the presence of 10 μM F14512 or 100 μM etoposide, diluting reaction mixtures 20-fold, and monitoring the decay of cleavage complexes over time. As seen in Figure 8, cleavage complexes formed in the presence of F14512 persisted 5−10-fold longer than equivalent complexes induced by etoposide. This suggests that F14512 forms a more stable ternary complex than etoposide.

In the second approach, the ability of the quinolone ciprofloxacin to compete with F14512 or etoposide for binding in the ternary complex was determined. Ciprofloxacin is an antibacterial topoisomerase II poison[64,65] that displays little ability to stimulate DNA cleavage mediated by the eukaryotic type II enzyme.[66] However, the quinolone interacts with eukaryotic topoisomerase II and shares a binding site with anticancer drugs.[66] Thus, it can inhibit the ability of these drugs to enhance DNA scission.[66]

As seen in Figure 9, ciprofloxacin competed with both F14512 and etoposide, but the ciprofloxacin:F14512 ratio required to decrease DNA cleavage was 5−10-fold higher than seen with etoposide. These experiments indicate that F14512 binds more tightly than etoposide in the ternary enzyme−drug−DNA complex. The enhanced stability of cleavage complexes formed in the presence of F14512 and the tighter drug binding provide a probable basis for the greater efficacy and potency of F14512 compared to etoposide.

Importance of the Spermine−Drug Linkage to the Enhanced Activity of F14512 against Topoisomerase IIα. Experiments in the previous section suggest that the spermine-mediated binding of F14512 to DNA is responsible for the enhanced activity of the drug. However, spermine−DNA interactions also neutralize nucleic acid charge and allow condensation of the double helix.[67−69] Furthermore, polyamines such as spermine and spermidine can alter interactions between type II topoisomerases and DNA and are used routinely as DNA condensation agents to convert intramolecular to intermolecular DNA strand passage reactions (i.e., DNA relaxation to catenation).[70]

Therefore, to determine whether the spermine moiety alters F14512 activity by a specific effect on drug interactions in the ternary complex or by a general effect on DNA charge/structure, we characterized the importance of the spermine−drug linkage to the activity of F14512. As a first step, drug binding in the ternary enzyme−drug−DNA complex was assessed when the complex was formed in the simultaneous presence of the unlinked etoposide core (i.e., DEPT, see Figure 1) and spermine. As assessed by

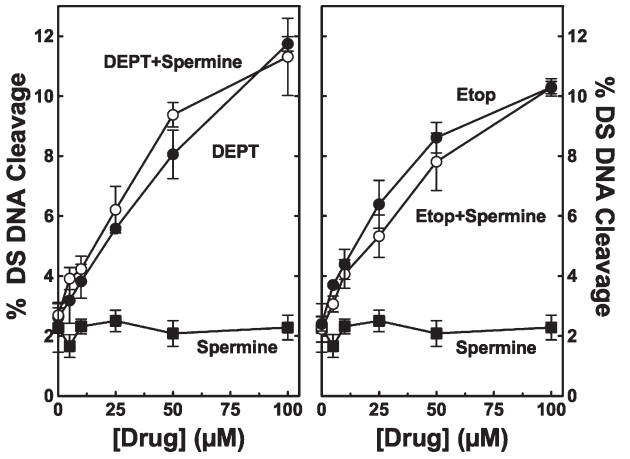


Figure 10. Covalent linkage of the C4 spermine moiety to the etoposide core is necessary for the enhanced DNA cleavage seen in the presence of F14512. The ability of DEPT (closed circles) or a 1:1 mixture of DEPT + spermine (open circles) (left panel), and etoposide (Etop, closed circles) or a 1:1 mixture of etoposide + spermine (open circles) (right panel) to enhance topoisomerase IIα-mediated DNA cleavage is shown. Control experiments assessing the effects of spermine alone on the DNA cleavage activity of topoisomerase IIα (closed squares) are shown in both panels. Error bars represent the standard deviation of three independent experiments.

the ciprofloxacin competition assay (Figure 9), the affinity of a 1:1 DEPT:spermine mixture for the topoisomerase IIα–DNA complex was similar to that of etoposide and was ~10-fold lower than that of F14512. This finding indicates that the drug–spermine linkage is critical for the tighter binding of F14512 in the ternary complex.

The effects of the drug–spermine linkage on the activity of F14512 also were determined. As seen in Figure 10, spermine (up to 100 μM) had little influence on levels of DNA cleavage mediated by human topoisomerase IIα.[71] Moreover, DNA cleavage enhancement observed in the presence of a 1:1 DEPT:spermine mixture was comparable to that of the drug core in the absence of the polyamine. A similar result was seen comparing a 1:1 etoposide:spermine mixture to the activity of etoposide alone. Taken together, these results provide strong evidence that, in the concentration ranges examined, spermine does not enhance topoisomerase II-mediated DNA cleavage by a general effect on DNA charge or structure. Furthermore, the drug–spermine linkage is critical for the high activity of F14512.

In conclusion, F14512 is a novel etoposide derivative that contains a spermine group in place of the C4 glycosidic moiety.[25] The presence of the spermine enhances the selectivity of the drug in cancers that overexpress an active polyamine transport system.[25,28,29] In addition, F14512 is a more potent and efficacious topoisomerase II poison than etoposide, and this enhanced activity correlates with tighter drug binding and an increased stability of the ternary topoisomerase II–drug–DNA complex. The linkage between the drug core and spermine, which converts etoposide to a DNA binder,[25] is critical for the enhanced activity of F14512. While the A-, B-, and E-ring stabilize drug binding through protein interactions, we suggest that polyamine–DNA interactions provide a second anchor for the drug in the ternary complex.

The present findings highlight the utility of a C4 DNA binding group and provide a rational basis for the development of novel and more active etoposide-based topoisomerase II poisons. Finally, the dual function of the C4 spermine moiety (i.e., enhanced cellular uptake in cancers with active polyamine transport and greater activity against topoisomerase II) supports the ongoing clinical development of F14512.

AUTHOR INFORMATION

Corresponding Author

*Tel: 615-322-4338. Fax: 615-343-1166. E-mail: neil.osheroff@vanderbilt.edu.

Funding Sources

This work was supported by National Institutes of Health research grant GM33944 (to N.O.) and National Science Foundation Grant MCB-0334785 (to D.E.G.). A.C.G. was a trainee under grant T32 CA09582 from the National Institutes of Health.

ACKNOWLEDGMENT

We are grateful to Adam C. Ketron and Katie J. Aldred for critical reading of the manuscript. We also thank Dr. Clemens Anklin (Bruker) for his invaluable assistance with developing the protocols for the STD ^{1}H NMR spectroscopy. The contributions of the CROE/IRPF members to the synthesis and research on F14512 also are acknowledged.

ABBREVIATIONS

DEPT, 4′-demethylepipodophyllotoxin; STD, saturation transfer difference; NOE, nuclear Overhauser enhancement.

REFERENCES

(1) Hande, K. R. (1998) Etoposide: four decades of development of a topoisomerase II inhibitor. *Eur. J. Cancer 34*, 1514–1521.

(2) Hande, K. R. (1998) Clinical applications of anticancer drugs targeted to topoisomerase II. *Biochim. Biophys. Acta 1400*, 173–184.

(3) Holden, J. A. (2001) DNA topoisomerases as anticancer drug targets: from the laboratory to the clinic. *Curr. Med. Chem. Anti-Cancer Agents 1*, 1–25.

(4) Baldwin, E. L., and Osheroff, N. (2005) Etoposide, topoisomerase II and cancer. *Curr. Med. Chem. Anti-Cancer Agents 5*, 363–372.

(5) Pommier, Y., and Marchand, C. (2005) Interfacial inhibitors of protein-nucleic acid interactions. *Curr. Med. Chem. Anti-Cancer Agents 5*, 421–429.

(6) McClendon, A. K., and Osheroff, N. (2007) DNA topoisomerase II, genotoxicity and cancer. *Mut. Res. 623*, 83–97.

(7) Deweese, J. E., and Osheroff, N. (2009) The DNA cleavage reaction of topoisomerase II: wolf in sheep's clothing. *Nucleic Acids Res. 37*, 738–748.

(8) Nitiss, J. L. (2009) Targeting DNA topoisomerase II in cancer chemotherapy. *Nat. Rev. Cancer 9*, 338–350.

(9) Baguley, B. C., and Ferguson, L. R. (1998) Mutagenic properties of topoisomerase-targeted drugs. *Biochim. Biophys. Acta 1400*, 213–222.

(10) Kaufmann, S. H. (1998) Cell death induced by topoisomerase-targeted drugs: more questions than answers. *Biochim. Biophys. Acta 1400*, 195–211.

(11) Sordet, O., Khan, Q. A., Kohn, K. W., and Pommier, Y. (2003) Apoptosis induced by topoisomerase inhibitors. *Curr. Med. Chem. Anti-Cancer Agents 3*, 271–290.

(12) Seiter, K. (2005) Toxicity of the topoisomerase II inhibitors. *Expert Opin. Drug Saf. 4*, 219–234.

(13) Sinkule, J. A. (1984) Etoposide: a semisynthetic epipodophyllotoxin. Chemistry, pharmacology, pharmacokinetics, adverse effects and use as an antineoplastic agent. *Pharmacotherapy 4*, 61–73.

(14) DeVore, R., Whitlock, J., Hainsworth, J. D., and Johnson, D. H. (1989) Therapy-related acute nonlymphocytic leukemia with monocytic features and rearrangement of chromosome 11q. *Ann. Intern. Med. 110*, 740–742.

(15) Felix, C. A. (2001) Leukemias related to treatment with DNA topoisomerase II inhibitors. *Med. Pediatr. Oncol. 36*, 525–535.

(16) Felix, C. A., Kolaris, C. P., and Osheroff, N. (2006) Topoisomerase II and the etiology of chromosomal translocations. *DNA Repair 5*, 1093–1108.

(17) McClendon, A. K., and Osheroff, N. (2006) The geometry of DNA supercoils modulates topoisomerase-mediated DNA cleavage and enzyme response to anticancer drugs. *Biochemistry 45*, 3040–3050.

(18) Sehested, M., Holm, B., and Jensen, P. B. (1996) Dexrazoxane for protection against cardiotoxic effects of anthracyclines. *J. Clin. Oncol. 14*, 2884.

(19) Azarova, A. M., Lyu, Y. L., Lin, C. P., Tsai, Y. C., Lau, J. Y., Wang, J. C., and Liu, L. F. (2007) Roles of DNA topoisomerase II isozymes in chemotherapy and secondary malignancies. *Proc. Natl. Acad. Sci. U.S.A. 104*, 11014–11019.

(20) Lyu, Y. L., Kerrigan, J. E., Lin, C. P., Azarova, A. M., Tsai, Y. C., Ban, Y., and Liu, L. F. (2007) Topoisomerase IIβ mediated DNA double-strand breaks: implications in doxorubicin cardiotoxicity and prevention by dexrazoxane. *Cancer Res. 67*, 8839–8846.

(21) Bachrach, U., and Seiler, N. (1981) Formation of acetylpolyamines and putrescine from spermidine by normal and transformed chick embryo fibroblasts. *Cancer Res. 41*, 1205–1208.

(22) Chen, K. Y., and Liu, A. Y. (1981) Differences in polyamine metabolism of the undifferentiated and differentiated neuroblastoma cells. Metabolic labeling of an 18,000-M(r) protein by [14C]putrescine and the conversion of putrescineto GABA. *FEBS Lett. 134*, 71–74.

(23) Cullis, P. M., Green, R. E., Merson-Davies, L., and Travis, N. (1999) Probing the mechanism of transport and compartmentalisation of polyamines in mammalian cells. *Chem. Biol. 6*, 717–729.

(24) Phanstiel, O., Kaur, N., and Delcros, J. G. (2007) Structure-activity investigations of polyamine-anthracene conjugates and their uptake via the polyamine transporter. *Amino Acids 33*, 305–313.

(25) Barret, J. M., Kruczynski, A., Vispe, S., Annereau, J. P., Brel, V., Guminski, Y., Delcros, J. G., Lansiaux, A., Guilbaud, N., Imbert, T., and Bailly, C. (2008) F14512, a potent antitumor agent targeting topoisomerase II vectored into cancer cells via the polyamine transport system. *Cancer Res. 68*, 9845–9853.

(26) Wilstermann, A. M., Bender, R. P., Godfrey, M., Choi, S., Anklin, C., Berkowitz, D. B., Osheroff, N., and Graves, D. E. (2007) Topoisomerase II - drug interaction domains: identification of substituents on etoposide that interact with the enzyme. *Biochemistry 46*, 8217–8225.

(27) Bender, R. P., Jablonksy, M. J., Shadid, M., Romaine, I., Dunlap, N., Anklin, C., Graves, D. E., and Osheroff, N. (2008) Substituents on etoposide that interact with human topoisomerase IIα in the binary enzyme-drug complex: contributions to etoposide binding and activity. *Biochemistry 47*, 4501–4509.

(28) Kruczynski, A., Vandenberghe, I., Pillon, A., Pesnel, S., Goetsch, L., Barret, J. M., Guminski, Y., Le Pape, A., Imbert, T., Bailly, C., and Guilbaud, N. (2009) Preclinical activity of F14512, designed to target tumors expressing an active polyamine transport system. *Invest. New Drugs 29*, 9–21.

(29) Annereau, J. P., Brel, V., Dumontet, C., Guminski, Y., Imbert, T., Broussas, M., Vispe, S., Breand, S., Guilbaud, N., Barret, J. M., and Bailly, C. (2008) A fluorescent biomarker of the polyamine transport system to select patients with AML for F14512 treatment. *Leuk. Res. 34*, 1383–1389.

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

(31) Chow, K. C., Macdonald, T. L., and Ross, W. E. (1988) DNA binding by epipodophyllotoxins and N-acyl anthracyclines: implications for mechanism of topoisomerase II inhibition. *Mol. Pharmacol. 34*, 467–473.

(32) Worland, S. T., and Wang, J. C. (1989) Inducible overexpression, purification, and active site mapping of DNA topoisomerase II from the yeast *Saccharomyces cerevisiae*. *J. Biol. Chem. 264*, 4412–4416.

(33) Kingma, P. S., Greider, C. A., and Osheroff, N. (1997) Spontaneous DNA lesions poison human topoisomerase IIα and stimulate cleavage proximal to leukemic 11q23 chromosomal breakpoints. *Biochemistry 36*, 5934–5939.

(34) McClendon, A. K., Rodriguez, A. C., and Osheroff, N. (2005) Human topoisomerase IIα rapidly relaxes positively supercoiled DNA: implications for enzyme action ahead of replication forks. *J. Biol. Chem. 280*, 39337–39345.

(35) Mayer, M., and Meyer, B. (2001) Group epitope mapping by saturation transfer difference NMR to identify segments of a ligand in direct contact with a protein receptor. *J. Am. Chem. Soc. 123*, 6108–6117.

(36) Fortune, J. M., and Osheroff, N. (1998) Merbarone inhibits the catalytic activity of human topoisomerase IIα by blocking DNA cleavage. *J. Biol. Chem. 273*, 17643–17650.

(37) Baldwin, E. L., Byl, J. A., and Osheroff, N. (2004) Cobalt enhances DNA cleavage mediated by human topoisomerase IIα in vitro and in cultured cells. *Biochemistry 43*, 728–735.

(38) O'Reilly, E. K., and Kreuzer, K. N. (2002) A unique type II topoisomerase mutant that is hypersensitive to a broad range of cleavage-inducing antitumor agents. *Biochemistry 41*, 7989–7997.

(39) Byl, J. A., Fortune, J. M., Burden, D. A., Nitiss, J. L., Utsugi, T., Yamada, Y., and Osheroff, N. (1999) DNA topoisomerases as targets for the anticancer drug TAS-103: primary cellular target and DNA cleavage enhancement. *Biochemistry 38*, 15573–15579.

(40) Fortune, J. M., Velea, L., Graves, D. E., and Osheroff, N. (1999) DNA topoisomerases as targets for the anticancer drug TAS-103: DNA interactions and topoisomerase catalytic inhibition. *Biochemistry 38*, 15580–15586.

(41) Bandele, O. J., and Osheroff, N. (2008) The efficacy of topoisomerase II-targeted anticancer agents reflects the persistence of drug-induced cleavage complexes in cells. *Biochemistry 47*, 11900–11908.

(42) Burden, D. A., Kingma, P. S., Froelich-Ammon, S. J., Bjornsti, M.-A., Patchan, M. W., Thompson, R. B., and Osheroff, N. (1996) Topoisomerase II-etoposide interactions direct the formation of drug-induced enzyme-DNA cleavage complexes. *J. Biol. Chem. 271*, 29238–29244.

(43) Kingma, P. S., Burden, D. A., and Osheroff, N. (1999) Binding of etoposide to topoisomerase II in the absence of DNA: decreased affinity as a mechanism of drug resistance. *Biochemistry 38*, 3457–3461.

(44) Long, B. H., Musial, S. T., and Brattain, M. G. (1984) Comparison of cytotoxicity and DNA breakage activity of congeners of podophyllotoxin including VP16−213 and VM26: a quantitative structure-activity relationship. *Biochemistry 23*, 1183–1188.

(45) Long, B. H., and Casazza, A. M. (1994) Structure-activity relationships of VP-16 analogues. *Cancer Chemother. Pharmacol. 34* (Suppl), S26–31.

(46) Kitamura, R., Bandoh, T., Tsuda, M., and Satoh, T. (1997) Determination of a new podophyllotoxin derivative, TOP-53, and its metabolite in rat plasma and urine by high-performance liquid chromatography with electrochemical detection. *J. Chromatogr., B: Biomed. Sci. Appl. 690*, 283–288.

(47) Byl, J. A., Cline, S. D., Utsugi, T., Kobunai, T., Yamada, Y., and Osheroff, N. (2001) DNA topoisomerase II as the target for the anticancer drug TOP-53: mechanistic basis for drug action. *Biochemistry 40*, 712–718.

(48) Sander, M., and Hsieh, T. (1983) Double strand DNA cleavage by type II DNA topoisomerase from *Drosophila melanogaster*. *J. Biol. Chem. 258*, 8421–8428.

(49) Shelton, E. R., Osheroff, N., and Brutlag, D. L. (1983) DNA topoisomerase II from *Drosophila melanogaster*. Purification and physical characterization. *J. Biol. Chem. 258*, 9530–9535.

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

(51) Zechiedrich, E. L., Christiansen, K., Andersen, A. H., Westergaard, O., and Osheroff, N. (1989) Double-stranded DNA cleavage/religation reaction of eukaryotic topoisomerase II: evidence for a nicked DNA intermediate. *Biochemistry 28*, 6229–6236.

(52) Bromberg, K. D., Burgin, A. B., and Osheroff, N. (2003) A two-drug model for etoposide action against human topoisomerase IIα. *J. Biol. Chem. 278*, 7406–7412.

(53) Deweese, J. E., Burgin, A. B., and Osheroff, N. (2008) Using 3′-bridging phosphorothiolates to isolate the forward DNA cleavage reaction of human topoisomerase IIα. *Biochemistry 47*, 4129–4140.

(54) Deweese, J. E., and Osheroff, N. (2009) Coordinating the two protomer active sites of human topoisomerase IIα: nicks as topoisomerase II poisons. *Biochemistry 48*, 1439–1441.

(55) Osheroff, N., Shelton, E. R., and Brutlag, D. L. (1983) DNA topoisomerase II from *Drosophila melanogaster*. Relaxation of supercoiled DNA. *J. Biol. Chem. 258*, 9536–9543.

(56) Osheroff, N. (1986) Eukaryotic topoisomerase II. Characterization of enzyme turnover. *J. Biol. Chem. 261*, 9944–9950.

(57) Lindsley, J. E., and Wang, J. C. (1993) On the coupling between ATP usage and DNA transport by yeast DNA topoisomerase II. *J. Biol. Chem. 268*, 8096–8104.

(58) Wang, H., Mao, Y., Zhou, N., Hu, T., Hsieh, T. S., and Liu, L. F. (2001) ATP-bound topoisomerase II as a target for antitumor drugs. *J. Biol. Chem. 276*, 15990–15995.

(59) Osheroff, N. (1989) Effect of antineoplastic agents on the DNA cleavage/religation reaction of eukaryotic topoisomerase II: inhibition of DNA religation by etoposide. *Biochemistry 28*, 6157–6160.

(60) Robinson, M. J., and Osheroff, N. (1991) Effects of antineoplastic drugs on the post-strand-passage DNA cleavage/religation equilibrium of topoisomerase II. *Biochemistry 30*, 1807–1813.

(61) Rodger, A., Taylor, S., Adlam, G., Blagbrough, I. S., and Haworth, I. S. (1995) Multiple DNA binding modes of anthracene-9-carbonyl-N1-spermine. *Bioorg. Med. Chem. 3*, 861–872.

(62) Pommier, Y., Covey, J. M., Kerrigan, D., Markovits, J., and Pham, R. (1987) DNA unwinding and inhibition of mouse leukemia L1210 DNA topoisomerase I by intercalators. *Nucleic Acids Res. 15*, 6713–6731.

(63) McClendon, A. K., Gentry, A. C., Dickey, J. S., Brinch, M., Bendsen, S., Andersen, A. H., and Osheroff, N. (2008) Bimodal recognition of DNA geometry by human topoisomerase IIα: preferential relaxation of positively supercoiled DNA requires elements in the C-terminal domain. *Biochemistry 47*, 13169–13178.

(64) Anderson, V. E., and Osheroff, N. (2001) Type II topoisomerases as targets for quinolone antibacterials: turning Dr. Jekyll into Mr. Hyde. *Curr. Pharm. Des. 7*, 337–353.

(65) Drlica, K., Hiasa, H., Kerns, R., Malik, M., Mustaev, A., and Zhao, X. (2009) Quinolones: action and resistance updated. *Curr. Top. Med. Chem. 9*, 981–998.

(66) Elsea, S. H., McGuirk, P. R., Gootz, T. D., Moynihan, M., and Osheroff, N. (1993) 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. *Antimicrob. Agents Chemother. 37*, 2179–2186.

(67) Lerman, L. S. (1971) A transition to a compact form of DNA in polymer solutions. *Proc. Natl. Acad. Sci. U.S.A. 68*, 1886–1890.

(68) Gosule, L. C., and Schellman, J. A. (1976) Compact form of DNA induced by spermidine. *Nature 259*, 333–335.

(69) Vijayanathan, V., Thomas, T., Shirahata, A., and Thomas, T. J. (2001) DNA condensation by polyamines: a laser light scattering study of structural effects. *Biochemistry 40*, 13644–13651.

(70) Marini, J. C., Miller, K. G., and Englund, P. T. (1980) Decatenation of kinetoplast DNA by topoisomerases. *J. Biol. Chem. 255*, 4976–4979.

(71) Pommier, Y., Kerrigan, D., and Kohn, K. (1989) Topological complexes between DNA and topoisomerase II and effects of polyamines. *Biochemistry 28*, 995–1002.