Intercalation into DNA Is Not Required for Inhibition of Topoisomerase I by Indolocarbazole Antitumor Agents

Christian Bailly, Laurent Dassonneville, Pierre Colson, Claude Houssier, Kazuhiro Fukasawa, Susumu Nishimura, and Tomoko Yoshinari

ABSTRACT
The DNA-intercalating antitumor drug NB-506 is a potent topoisomerase poison currently undergoing phase I/II clinical trials. It contains a planar indolocarbazole chromophore substituted with a glucose residue. Up until now, it was thought that intercalation of the drug into DNA was essential for the stabilization of topoisomerase I-DNA covalent complexes. But, in the present study, we show that a regio-isomeric form of NB-506 has lost its capacity to intercalate into DNA, but remains an extremely potent topoisomerase I poison. The new analogue contains two hydroxyl groups at positions 2,10 instead of positions 1,11 in NB-506. The relocation of the two OH groups reduces considerably the strength of binding to DNA and prevents the drug from intercalating into the DNA double helix. However, the topoisomerase I inhibition capacity of the new analogue remains very high. The two drug isomers are equally potent at maintaining the integrity of the topoisomerase I-DNA coherent complexes, but stimulate cleavage at different sites on DNA. NB-506 stabilizes topoisomerase I preferentially at sites having a pyrimidine (T or C) and a G on the 5' and 3' sides of the cleaved bond, respectively. The 2,10-isomer induces topoisomerase I-mediated cleavage only at TG sites and, thus, behaves exactly as the reference topoisomerase I poison camptothecin. Finally, cytotoxicity measurements performed with a panel of murine and human cancer cell lines reveal that the newly designed drug is considerably (up to 100-fold) more toxic to tumor cells than the parent drug NB-506. We conclude that the DNA-binding and topoisomerase I poisoning activities of NB-506 can be viewed as two separate mechanisms.

INTRODUCTION
NB-506 (Fig. 1) is a promising antitumor agent targeting topoisomerase I (1). This glycosylated indolocarbazole drug derives from the antibiotic BE13793C, isolated from culture supernatants of a Streptoverticillium species (2). NB-506 and related analogues, such as ED-110, are extremely toxic against various tumor cell lines (including cells resistant to vincristine and adriamycin) and are also very potent at inhibiting the growth of human solid tumors in mice (3–6). For these reasons, NB-506 which is the most active compound in the series, was recently advanced into clinical trials. The results of the first Phase I trials are promising and indicate exciting development for this compound in years to come (7, 8).

Like the CPTs3, NB-506 and related indolocarbazole compounds are potent inhibitors of topoisomerase I both in vitro and in cellular systems (9, 10). But, unlike the CPTs, NB-506 binds tightly to DNA even in the absence of topoisomerase I. Studies with indolocarbazoles related to NB-506 (e.g., rebeccamycin analogues) have revealed that the planar indolocarbazole chromophore can intercalate between two consecutive bp in the DNA double helix, thus placing the appended glucose residue into one of the helical grooves, most likely the minor groove. The sugar residue plays an essential role for the interaction with DNA and the recognition of specific sequences, as well as for the interference with topoisomerase I (11–14). Structure-activity relationship studies have revealed that the removal of the sugar reduces considerably the interaction of the drug with DNA and concomitantly decreases the topoisomerase I inhibition properties (15). Indolocarbazole analogues lacking the sugar moiety exhibit very weak topoisomerase I poisoning activities (12). Similarly, analogues bearing bulky groups on the indolocarbazole chromophore (e.g., halogeno-acetyl) or drugs for which the sugar residue is linked to the two indole nitrogens (as with the related drug staurosporine) generally show very weak interaction with DNA and produce minimal effects on topoisomerase I (16, 17). Therefore, these structure-activity relationships studies have lead to dogma that the DNA binding and topoisomerase I inhibition properties of drugs like NB-506 are tightly associated. In other words, it was thought that the intercalation of the drug into DNA was necessary for the drug to act as a topoisomerase I poison. The present study shows that this idea is not valid: intercalation into DNA need not accompany the binding of topoisomerase I inhibitors to DNA.

In this study, we present the DNA binding and topoisomerase I poisoning activities of NB-506 (compound 1) and its regio-isomer (compound 2; Fig. 1). The two drugs differ only by the relative positions of the two hydroxyl groups on the indolocarbazole chromophore; the rest of the molecule is absolutely identical. NB-506 has the two OH groups at positions 1,11, whereas they are at positions 2,10 in compound 2. At first sight, this is a minor modification, but the results presented here show unambiguously that the relocation of the two hydroxyl groups has a dramatic effect on the DNA binding capacities of compound 2. Interestingly, complementary biochemical data attest that the newly designed analogue remains a very potent topoisomerase I poison despite its strongly reduced interaction with DNA. In addition, the 2,10-isomer (compound 2) is significantly more cytotoxic than the 1,11-isomer (compound 1) against a panel of tumor cell lines (P388, colon 26, PC-13, HCT-116, DLD-1, and MKN-45). The results provide key information for a better understanding of the rules that govern the recognition of the topoisomerase I-DNA complex by NB-506-type drugs and raise new ideas for the rational design of topoisomerase I-targeted antitumor agents.

MATERIALS AND METHODS
Drugs and Chemicals. The two drugs were provided by Banyu Pharmaceuticals (Tsukuba, Japan). Their chemical synthesis has been reported (18–20). CPT was purchased from Sigma Chemical Co. (La Verpillière, France). The drugs were first dissolved in DMSO at 10 mM and then further diluted with water. The final DMSO concentration never exceeded 0.3% (v/v) in the cleavage reactions. Under these conditions, DMSO, which is also used in the controls, does not affect the topoisomerase activity. The stock solutions of drugs were kept at −20°C and freshly diluted to the desired concentration at the final concentration. The drugs were kept at 20°C and freshly diluted to the desired concentration.
E. L. D. ELD measurements were performed using a computerized optical measurement system built by Houssier and O‘Konski (22). The procedures outlined previously were followed (23). All experiments were conducted at 20°C with a 10-mm pathlength Kerr cell having 1.5-mm electrode separation in 1 mM sodium cacodylate buffer (pH 6.5). The DNA samples were oriented under an electric field strength of 13 kV/cm, and the drug under test was present at 10 μM together with the DNA or polynucleotide at 100 μM, unless otherwise stated. This electro-optical method has proved most useful as a means of determining the orientation of drugs bound to DNA and has the additional advantage that it senses only the orientation of the polymer-bound ligand: free ligand is isotropic and does not contribute to the signal (24).

To investigate the geometry of drug binding to DNA by ELD, the reduced dichroism ∆A/A of a ligand-DNA complex measured in the ligand absorption band must be analyzed with respect to the reduced dichroism calculated for the same DNA or polynucleotide at 260 nm in the absence of drug, (∆A/A)DNA. The reduced DR is defined as follows: DR = [(ΔA[A]DNA/[(ΔA/A)DNA]]. The numerator refers to the reduced dichroism of the drug-DNA complex measured at the absorption maximum of the ligand bound to DNA. The denominator is always negative under the experimental conditions used. The DR is expected to be +1 if the transition moment of the drug chromophore is parallel to the DNA bases, as in the case of complete intercalative binding. For groove binders, the angle between the double helical axis and the long axis of the chromophore lies below 54°, which gives rise to positive dichroism and, thus, to a negative DR value. Under these conditions, the reduced DRs for any given drug-DNA and drug-polynucleotide complexes can be mutually compared with good relative accuracy, independent of the polymer size (24, 25).

DNA Purification and Labeling. The plasmid pBS (Stratagene, La Jolla, CA) was isolated from Escherichia coli by a standard SDS-sodium hydroxide lysis procedure and purified using Qiagen columns. The 117-mer fragment was prepared by 3'-[32P] end-labeling of the EcoRI-PvU1I digest of the pBS plasmid using α-[32P]-dATP (Amersham, Buckinghamshire, England) and AMV reverse transcriptase. The digestion products were separated on a 6% polyacrylamide gel under native conditions in TBE-buffered solution (89 mM Tris-borate (pH 8.3) and 1 mM EDTA). After autoradiography, the band of DNA was excised, crushed, and soaked in water overnight at 37°C. This suspension was filtered through a Millipore 0.22-μm filter, and the DNA was precipitated with ethanol. After washing with 70% ethanol and vacuum drying of the precipitate, the labeled DNA was resuspended in 10 mM Tris (adjusted to pH 7.0) containing 10 mM NaCl.

Footprinting Experiments. Cleavage reactions by Dnase I were performed essentially according to the previously detailed protocols (26). Briefly, reactions were conducted in a total volume of 10 μl. Samples (3 μl) of the 32P-labeled DNA fragment were incubated with 5 μl of the buffer solution containing the desired drug concentration. After a 20-min incubation at 37°C to ensure equilibration of the binding reaction, the digestion was initiated by the addition of 2 μl of Dnase I (0.01 unit/ml enzyme in 20 mM NaCl, 2 mM MgCl2, and 2 mM MnCl2 (pH 7.3)). At the end of the reaction time (routinely 4 min at room temperature), the digestion was stopped by freeze-drying. After lyophilization, each sample was resuspended in 4 μl of an 80% formamide solution containing tracking dyes before electrophoresis. A Molecular Dynamics 445SI PhosphorImager was used to collect all data, which were analyzed using the ImageQuant version 4.1 software. Each resolved band on the autoradiograph was assigned to a particular band within the DNA fragment by comparison of its position, relative to sequencing standards.

DNA Relaxation Experiments. Supercoiled pKM27 DNA (0.5 μg) was incubated with 6 units of human topoisomerase I at 37°C for 1 h in relaxation buffer [50 mM Tris (pH 7.8), 50 mM KCl, 10 mM MgCl2, 1 mM EDTA, and 1 mM DTT] in the presence of varying concentrations of the drug under study. Reactions were terminated by adding SDS to 0.25% and proteinase K to 250 μg/ml. DNA samples were then added to the electrophoresis dye mixture (2). Each resolved lane of the autoradiograph was assigned to a particular band within the DNA fragment by comparison of its position, relative to sequencing standards.

Stimulation of Topoisomerase I-mediated DNA Cleavage. The plasmid pKM27 (28) was linearized with EcoRI and labeled with α-[32P]-dATP in the presence of the Klenow fragment of DNA polymerase I. The labeled DNA was then digested to completion with AvaI. The cleavage reaction mixture contained 20 mM Tris-HCl (pH 7.4), 60 mM KCl, 0.5 mM EDTA, 0.5 mM DTT, 2 × 104 dpm of α-[32P]-pKM27 DNA, and the indicated drug concentrations.
The reaction was initiated by the addition of human topoisomerase I (20 units in 200-μl reaction volume) and allowed to proceed for 10 min at 37°C. Reactions were stopped by adding SDS to a final concentration of 0.25% and proteinase K to 250 μg/ml, followed by incubation for 30 min at 50°C. Samples were denatured by the addition of 10 μl denaturing loading buffer consisting of 0.45 M NaOH, 30 mM EDTA, 15% (w/v) sucrose, and 0.1% brom cresol green before loading onto a 1% agarose gel in TBE buffer containing 0.1% SDS. Electrophoresis was conducted at 2 V/cm for 18 h.

Sequencing of Topoisomerase I-mediated DNA Cleavage Sites. Each reaction mixture contained 2 μl of 3' end [32P]-labeled DNA (~1 μM), 5 μl of water, 2 μl of 10 × topoisomerase I buffer, and 10 μl of drug solution at the desired concentration, usually 10–50 μM. After at least 30 min of incubation to ensure equilibration, the reaction was initiated by the addition of 20 units of calf thymus topoisomerase I. Samples were incubated for 40 min at 37°C before adding SDS to 0.25% and proteinase K to 250 μg/ml to dissociate the drug-DNA-topoisomerase I cleavable complexes. The DNA was precipitated with ethanol and then resuspended in 5 μl of formamide-TBE loading buffer, denatured at 90°C for 4 min, then chilled in ice for 4 min before loading onto the sequencing gel.

Tumor Cells. HCT116 (human colon cancer) cells and DLD-1 (human colon cancer) cells were provided by Drs. N. Shindo-Okada and H. Fukazawa (National Cancer Center Research Institute, Tokyo, Japan), respectively. MKN-45 (human gastric cancer) and PC-13 (human lung cancer) cells were purchased from Immuno Biological Laboratories (Gunma, Japan). Colon 26 (murine colon cancer) and P388 (murine leukemia) cells were provided by Dr. T. Tsuruo (Applied Microbiology, University of Tokyo, Tokyo, Japan). All of the cells were cultured in DMEM or RPMI 1640 supplemented with 10% fetal bovine serum.

Cleavable Complex Formation in Cultured Cells. HCT116 cells (2.5 × 10^5) were prelabeled by the incubation with DMEM-10% FBS containing 0.5 μCi/ml [3H]thymidine at 37°C and 5% CO₂ overnight. The cells were washed with fresh medium and then incubated with topoisomerase I inhibitors for 1 h. Then, the cells were directly lysed with a 1.5% SDS/5 mM EDTA solution and subjected to K⁺/SDS precipitation assay (29).

RESULTS

Drug-DNA Interaction. Initial attempts to evaluate the drug-DNA interaction were made by titrating measured quantities of a stock solution of calf thymus DNA into a known volume of the drug solution and monitoring the resulting changes in the absorption spectrum of the ligand. As shown in Fig. 2, a significant bathochromic shift (11 nm) and 29% hypochromism are observed when DNA is added to compound 1, whereas, under identical conditions (neutral pH, 16 mM Na⁺), the absorption spectrum of compound 2 is totally unaffected by the addition of DNA. The absorption maximum of the free drug compound 2 at 347 nm remains unchanged, and the hypochromism is extremely weak (3%), suggesting that the 2,10-isomer has no significant interaction with the DNA double helix. In the same buffer, a DNA binding constant of 2.64 × 10^3 (M⁻¹) was accurately measured for compound 1 using the intrinsic fluorescence of the indolocarbazole chromophore (14). The affinity constant for compound 2 is too weak to be measured precisely; it must be in the order of ~10^3 M⁻¹.

Two alternative procedures were tried to compare the strength of...
the drug-DNA interaction. First, we determined the ability of the test drugs to alter the thermal denaturation profile of DNA. The duplex stabilizing effect (ΔTm) of compound 1 is relatively weak, but superior to that of compound 2 (Fig. 3a). Second, we used fluorescence spectroscopy to examine the abilities of the test drugs to compete with a DNA-binding ligand for available binding sites. In these experiments, the fluorescent probe used was the intercalating drug ethidium bromide, which is highly fluorescent in the presence of DNA. Only compound 1 significantly reduces the fluorescence of the ethidium-DNA complexes. Almost no quenching was observed with compound 2 (Fig. 3b). The results of the Tm and fluorescence quenching experiments are, thus, totally consistent with the absorption measurements and concur that the 2,10-isomer 2 has minimal, if any, interaction with DNA.

CD and ELD were used to characterize the mode of binding of the drugs to DNA. Addition of calf thymus DNA induces significant changes in the CD spectrum of compound 1. The band at 340 nm decreases as the DNA concentration is raised; meanwhile, the CD in the 310–330-nm region increases significantly (Fig. 4a). In sharp contrast, the CD spectrum of compound 2 is not affected at all by the addition of DNA (Fig. 4b). In the presence of DNA, the variation in CD intensity at 340 nm is very different for the two drugs (Fig. 4c), indicating, thus, that their DNA binding modes strongly differ. The ELD measurements confirmed this belief. The reduced dichroism in the 300–340-nm region, where the indolocarbazole chromophore absorbs the light, reaches 0.45 with compound 1, whereas it does not exceed 0.15 with compound 2 (Fig. 5a). The intensity of the ELD signal is a function of the degree of alignment of the DNA molecules in the electric field. When the drug molecules are fully bound to DNA (i.e., for P:D ratios ≥20; Fig. 5b), the reduced dichroism depends on field strength similarly at 260 nm for the DNA bases and at 340 nm for compound 1 (Fig. 5c). This indicates that the indolocarbazole ring of compound 1 is tilted close to the plane of the DNA bases, consistent with an intercalative mode of binding. The situation strongly differs with compound 2. In this case, the reduced dichroism measured at 350 nm is much less negative than that of DNA. Therefore, on the basis of the CD and ELD experiments, we can firmly conclude that the 2,10-isomer 2 does not intercalate into DNA. Additional ELD experiments with a series of synthetic polynucleotides of different base compositions confirmed that compound 1, but not compound 2, behaves as a typical DNA intercalating agent. With compound 1, the DR
(defined in “Materials and Methods”) was always close to +1 (data not shown; i.e., the expected value for a drug parallel to the DNA bp plane). With compound 2, the DR never exceeded 0.5, whatever the sequence. The ELD data with compound 2 are totally incompatible with an intercalation binding mode.

Next, we evaluated the sequence selectivity of the drugs. The results of the footprinting experiments presented in Fig. 6 reflect the spectroscopic binding data described above. As anticipated, no footprint was seen with compound 2 and, on the opposite, the 1,11-isomer protected the sequence 5'-CCAGGGT from cutting by the nuclease. The footprint detected with compound 1 around nucleotide position 70 on the 117-bp DNA fragment is in accordance with previous footprinting studies with rebeccamycin analogues, indicating that binding occurs preferentially at sequences containing CG or TG sites (13). It is worth noting that there is no correspondence between the topoisomerase I cleavage sites stimulated by compound 1 (described below) and the position of the preferential drug binding site on the same DNA fragment. One of the most pronounced topoisomerase I cleavage sites (at position 26) occurs some distance from the main drug binding site at the GC-rich sequence around position 70 (compare Fig. 7 with Fig. 9). This is another indication that the two molecular events, DNA binding and topoisomerase I inhibition, may not be related.

Topoisomerase I Inhibition. The topoisomerase I inhibitory properties of the drugs were first examined using a DNA relaxation assay. Negatively supercoiled plasmid pKMp27 was incubated with topoisomerase I in the presence of increasing concentration of the two drugs. The DNA samples were treated with SDS and proteinase K to remove any covalently bound protein and were resolved in a 1% agarose gel without ethidium bromide. As shown in Fig. 7A, supercoiled DNA is fully relaxed by topoisomerase I in the absence of drug (compare lanes DNA and Topo I). In the presence of the two drugs, the intensity of the band corresponding to the nicked form of DNA has increased significantly. This effect, observed with CPT, reflects the stabilization of topoisomerase I-DNA cleavable complexes. Interestingly, we noted that at concentrations >10 μM, compound 1 inhibited the relaxation of DNA by topoisomerase I, whereas the relaxation was still complete with compound 2, even when using a drug concentration as high as 100 μM.

To better differentiate the specific (poisoning) and nonspecific effects, we repeated the experiments using agarose gels prestained with ethidium bromide (Fig. 7B). In this case, the relaxed DNA migrates faster than the supercoiled plasmid because of ethidium-induced DNA unwinding effects. Here again, a marked increase in the intensity of the band corresponding to nicked DNA molecules can be detected with the two drugs. It seems clear that both drugs efficiently stabilize topoisomerase I-DNA complexes despite their distinct DNA...
binding capacities. Interestingly, a strong shift in the mobility of supercoiled plasmid (form I) was observed with increasing concentrations of compound 1, but not with compound 2 (Fig. 7B). This is another evidence for their very different DNA binding properties. The effect of compound 1 on the electrophoretic mobility of supercoiled DNA can be attributed to a decrease in plasmid DNA-linking number due to intercalation (the same effect was observed in the absence of the enzyme). These results indicate that compound 1 can behave both as specific topoisomerase I inhibitor, trapping the cleavable complexes and as nonspecific inhibitors of a DNA-processing enzyme acting via DNA binding. In sharp contrast, the 2,10-isomer 2 behaves exclusively as a specific topoisomerase I poison, as it is the case with CPT.

Next, we used a 32P-labeled linear plasmid DNA as a substrate for human topoisomerase I. The 3' end-labeled EcoRI-AvaI restriction fragment of pKM27 was incubated with the enzyme in the presence and absence of the indolocarbazoles, and the resulting DNA cleavage products were analyzed by agarose gel electrophoresis under alkaline conditions. The profiles of DNA cleavage, presented in Fig. 8, are different with the two drugs. One of the cleavage sites (open arrowhead) is much more enhanced in the presence of compound 1 than with compound 2. Conversely, at another site (filled arrowhead), the cleavage is more intense with compound 2 than compound 1. The position of the hydroxyl groups seems to influence the cleavage of DNA by the enzyme. It is important to note, also, that the cutting profiles obtained with the two indolocarbazoles are significantly different from that obtained with CPT.

To investigate further the effect of the OH groups on the sequence-specific cleavage of DNA by topoisomerase I, we used the 117-bp fragment from plasmid pBS, as used in the above footprinting experiments. The DNA restriction fragment (32P-labeled at the 3' end of the EcoRI site) was incubated with the test drug and the enzyme, and the resulting cleavage products were resolved on sequencing gels so as to identify the sequence of the drug-induced topoisomerase I cleavage sites. A typical example of a gel is shown in Fig. 9. The positions of seven cleavage sites were determined with nucleotide resolution (Table 1). An eighth site can be seen at the top of the gel around nucleotide position 105, but it lies beyond the area accessible to densitometric analysis. The weak site 1 at a TA step is specific to CPT. Sites 3, 5, and 6 are only detected with compound 1; they correspond to CG sites. This compound also promotes cleavage at the TG sites, especially at site 2, much more weakly at sites 4 and 7. In sharp contrast, the topoisomerase I cleavage sites stimulated by compound 2 are restricted to the TG sites. The cutting profiles observed with compound 2 are almost identical to those seen...
TOPOISOMERASE INHIBITION BY ANTITUMOR INDOLOCARBAZOLES

Table 1  Sequences of the cleavage sites stimulated by the drugs

<table>
<thead>
<tr>
<th>Site</th>
<th>Position (5' → 3')</th>
<th>Sequence</th>
<th>Type</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22</td>
<td>TGTAAT</td>
<td>ACAGCT</td>
<td>++</td>
</tr>
<tr>
<td>2</td>
<td>26</td>
<td>TGAATT</td>
<td>GTAATA</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>41</td>
<td>TAAAC</td>
<td>GACGTC</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>48</td>
<td>GAGTTT</td>
<td>GTAAAA</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>54</td>
<td>AGTCAC</td>
<td>GACGTT</td>
<td>++</td>
</tr>
<tr>
<td>6</td>
<td>73</td>
<td>GGTACG</td>
<td>GCCAGG</td>
<td>++</td>
</tr>
<tr>
<td>7</td>
<td>81</td>
<td>TAAGCT</td>
<td>GGGTTA</td>
<td>++</td>
</tr>
</tbody>
</table>

*Positions of the drug-stimulated cleavage sites within the 117-mer fragment from pBS.

† Arrows point out the cut that occurs between positions −1 and +1.

§ +, ++, and +++ correspond to weak, medium, and strong cleavage, respectively.

Fig. 10. Drug-induced formation of DNA-protein complexes in HCT116 human colon cancer cells. Compound 1 (○); compound 2 (□).

The results of the sequencing studies presented in Fig. 9 and Table 1 are important because they reveal unambiguously that the OH groups of NB-506 serve to control the activity of the DNA cleaving enzyme, but also because they reconcile the results of two previous studies with indolocarbazoles. Indeed, recently Fukasawa et al. (34) reported that NB-506 stimulates DNA cleavage by topoisomerase I, but which interacted strongly with DNA. Nevertheless, all of the studies suggest that topoisomerase I is a very important, if not essential, target for indolocarbazoles.

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Compound 2 has lost the capacity to intercalate into DNA, however, it remains highly cytotoxic, even more cytotoxic than NB-506 against a panel of tumor cells. This does not mean that the DNA-binding capacities of NB-506 are detrimental to the anticancer activity. The intercalation into DNA may well contribute to the biological activity, at least partially. We have previously obtained highly cytotoxic indolocarbazole drugs endowed with modest effects on topoisomerase I, but which interacted strongly with DNA. Nevertheless, all of the studies suggest that topoisomerase I is a very important, if not the essential, target for indolocarbazoles.

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preferentially at sites having a C and G on the 5’ and 3’ sides of the cleaved bond, respectively, whereas sequencing studies with rebeccamycin analogues (which lack the key OH groups on the drug chromophore) showed that topoisomerase I-mediated cleavage occurred predominantly at 5’-TG-3’ sites (12). The reason for the slight discrepancy between the two studies is now elucidated. The selectivity for CG or TG sites depends on the absence or presence (and the position) of the two OH groups in the test drugs.

The newly designed NB-506 analogue, compound 2, is a potent topoisomerase I inhibitor with practically no binding to DNA (at least in the absence of topoisomerase I). In this respect, this compound appears very similar to CPT, which also fails to bind to DNA in the absence of the enzyme (but DNA contributes to its effects on topoisomerase I; Ref. 35). The analogy between the two drugs is even more striking if one considers that they both stimulate cleavage by topoisomerase I; Ref. 35). The analogy between the two drugs is even more striking if one considers that they both stimulate cleavage by topoisomerase I; Ref. 35). The analogy between the two drugs is even more striking if one considers that they both stimulate cleavage by topoisomerase I; Ref. 35). The analogy between the two drugs is even more striking if one considers that they both stimulate cleavage by topoisomerase I; Ref. 35).

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