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ANTITUMOR ACTIVITY AND BIOCHEMICAL EFFECTS OF TOPSENTIN

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Abstract—Topsentin, a bis(indolyl)imidazole marine natural product, inhibited the proliferation of cultured human and murine tumor cells at micromolar concentrations (IC_{50} values ranged from 4 to 40 μM) and was active against *in vivo* P388 leukemia (%T/C = 137, 150 mg/kg, QD1–5) and B16 melanoma (%T/C = 144, 37.5 mg/kg, QD1–9) tumors. Effects of 30 μM topsentin (1-hr exposures) on incorporation of radiolabeled precursors by P388 cells indicated inhibition of DNA synthesis (91%) and to a lesser extent RNA synthesis (57%), whereas synthesis of protein was unaffected (0%). Fluorescence spectral changes and competitive binding experiments with ethidium bromide indicated that topsentin interacted with DNA. No evidence for intercalation was observed in DNA unwinding studies, but competitive binding experiments with Hoechst 33342 and CC-1065 indicated that topsentin bound to DNA in the minor groove.

Nonintercalative DNA groove-binding drugs exhibit a wide spectrum of antibacterial, antiprotozoal, antiviral, and antitumor activity. Drugs capable of interacting with the minor groove of DNA are structurally diverse compounds, and not surprisingly several distinct modes of binding have been observed. Netropsin and distamycin A reversibly bind to the minor groove of AT regions of B-DNA [1]. Similarly, 4′,6-diamidine-2-phenylindole (DAPI§), bisbenzimide dyes, and the anti-trypanosomal drug, berenil, bind preferentially to AT-rich DNA within the minor groove of the helix [2]. A preference for GC sequences is shown by antitumor antibiotics of the aureolic acid group (mithramycin, olivomycin, and chromomycin), which also bind in the minor groove [3]. In contrast to these reversible physical interactions, CC-1065 and pyrrolo[1,4]benzodiazepines (anthramycin, tomaymycin, sibiromycin) bind covalently to DNA and the resulting drug adducts occupy the minor groove [4, 5]. Certain compounds that intercalate may also interact with the minor groove of DNA. Although the chromophore of actinomycin D intercalates between GC base pairs, the cyclic amino acid side chains are located in the minor groove and the carbonyl oxygen of the threonine moiety is hydrogen-bonded to the NH_2 of guanine [6]. The cyclic depsipeptide moieties of echinomycin and triostin A bind to the minor groove with bisintercalation of their quinoxiline rings [7]. Recently, an X-ray diffraction analysis of the binding of nogalamycin to a thiophospho-containing DNA hexamer indicated an even more complex ligand–DNA interaction. Apparently, local transient melting of the DNA allows intercalation of the nogalamycin chromophore with the nogalose and aminoglucose sugars simultaneously binding in the minor and major grooves at opposite sides of the helix [8].

Many compounds that bind the minor groove of DNA are potent antitumor agents. These agents change the physical characteristics of DNA and alter its ability to function as a template for DNA and RNA polymerases, but the various modes of interaction with DNA suggest that the biological activity of these drugs may not be mediated by a common mechanism. CC-1065 inhibits DNA polymerase-α and *in vitro* synthesis of DNA by L1210 cells, but S-phase cells are the most resistant to the lethal effect of the drug [4]. In addition, concentrations of CC-1065 that block division of tumor cells do not block progression through S-phase; instead an arrest in G_2-M was observed. Effects on mitotic events following covalent binding of CC-1065 to DNA suggest that repair-related mechanisms may be important in the cytotoxicity of this agent and other groove binders that form covalent adducts. Distamycin A, bisbenzimide, and DAPI inhibit the DNA relaxing activity of mammalian topoisomerase I and, depending on concentration, stimulate or inhibit the catalytic activity of topoisomerase II [9, 10]. However, unlike camptothecin or doxorubicin [11], inhibition of the catalytic activity of either topoisomerase I or II in isolated enzyme reactions is not associated with DNA strand breakage. In addition to disrupting the catalytic activity of topoisomerases, minor groove binders also affect other DNA-directed enzymes. For example, the activities of DNA and RNA polymerases, DNAse I, and S1 nuclease have all

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§ Abbreviations: DAPI, 4′,6-diamidine-2-phenylindole; IC_{50}, concentration of agent that resulted in 50% of untreated culture absorbance; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; PBS, pH 7.0, 0.8% NaCl, 0.22% $Na_2HPO_4 \cdot 7H_2O$, 0.02% KH_2PO_4, 0.02% KCl, w/w; and %T/C, ratio of median life span of treated to control tumor bearing mice expressed as a percentage.

been reported to be inhibited by compounds that bind the minor groove of DNA [2].

In this study, the antiproliferative effects of topsentin, a new marine natural product, were quantitated with cells derived from human and murine solid tumors and leukemias, and the antitumor activity of the compound was assessed with *in vivo* P388 and B16 murine tumor models. Incorporation studies indicated that topsentin inhibited macromolecular synthesis with a pattern similar to known DNA-active agents, and the nature of the interaction between topsentin and DNA was assessed by fluorescence spectral changes and competitive binding studies.

MATERIALS AND METHODS

Cell culture. P388 murine leukemia cells obtained from Dr. J. Mayo, National Cancer Institute, Bethesda, MD, were maintained in RPMI medium 1640 supplemented with 10% horse serum. HL-60 promyelocytic leukemia cells [12], HT-29 colon carcinoma [13], and A549 lung carcinoma [14] cells, obtained from the American Type Culture Collection, Rockville, MD, and HL-60/AR cells [15], obtained from Dr. Steven Grant, Columbia University, NY, were maintained in RPMI 1640 supplemented with 10% fetal bovine serum. All cell lines were cultured in plastic tissue culture flasks and kept in an incubator at 37° in humidified air containing 5% CO_2. Antibiotic-free stock cultures of P388, HL-60 and HL-60/AR cells were subcultured to 10^5 cells/mL by dilution in fresh growth medium at 2- to 5-day intervals. The ability of agents to induce granulocytic differentiation in HL-60 cell cultures was determined as described previously [16]. Antibiotic-free stock cultures of HT-29 and A549 were subcultured (2×10^5 cells/T-25 flask) at weekly intervals by disassociation with 0.05% trypsin and 0.02% EDTA (15–20 min, 37°). Every 3–4 months, stock cultures were reinitiated from frozen cells [frozen in liquid nitrogen in RPMI 1640 medium supplemented with 20% serum and 8% dimethyl sulfoxide (DMSO)] that were free of *Mycoplasma* as determined with Hoechst 33258 stain [17]. All experimental cultures were initiated in medium containing gentamicin sulfate (50 μg/mL; Schering Corp., Kenilworth, NJ).

To assess the antiproliferative effects of agents against P388, HL-60 and HL-60/AR cells, 200 μL cultures (96-well tissue culture plates, Nunc, Denmark) were established at 1×10^5 cells/mL in drug-free medium or medium containing agents at various concentrations. After 48-hr exposures, P388 cells were enumerated using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) as described below [18]. After 72-hr exposures, HL-60 and HL-60/AR cells were also enumerated with MTT. To assess the antiproliferative effect of agents against HT-29 and A549 cells, 200 μL cultures were initiated with 4×10^3 cells/cm^2 of culture area in 96-well tissue culture plates. After 24 hr, 100 μL of the culture fluids was removed and replaced with fresh drug-free growth or medium containing the agent to be tested at twice the final concentration. Following an additional 96-hr incubation, cells were enumerated using MTT.

To quantitate the effects of agents on cell proliferation, 75 μL of warm growth medium containing 5 mg/mL MTT was added to each well, and cultures were returned to the incubator and left undisturbed for 90 min. To spectrophotometrically quantitate formation of reduced formazan, plates were centrifuged (900 *g*, 5 min), culture fluids were removed by aspiration, and 200 μL of acidified isopropanol (2 mL concentrated HCl/L isopropanol) was added per well. The absorbance of the resulting solutions was measured at 570 nm with a plate reader (MR700 Microplate Reader, Dynatech Laboratories, Chantilly, VA). The absorbance of test wells was compared with the absorbance of drug-free wells, and the concentration of agent that resulted in 50% of the absorbance of untreated cultures was determined by linear regression of logit-transformed data [19]. A linear relationship between P388, HL-60, HL-60/AR, HT-29, and A549 cell number and formazan production was found over the range of cell densities observed in this study.

Antitumor efficacy. Compounds were dissolved in 95% ethanol, diluted in sterile 0.9% NaCl, and injected i.p. in a total volume of 0.5 mL. The efficacy of agents was expressed as the median life span of treated mice normalized as a percentage of the life span of untreated tumor-bearing mice (%T/C). The P388 lymphocytic leukemia and B16 melanoma tumor were obtained and passaged as described previously [16]. Intraperitoneal P388 tumors were inoculated as ascites (10^6 cells/0.1 mL) 1 day prior to treatment, into male CD2F1 mice (Charles River, Raleigh, NC), six per group, and compounds were administered daily for 5 days (QD1-5). Time to death (mean ± SD, N = 18) for untreated mice was 10.2 ± 1.0 days. Fluorouracil (40 mg/kg; QD1–9, i.p., N = 6) administration resulted in a %T/C of 230. B16 melanoma was established by i.p. injection of 0.5 mL of a 10% tumor brei into groups of ten female B6D2F1 (Charles River) mice 1 day before therapy began. Mean time to death (±SD, N = 20) for tumor-bearing animals was 17.5 ± 2.5 days, and i.p. *cis*-platinum(II)diamine dichloride administration (1 mg/kg, QD1–9, N = 10) resulted in a %T/C of 156.

Incorporation studies. The effects of topsentin on the apparent rates of protein, RNA, and DNA synthesis were assessed by measuring cellular incorporation of tritiated leucine, uridine, and thymidine from culture fluids. P388 cultures were established in 20-mL plastic vials (2×10^5 cells/mL, 10 mL cultures), gassed with 5% CO_2 in air, and incubated at 37° for 30 min in a shaking waterbath. To initiate incorporations, 100 μL of PBS (pH 7.0, 0.8% NaCl, 0.22% $Na_2HPO_4 \cdot 7H_2O$, 0.02% KH_2PO_4, 0.02% KCl, w/w) containing [^{3}H]leucine (200 μCi/mL), [^{3}H]uridine (100 μCi/mL) or [^{3}H]thymidine (100 μCi/mL) was added. At 5-min intervals, 1 mL of the cell suspension was removed and diluted with 10 mL of PBS containing the appropriate unlabeled precursor at 100 μM. Cells were collected on 0.45 μm nitrocellulose filters (Millipore, Bedford, MA) by vacuum filtration, and macromolecules precipitated by the addition of cold

5% trichloroacetic acid. Filters were extracted three times with 5 mL of cold 5% trichloroacetic acid, washed with 5 mL of water, and rinsed with 5 mL of 70% ethanol. Filters were transferred to 20-mL scintillation vials and 12 mL of scintillation fluor (Aquasol, NEN Research Products, Boston, MA) was added per vial. Radioactivity was determined by liquid scintillation spectrometry. Quenching was similar in all samples as determined by the ratio of counts in a narrow tritium channel and a wide ^{14}C channel. Tritiated compounds were obtained from NEN Research Products, and were the highest specific activity available.

DNA binding studies. The fluorescence spectral characteristics of topsentin in the absence or presence of 1 mg/mL DNA were measured with an LS-50 fluorescence spectrometer (Perkin-Elmer Corp., Norwalk, CT). Fluorescence spectra reported are uncorrected. The effects of topsentin on the binding of ethidium bromide to DNA were assessed by measuring the increase in ethidium fluorescence as DNA was added to the cuvette. Volumes of a calf thymus DNA solution (2 mg/mL) were added to solutions of ethidium bromide (1 μM) in the absence or presence of topsentin (100 μM). Slit widths were 10 nm for all experiments, and excitation and emission wavelengths were as indicated in the text. The total increase in volume due to the addition of DNA was less than 7% and increases in fluorescence were not corrected for volume changes. The effects of topsentin (100 μM) on the binding of Hoechst 33342 (1 μM) were determined as described for ethidium bromide, except that the excitation wavelength was 310 nm and increases in emission were monitored at 512 nm (10-nm slit widths). The effects of CC-1065 on topsentin binding to DNA were assessed by adding DNA (final concentration of 1 mg/mL) to solutions of topsentin (100 μM) in the absence or presence of various concentrations of CC-1065. The relative amounts of topsentin bound to DNA were assessed by fluorescence spectral changes (excitation = 401 nm, emission = 512 nm, slit widths = 10 nm). CC-1065 was dissolved in DMSO and solvent controls were included in all experiments.

DNA unwinding. The ability of topsentin to unwind supercoiled closed circular DNA was determined as previously described [20]. Reaction mixtures (20 μL total volume) containing 50 mM Tris–HCl, pH 7.4, 120 mM KCl, 0.5 mM EDTA, 0.5 mM dithiothreitol, 10 mM $MgCl_2$, 50 μg/mL BSA, and 0.2 μg ColE1 DNA were incubated with various amounts of topsentin or ethidium bromide for 1 hr. DNA topoisomerase I (5 units of calf thymidine enzyme, Bethesda Research Laboratories, Gaithersburg, MD) was added and the mixture was incubated at 30° for 30 min before the reaction was terminated by the addition of 2 μL of 5% sodium dodecyl sulfate. DNA was extracted with phenol, and supercoiled and relaxed forms of DNA were separated by electrophoreses in a 1% horizontal agarose gel. Gels were stained with ethidium bromide and photographed under UV illumination.

Experimental agents. Topsentin (>95% pure) used for this study was obtained from a deep water marine sponge, *Spongosorites* sp., as previously described [21]. The purity of topsentin ($R_f = 0.75$) was assessed by analytical reversed-phase thin-layer chromatography (MKC-18F RP plates, 200 nm, Whatman, Clifton, NJ) with 20% aqueous methanol as the development system. CC-1065 was the gift of Dr. J. P. McGovern, The Upjohn Co., Kalamazoo, MI. All other chemicals were obtained from commercial sources.

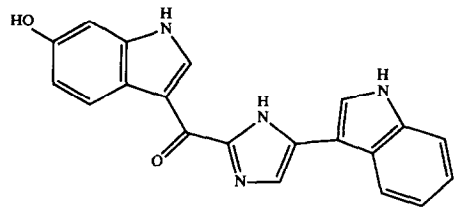

Fig. 1. Structure of topsentin, a bis(indolyl)imidazole isolated from a Caribbean deep-sea sponge of the family Halichondriidae.

RESULTS

Organic extracts of *Spongosorites* sp. sponges collected in the Caribbean showed significant inhibition of cultured P388 cell proliferation and herpes simplex virus, type 1 (HSV-1) cytopathic effect. This activity was found by TLC HSV-1 and P388 [22] bioautography methods to be associated with a bright yellow compound present in the crude extract. Purification lead to the isolation of a bis-(indolyl)imidazole, designated topsentin (Fig. 1). Topsentin and certain synthetic analogs inhibit the growth of P388 cells, HSV-1, vesicular stomatitis virus and corona virus A-59 [23]. Topsentin has also been isolated from a Mediterranean sponge, *Topsentia genitrix*, and reported to be weakly toxic to fish [23].

Antitumor activity. Topsentin inhibited the proliferation of cultured murine and human tumor cells and showed modest activity in murine P388 and B16 *in vivo* tumor models. Of the cell lines tested, murine P388 leukemia cells were most sensitive to the antiproliferative effect of topsentin, while the potency for inhibition of replication of cells derived from human lung and colon tumors or a leukemia was approximately an order of magnitude greater (Table 1). Over a range of concentrations from nontoxic to lethal, HL-60 cells exposed to topsentin did not differentiate as determined by nitroblue tetrazolium reduction (data not shown). Against ascitic P388 tumors, topsentin showed modest activity on a QD1–5 schedule with a best response (150 mg/kg) of 137% (Table 2). Topsentin also showed similar activity with the i.p. B16 model (%T/C = 144, 37.5 mg/kg) with daily administration (QD1–9).

Macromolecular biosynthesis. An initial characterization of the toxic effects of topsentin was made by assessing incorporation of radiolabeled precursors of DNA, RNA, and protein into acid-precipitable material of P388 cells. After 1-hr exposures to 30 μM topsentin, incorporation of [^{3}H]thymidine and

Table 1. Antiproliferative effects of topsentin*

Cell line	IC_{50} (μM)
P388 leukemia	4.1 ± 1.4
HT-29 colon	20.5 ± 2.1
A549 lung	41.1 ± 8.5
HL-60 leukemia	15.7 ± 4.3
HL-60/AR leukemia	33.3 ± 3.6

* Cells were incubated for 48 hr (P388), 72 hr (HL-60, HL-60/AR), or 96 hr (HT-29, A549) at 37° in a humidified atmosphere containing 5% CO_2 in air. Cells were enumerated with MTT as described in Materials and Methods, and data are expressed as the micromolar concentration of topsentin that resulted in an absorbance equal to 50% of that obtained from an equivalent untreated culture.

Results are the means (±SD) of 3–8 separate experiments.

Table 2. Antitumor activity of topsentin*

Dose (mg/kg, i.p.)	%T/C in tumor model	
	P388	B16
300	126	ND
150	137	136
75	132	139
37.5	111	144
18.7	ND	125

* Intraperitoneal P388 or B16 tumors were established 1 day prior to treatment with topsentin on a daily schedule (QD1–5 or QD1–9) as described in Materials and Methods. Data are expressed as the median time to death of six (P388) or ten (B16) treated animals normalized to the time of death of untreated tumor-bearing animals (%T/C). ND represents not determined.

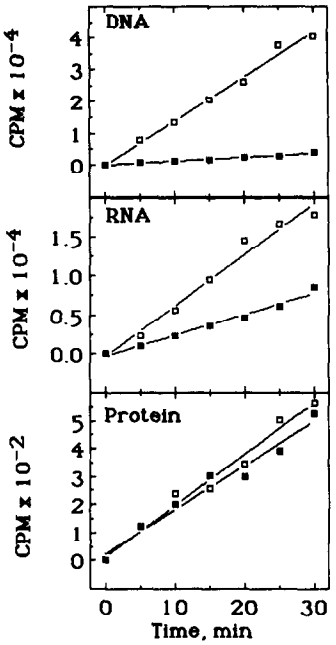

Fig. 2. Inhibition of whole cell DNA, RNA, and protein synthesis in P388 cells. P388 cultures (2×10^5 cells/mL, 10-mL cultures) were established in medium in the presence or absence of 30 μM topsentin. After 1 hr, incorporation of radiolabeled compounds was initiated and cells were collected on filters at the times indicated. Filters were extracted, and radioactivity incorporated by untreated (□) and topsentin-treated (■) cultures was determined. Results represent the total cpm incorporated per 2×10^5 cells.

Table 3. Inhibition of whole-cell DNA, RNA, and protein synthesis in P388 cells*

Agent	% Inhibition		
	DNA	RNA	Protein
Topsentin, 30 μM	91	57	0
Topsentin, 3 μM	20	13	0
Daunomycin, 0.5 μM	49	49	0
Actinomycin D, 10 μM	94	95	42
Ara-C, 10 μM	98	0	0
Emetine, 1 μM	72	49	100

* Cells were incubated for 1 hr at 37° with concentrations of the agents indicated and then exposed to tritiated thymidine, uridine, or leucine for 5–30 min as described in Fig. 2. Data are expressed as the percent inhibition of the rate of incorporation of precursor in treated cultures normalized to that incorporated in untreated cultures.

[^{3}H]uridine were inhibited 91 and 60%, respectively, while incorporation of [^{3}H]leucine was unaffected (Fig. 2). At a lower concentration of topsentin (3 μM), effects were less, but a similar pattern was observed (Table 3). Inhibition of nucleic acid polymerization with less effects on protein synthesis was also observed following exposure of P388 cells to DNA binding agents such as daunomycin or actinomycin D, whereas agents that do not interact with DNA such as Ara-c or emetine show markedly different patterns (Table 3).

DNA binding studies. Since incorporation experiments suggested that topsentin interacted with DNA, fluorescence spectral changes and competitive binding experiments with known DNA active compounds were undertaken. Changes in the fluorescence spectra of topsentin indicate that this agent interacts with DNA. The excitation and emission maxima of topsentin were 273 and 348 nm, respectively, while in the presence of 1 mg/mL DNA a shift in the emission maximum to 512 was observed. In the presence of DNA, changes in the excitation spectrum of topsentin were also observed, as the excitation maximum shifted to 401 nm and a local maximum was observed at 301 nm. As shown in Fig. 3, the addition of DNA to topsentin also resulted in an increase in the intensity of emitted light, with maximal changes observed at a molar base pair to topsentin ratio of 95 to 1. Competitive binding experiments with ethidium bromide confirmed that topsentin interacted with DNA (Fig. 4), but topsentin did not alter the electrophoretic mobility of DNA

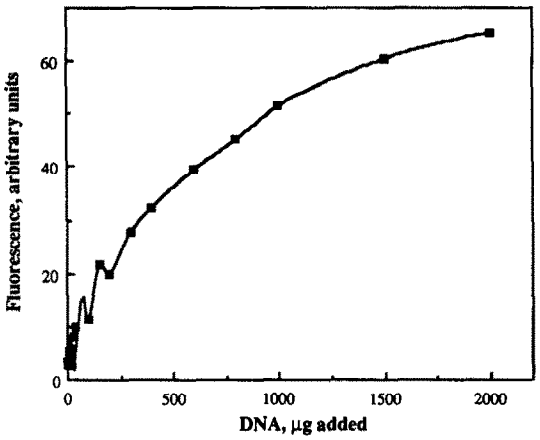

Fig. 3. Effects of DNA on the fluorescence of topsentin. DNA was added to a cuvette containing 10 μM topsentin (initial volume), and appropriate volumes of a 2 mg/mL stock solution of DNA were added to result in the total amounts of DNA as indicated. Excitation wavelength was 301 nm and emission was measured at 512 (slit widths were 10 nm).

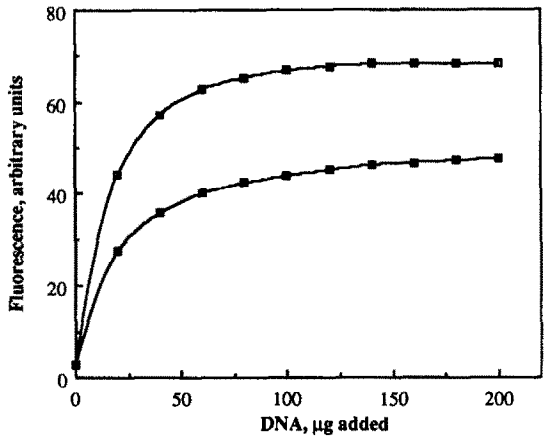

Fig. 4. Effects of topsentin on the increase in ethidium bromide fluorescence observed in the presence of DNA. DNA was added to cuvettes containing 1 μM ethidium bromide in the absence (□) or presence (■) of 100 μM topsentin, as described in Fig. 3. Excitation wavelength was 530 nm and emission was measured at 590 nm (slit widths were 10 nm); fluorescence was not corrected for dilution. Virtually identical results were obtained when excitation and emission were monitored at 295 and 590 nm, respectively.

suggesting that intercalation did not occur (data not shown). More rigorous DNA-unwinding experiments indicated that topsentin did not intercalate (Fig. 5). Topsentin reduced the apparent binding of Hoechst 33342 to calf thymus DNA (Fig. 6, top panel) and CC-1065 blocked increases in topsentin fluorescence in the presence of DNA (Fig. 6, bottom panel). However, DMSO alone did alter the fluorescence spectral characteristics of topsentin.

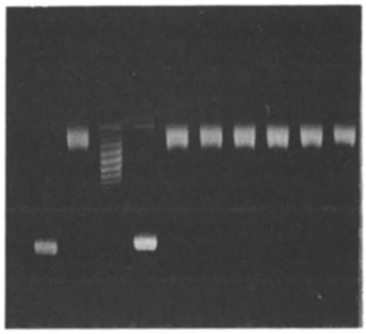

Fig. 5. DNA unwinding in the presence of ethidium bromide or topsentin. Supercoiled closed circular DNA was treated with agents, relaxed with topoisomerase I, phenol extracted and separated by electrophoresis in agarose gels. Lane 1: supercoiled ColE1 DNA standard. Lane 2: supercoiled ColE1 DNA relaxed with topoisomerase I. Lanes 3 and 4: supercoiled ColE1 DNA standard exposed to 1 and 2.5 μM ethidium bromide, respectively, before treatment with topoisomerase I. Lanes 5–10: supercoiled ColE1 DNA exposed to 12, 24, 48, 96, 192, and 384 μM topsentin, respectively, before treatment with topoisomerase I.

DISCUSSION

Topsentin is a new cytotoxic bis(indolyl)imidazole marine natural product that possesses modest antitumor activity. Incorporation experiments with whole cells indicated that topsentin interfered with nucleic acid synthesis in a manner similar to agents known to bind DNA. Changes in the fluorescence spectra of topsentin in the presence of DNA and the ability of the compound to inhibit ethidium bromide binding to DNA demonstrated that topsentin interacted with this nucleic acid. Topsentin did not change the electrophoretic mobility of supercoiled circular DNA, suggesting that intercalation did not occur. In addition, when supercoiled DNA was exposed to various concentrations of topsentin, completely relaxed with topoisomerase I, and bound topsentin removed by phenol extraction, no supercoiled DNA became apparent after electrophoresis in agarose gels. After relaxation and removal of bound drug, the final state of supercoiling depends on the number of negative supercoils removed before relaxation. The lack of supercoiled DNA following treatment with topsentin, at concentrations of DNA far in excess of those required for cytotoxicity, indicates that this agent did not intercalate. Instead, competition binding experiments with Hoechst 33342 and CC-1065 indicate that topsentin interacts with the minor groove of DNA. Direct interaction between topsentin and CC-1065 or Hoechst 33342 could explain the observations made, but results indicating an interaction between topsentin and DNA with structurally unrelated compounds suggest that this possibility is unlikely.

The marine environment has yielded novel classes of compounds with diverse biological activities [24], but remains the largest and least chemically investigated source of new chemotherapeutic agents. Bioassay-guided isolation of compounds from a

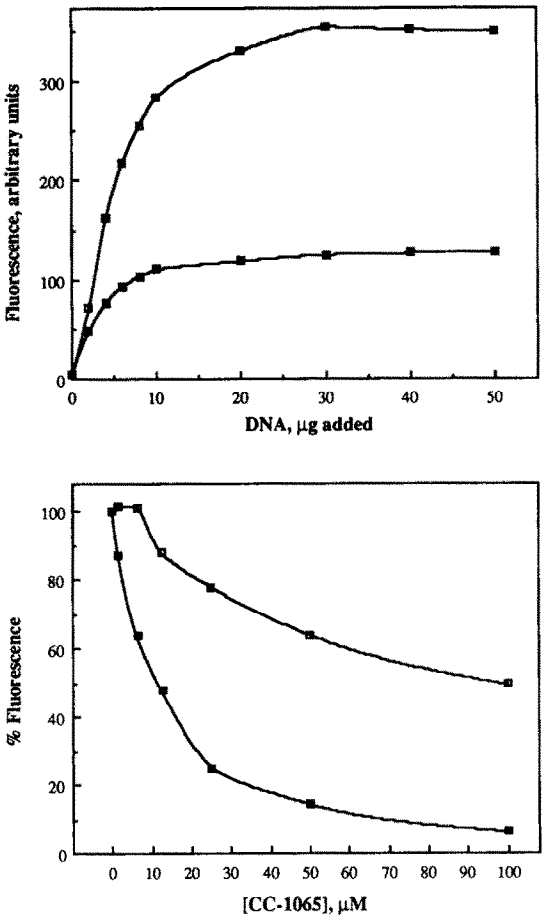

Fig. 6. Competitive binding experiments with known minor groove binders. (Top panel) Effect of topsentin on apparent binding of Hoechst 33342 (10 μM) to DNA. Binding of Hoechst 33342 to DNA in the absence (□) or presence (■) of 100 μM topsentin was assessed by increases in fluorescence monitored at the excitation (310 nm) and emission (512 nm) maxima of the Hoechst 33342–DNA complex. (Bottom panel) Effects of CC-1065 on apparent binding of topsentin to DNA. The fluorescence at the excitation and emission maxima of the topsentin–DNA complex [a mixture of topsentin (10 μM) and DNA (1 mg/mL)] was measured in the presence (■) or absence (□) of the concentration of CC-1065 indicated (dissolved in DMSO).

marine *Spongosorites* sp. sponge has resulted in the identification of topsentin, a new antitumor natural product that binds DNA in the minor groove. Further characterization of the mechanism of action of topsentin may provide a basis for structural modifications that lead to increased potency and antitumor utility.

REFERENCES

1. Pelton JG and Wemmer DE, Structural characterization of a 2:1 distamycin A·d(CGCAAATTGGC) complex by two-dimensional NMR. *Proc Natl Acad Sci USA* **86**: 5723–5727, 1989.
2. Pullman B, Molecular mechanisms of specificity in DNA–antitumour drug interactions. *Adv Drug Res* **18**: 1–113, 1989.
3. Cons BMG and Fox KR, High resolution hydroxyl radical footprinting of the binding of mithramycin and related antibiotics to DNA. *Nucleic Acids Res* **17**: 5447–5457, 1989.
4. Reynolds VL, McGovern JP and Hurley LH, The chemistry, mechanism of action and biological properties of CC-1065, a potent antitumor antibiotic. *J Antibio (Tokyo)* **XXXIX**: 319–334, 1986.
5. Petrusek RL, Anderson GL, Garner TF, Fannin QL, Kaplan KJ, Zimmer SG and Hurley LH, Pyrrolo[1,4] benzodiazepine antibiotics. Proposed structures and characteristics of the *in vitro* deoxyribonucleic acid adducts of anthramycin, tomaymycin, sibiromycin, and neothramycins A and B. *Biochemistry* **20**: 1111–1119, 1981.
6. Sobell HM and Jain SC, Stereochemistry of actinomycin binding to DNA. II. Detailed molecular model of actinomycin–DNA complex and its implications. *J Mol Biol* **68**: 21–34, 1972.
7. Ughetto G, Wang AH-J, Quigley GJ, van der Marel GA, van Boom JH and Rich A, A comparison of the structure of echinomycin and triostin A complexed to a DNA fragment. *Nucleic Acids Res* **13**: 2305–2323, 1985.
8. Liaw YC, Gao YG, Robinson H, van der Marel GA, van Boom JH and Wang AHJ, Antitumor drug nogalamycin binds DNA in both grooves simultaneously: Molecular structure of nogalamycin–DNA complex. *Biochemistry* **28**: 9913–9918, 1989.
9. McHugh MH, Woynarowski JM, Sigmund RD and Beerman TA, Effect of minor groove binding drugs on mammalian topoisomerase I activity. *Biochem Pharmacol* **38**: 2323–2328, 1989.
10. Woynarowski JM, McHugh M, Sigmund RD and Beerman TA, Modulation of topoisomerase II catalytic activity by DNA minor groove binding agents distamycin, Hoechst 33258, and 4′,6-diamidine-2-phenylindole. *Mol Pharmacol* **35**: 177–182, 1989.
11. Bodley AL and Liu LF, Role of DNA topoisomerases in drug cytotoxicity and drug resistance. In: *Mechanisms of Drug Resistance in Neoplastic Cells* (Eds. Wooley PV III and Tew KD), pp. 137–162. Academic Press, San Diego, 1988.
12. Collins SJ, Gallo RL and Gallagher RE, Continuous growth and differentiation of human myeloid leukemic cells in suspension culture. *Nature* **270**: 347–349, 1977.
13. Fogh J and Trempe G, New human tumor cell lines. In: *Human Tumor Cells In Vitro* (Ed. Fogh J), pp. 115–141. Plenum, New York, 1975.
14. Giard DJ, Aaronsonk SA, Todaro GJ, Arnstein P, Kersey JH, Dosik H and Parks WP, *In vitro* cultivation of human tumors. Establishment of cell lines derived from a series of solid tumors. *J Natl Cancer Inst* **51**: 1417–1423, 1973.
15. Bhalla K, Hindenburg A, Taub RN and Grant S, Isolation and characterization of an anthracycline-resistant human leukemic cell line. *Cancer Res* **45**: 3657–3662, 1985.
16. Burres NS and Clement JJ, Antitumor activity and mechanisms of action of the novel marine natural products mycalamide-A and -B and onnamide. *Cancer Res* **49**: 2935–2940, 1989.
17. Chen RR, *In situ* detection of mycoplasma contamination in cell cultures by fluorescent Hoechst 33258 stain. *Exp Cell Res* **104**: 255–262, 1977.
18. Alley MC, Scudiero DA, Monks A, Hursey ML, Czerwinski MJ, Fine DL, Abbott BJ, Mayo JG, Shoemaker RH and Boyd MR, Feasibility of drug screening with panels of human tumor cell lines using

a microculture tetrazolium assay. *Cancer Res* **48**: 589–601, 1988.

19. Finney DJ, *Statistical Method in Biological Assay*, 3rd Edn, pp. 316–348. Charles Griffin Company, London, 1978.
20. Burres NS, Sazesh S, Gunawardada GP and Clement JJ, Antitumor activity and nucleic acid binding properties of dercitin, a new acridine alkaloid isolated from a marine *Dercitus* species sponge. *Cancer Res* **49**: 5267–5274, 1989.
21. Tsujii S, Rinehart KL, Gunasekera SP, Kashman Y, Cross SS, Lui MS, Pomponi SA and Diaz MC, Topsentin, bromotopsentin, and dihydrodeoxybromotopsentin: Antiviral and antitumor bis(indolyl)imidazoles from Caribbean deep-sea sponges of the family halichondriidae. Structural and synthetic studies. *J Org Chem* **53**: 5446–5453, 1988.
22. Burres NS, Hunter JE and Wright AE, A mammalian cell agar-diffusion assay for the detection of toxic compounds. *J Nat Prod* **52**: 522–527, 1989.
23. Bartik K, Braekman J-C, Daloze D, Stoller C, Huysecom J, Vandevyver G and Ottinger R, Topsentins, new toxic bis-indole alkaloids from the marine sponge *Topsentia genitrix*. *Can J Chem* **65**: 2118–2121, 1987.
24. Faulkner DJ and Fenical WH, *Marine Natural Products Chemistry*. Plenum Press, New York, 1976.