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Stereo(C7)-dependent Topoisomerase II Inhibition and Tumor Growth Suppression by a New Quinolone, BO-2367

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A new antimicrobial quinolone (−)BO-2367, (−)-7-[(1R*,2R*,6R*)-2-amino-8-azabicyclo[4.3.0.]-non-3-en-8-yl]-1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid, strongly inhibited both mammalian and bacterial topoisomerase II. The IC_{50} values of (−)BO-2367 against the DNA relaxation activity of L1210 topoisomerase II and the supercoiling activities of *Escherichia coli* gyrase and *Micrococcus luteus* gyrase were 3.8, 0.5, and 1 μM, respectively. This compound enhanced double-stranded DNA cleavage mediated by topoisomerase II not only with purified enzyme, but also with intact L1210 cells. All these activities of (−)BO-2367 were more than 2-fold stronger than those of VP-16. Intriguingly, (+)BO-2367, which has an enantiomeric substituent at the C7 position of (−)BO-2367, did not affect the activity of the mammalian topoisomerase II, while it inhibited *E. coli* gyrase. Intraperitoneal injection of (−)BO-2367 at 0.08 mg/kg increased the lifespan of CDF_1 female mice bearing ascitic L1210 leukemia by 2.4 times, and subcutaneous injection at 1.25 mg/kg completely inhibited the growth of colon 26 carcinoma implanted subcutaneously. These results suggest that (−)BO-2367 is a potent antitumor agent which targets topoisomerase II. These enantiomers should be a useful tool for studying drug-topoisomerase II interactions.

Key words: Topoisomerase II — DNA gyrase — Quinolone — DNA cleavage — Anticancer agent

DNA topoisomerases II is one of the key enzymes that catalyze the topological change of DNA in nucleic acid metabolism, including DNA replication, RNA transcription, and chromosomal segregation. Therefore it is an attractive intracellular target for anticancer chemotherapy.[1–3] In fact, the well-known antitumor agents VP-16, mAMSA, adriamycin, ellipticine, and mitoxantrone are topoisomerase II inhibitors.[4] Bacterial topoisomerase II (DNA gyrase) has been a major target in the field of antibacterial chemotherapy. Many quinolone antibacterial agents based on this mechanism have been synthesized, and applied clinically.

Eukaryotic topoisomerase II relaxes supercoiled DNA, while bacterial topoisomerase II supercoils relaxed DNA. Despite the difference in the physiological action of these enzymes, DNA sequence analysis has revealed homology between eukaryotic topoisomerase II and DNA gyrase.[5, 6] Furthermore, some inhibitors, such as quinolones (for DNA gyrase) and VP-16 (for mammalian topoisomerase II), inhibit their target enzymes by very similar modes of action: both inhibitors form ternary complexes with DNA and the enzyme, and thereby cause double-stranded cleavage of the DNA.[7, 8] Thus, quinolones may have the potential to inhibit mammalian topoisomerase II.

We evaluated the effect on mammalian topoisomerase II of forty-two antimicrobial quinolones newly synthesized in our institute. It was discovered that (−)BO-2367 inhibited mammalian topoisomerase II more potently than VP-16 and prevented tumor growth in mice. In this paper, the inhibitory activity of (−)BO-2367 on L1210 topoisomerase II and the compound's antitumor activity *in vivo* are reported.

MATERIALS AND METHODS

Cells and mice Murine leukemia L1210 cells were purchased from Flow Laboratories Co., Hamden, CO. Murine leukemia P388 cells and colon 26 cells were kindly given by Dr. T. Tsuruo, Institute for Applied Microbiology, University of Tokyo. CDF_1 female mice were purchased from Charles River, Kanagawa.

Enzymes and DNA Topoisomerase II was purified from L1210 cells as described previously.[9] One unit of the enzyme was defined as the activity to relax completely 0.4 μg of supercoiled pBR322 DNA. Gyrase A and B subunits were separately purified from the lysate of *Escherichia coli* (NIHJ JC-2) according to the method reported by Otter and Cozzarelli.[10] *Micrococcus luteus*

Abbreviations: VP-16, 4′-demethylepipodophyllotoxin-9-(4,6-O-ethylidene-β-D-glucopyranoside); mAMSA, 4′-(9-acridinylamino)methanesulfon-*m*-anisidide; Tris, Tris[hydroxymethyl]-aminomethane; ATP, adenosine 5′-triphosphate; EDTA, ethylenediaminetetraacetic acid; SDS, sodium dodecyl sulfate; PBS^{2-}, Dulbecco's phosphate-buffered saline (Ca^{2+}, Mg^{2+}-free); TBE, 90 m*M* Tris, 90 m*M* boric acid, and 4 m*M* EDTA.

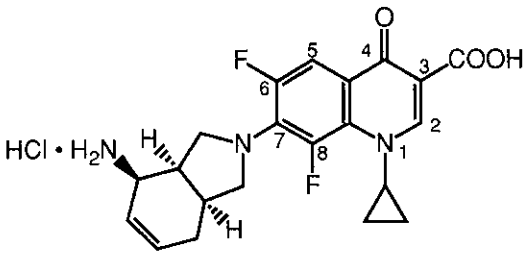

Fig. 1. Structure of (−)BO-2367.

gyrase was purchased from Bethesda Research Laboratories Life Technologies, Inc., Gaitherburg, MD. Relaxed DNA was prepared by incubating the supercoiled pBR322 plasmid DNA with L1210 topoisomerase I in the presence of T4 DNA ligase purchased from Takara Shuzo Co., Ltd., Kyoto.

BO-2367 and other reagents Synthetic methods for (−)BO-2367 (Fig. 1) and the (+)-enantiomer will be reported in detail elsewhere. Briefly, both enantiomers were synthesized by reacting the optically resolved side-chain amine, 2-amino-8-azabicyclo[4.3.0]non-3-ene tartrate, with a quinolinecarboxylic acid moiety, 1-cyclopropyl-6,7,8-trifluoroquinolinecarboxylic acid. Optical resolution of the side-chain amine was carried out by using the combination of the free base and the N-monoacylated form with tartaric acid. The $[\alpha]_D^{20}$ values of the obtained (−)- and (+)-enantiomers were −192° and +202° (c=0.5, 0.01 N HCl), respectively.

Norfloxacin and ciprofloxacin were synthesized at our institute. VP-16 was obtained from Nippon Kayaku Co., Ltd., Tokyo. Proteinase K was purchased from E. Merck, Darmstadt, Germany. Other reagents were from Sigma Chemical Co., St. Louis, MO.

Topoisomerase II-mediated DNA relaxation Supercoiled pBR322 plasmid DNA (0.4 μg) was relaxed with 1 unit of topoisomerase II in 20 μl of the relaxation assay buffer (50 mM Tris-HCl, pH 7.9, 120 mM KCl, 20 mM $MgCl_2$, 0.5 mM dithiothreitol, 0.5 mM EDTA, 1 mM ATP and 30 μg/ml bovine serum albumin) in the presence or absence of test compounds at 30°C for 15 min. The reaction was stopped by adding 5 μl of 0.5% SDS solution containing 50 mM EDTA and the DNA was electrophoresed in 1.2% agarose gel with TBE (0.5×) buffer at 1.6 V/cm for 11 h. The DNA was stained with ethidium bromide and photographed using a UV light. The amount of supercoiled DNA in the agarose gel was quantified by using a densitometer, flying spot scanner C-9000, Shimadzu Co., Kyoto.

Topoisomerase II-mediated DNA cleavage Cleavage reaction was carried out by incubating 0.4 μg of supercoiled pBR322 DNA with 10 units of topoisomerase II in 20 μl of the relaxation assay buffer at 30°C. After 15-min incubation, the reaction was stopped with 3 μl of 5% SDS and the denatured enzyme was digested by adding 3 μl of 10 mg/ml proteinase K. The DNA was subjected to 1.2% agarose gel electrophoresis in TBE (0.5×) buffer supplemented with 0.5 μg/ml ethidium bromide.

Cleavable complex formation in cells The formation of cleavable complex was determined by the K^+/SDS method described previously.[9] Briefly, L1210 cells were pre-labeled with [^{3}H]thymidine and then exposed to samples for 1 h at 37°C. The cells were lysed with 1.5% SDS solution containing 5 mM EDTA. DNA in the lysate was homogeneously sheared by pipetting at 65°C and then KCl was added to the lysate at the concentration of 65 mM. The K^+/SDS precipitate was formed on ice and submitted to liquid scintillation counting after removing the protein-unbound radioactive DNA.

Pulse field gel electrophoresis L1210 cells were incubated with a test sample at 37°C for 1 h. After washing with PBS^{2-} containing the sample, the cells were enclosed in 0.6% low-melting-point agarose and gently shaken in a solution containing 1% sodium N-lauroyl sarcosinate, 1 mg/ml proteinase K and 0.5 M EDTA (pH 9.0) at 50°C for 48 h. The soft gel was washed 4 times with 10 mM Tris-HCl buffer (pH 7.8) containing 1 mM EDTA and inserted into 1.2% agarose gel. The electrophoresis was performed using a Pulsaphor™ system LKB 2015, Pharmacia LKB Biotechnology, Uppsala, Sweden. The samples were run at 100 V in TBE (0.5×) with linear gradient pulses from 50 to 120 s in double homogeneous fields with hexagonal electrodes for 40 h. The chromatin of *Saccharomyces cerevisiae* was used for size markers.

Gyrase-mediated DNA supercoiling Reaction mixtures (25 μl each) containing 35 mM Tris-HCl (pH 7.5), 20 mM $MgCl_2$, 20 mM KCl, 0.1 mM EDTA, 10 mM 2-mercaptoethanol, 2 mM spermidine, 10 μg/ml bovine serum albumin, 0.5 μg of relaxed pBR322 DNA, 1 unit of *E. coli* or *M. luteus* gyrase, and test samples were incubated at 37°C for 30 min. The reaction was terminated by adding 5 μl of 0.5 % SDS and 10 mM EDTA and the DNA was analyzed by the same procedure as the relaxation assay. The IC_{50} values were calculated from the percentage of supercoiled DNA obtained by densitometric analysis.

Cytotoxicity L1210 or P388 cells were cultured with RPMI 1640 containing 10% fetal bovine serum (and 20 μM 2-mercaptoethanol for P388) at a density of 3×10^4 cells/ml. After culture for 3 days with test samples, the number of living cells was counted with a Coulter counter.

Antitumor activity in mice L1210, P388 and colon 26 cells were cultured *in vitro* as described under "Cells and Mice." CDF_1 mice (5 weeks old) were injected i.p. with

1×10^6 cells/mouse of L1210 cells or 1×10^4 cells/mouse of P388 cells on day 0 and were treated i.p. with graded doses of compounds once daily for 10 days from day 1 to 10. Survival was recorded for 60 days. As for colon 26, 1×10^6 cells were implanted s.c. into a flank of CDF_1 mice on day 0 and a test compound was injected s.c. once daily for 20 consecutive days beginning one day after the implantation. Mice were killed on day 28 and the tumors were weighed. Statistical analysis was done with Student's *t* test and the Mann-Whitney U-test.

RESULTS

Inhibitory effects of (−)BO-2367 and (+)BO-2367 on the DNA-relaxing activity of mammalian topoisomerase II To determine the effects of (−)- and (+)BO-2367 on the catalytic activity of mammalian topoisomerase II, the inhibitory effects on DNA relaxation were measured (Fig. 2) and the IC_{50} values were evaluated by densitometric analyses. The IC_{50} values of (−)- and (+)BO-2367 were 3.8 μM and 62 μM, respectively, indicating that the inhibitory effect of BO-2367 on topoisomerase II-mediated relaxation was specific to the (−)-enantiomer. The effect of (−)BO-2367 was about 2-fold stronger than that of VP-16 (IC_{50}: 7.4 μM).

Norfloxacin and ciprofloxacin, both of which are potent antibacterial quinolones, did not interfere with the relaxing activity of topoisomerase II at 150 μM (data not shown).

Enhancement of topoisomerase II-mediated DNA cleavage by (−)- and (+)BO-2367 In the presence of topoisomerase II, (−)BO-2367 induced double-stranded DNA cleavage at concentrations greater than 3 μM, while (+)BO-2367 did not induce any cleavage even at 30 μM (Fig. 3a). Since the concentration-response relation of each compound was different (Fig. 3b), the activities on the cleavage reaction were not simply compared in terms of the EC values (the values of effective concentration inducing a designated amount of cleaved DNA). When compared at each concentration tested, the effect of (−)BO-2367 on the cleavage was 2 times or more as potent as that of VP-16.

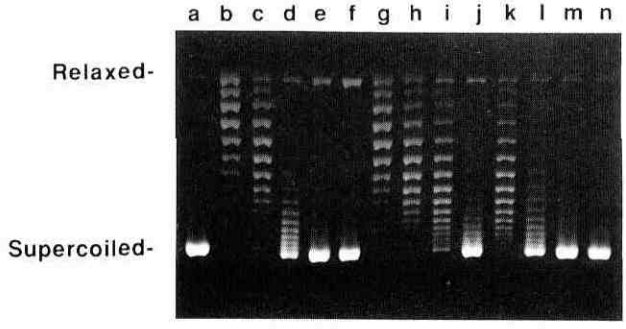

Fig. 2. Effects of (−)BO-2367 and (+)BO-2367 on DNA-relaxing activity of L1210 topoisomerase II. Supercoiled pBR-322 DNA was incubated with 1 unit of L1210 topoisomerase II in the presence or absence of samples. Lane a, no enzyme control; lane b, enzyme control; lane c–f, 1, 3, 10, 30 μM (−)BO-2367; lane g–j, 3, 10, 30, 100 μM (+)BO-2367; lane k–n, 3, 10, 30, 100 μM VP-16.

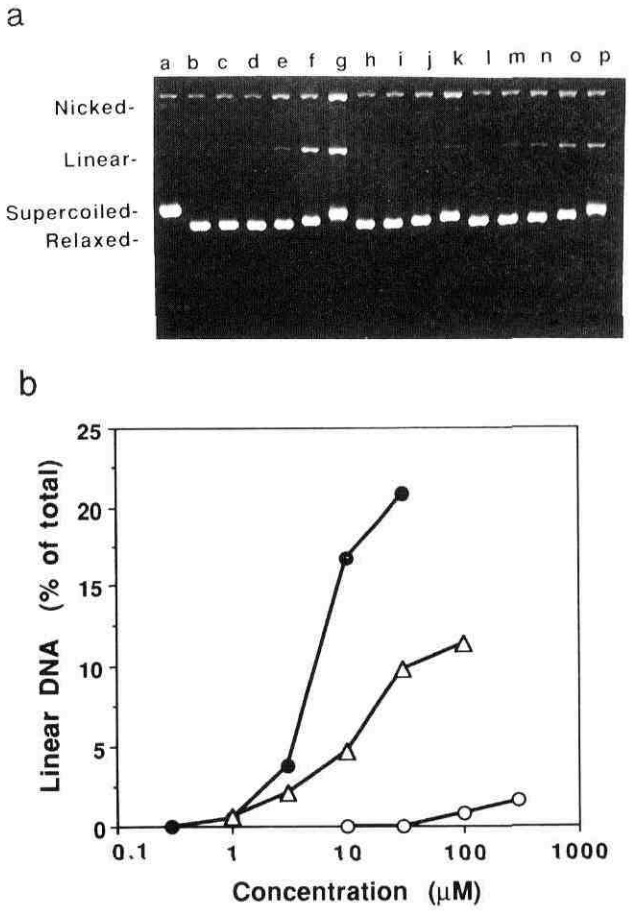

Fig. 3. Effects of (−)BO-2367 and (+)BO-2367 on topoisomerase II-mediated DNA cleavage. Supercoiled pBR322 DNA were incubated with 10 units of L1210 topoisomerase II in the presence or absence of samples. After treatment with SDS-proteinase K, the DNA was analyzed on 1% agarose gel containing 0.5 μg/ml ethidium bromide. (a) Lane a, no enzyme control; lane b, enzyme control; lane c–g, 0.3, 1, 3, 10, 30 μM (−)BO-2367; lane h–k, 10, 30, 100, 300 μM (+)BO-2367; lane l–p, 1, 3, 10, 30, 100 μM VP-16. (b) The linear DNA formation induced by (−)BO-2367 (●), (+)BO-2367 (○), and VP-16 (△) in Fig. 3a was quantified by densitometry.

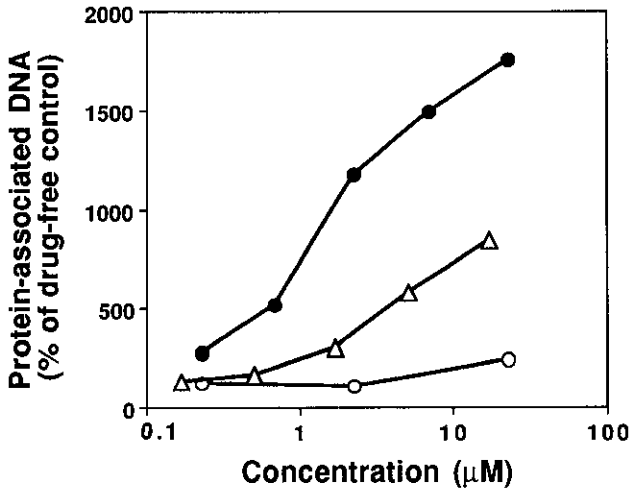

Fig. 4. Effects of (−)BO-2367 and (+)BO-2367 on the formation of cleavable complexes in L1210 cells. L1210 cells (1×10^5) labeled with [^{3}H]dThd were incubated with (−)BO-2367 (●), (+)BO-2367 (○) and VP-16 (△) at various concentrations for 1 h. The ordinate indicates the radioactivity of K^+/SDS precipitates of drug-treated cells as a percentage of that of untreated cells.

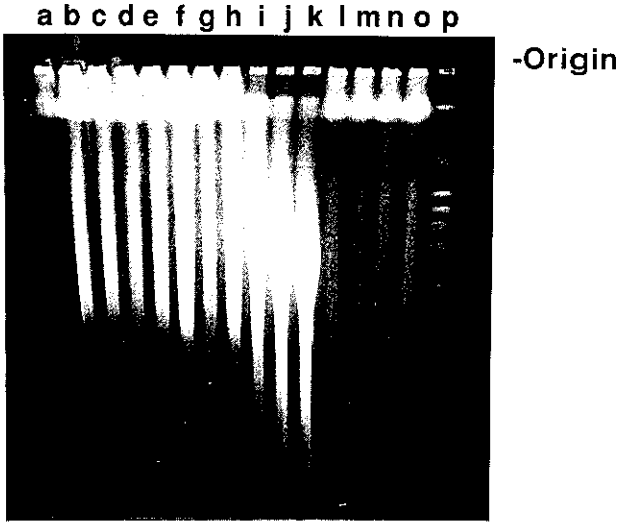

Fig. 5. Induction of double-strand DNA cleavage by (−)BO-2367 and (+)BO-2367 in L1210 cells. L1210 cells were incubated with various concentrations of drugs for 1 h and the cellular DNA was analyzed by using pulse field gel electrophoresis. Lane a, no-drug control; lane b–f, 0.51, 1.7, 5.1, 17, 51 μM VP-16; lane g–k, 0.69, 2.3, 6.9, 23, 69 μM (−)BO-2367; lane l–o, 2.3, 6.9, 23, 69 μM (+)BO-2367. Lane p, chromatin DNA of *S. cerevisiae* as size markers; from top to bottom, 1900, 1640, 1120/1100, 945, 915/815, 785/745, 680, 660/555 base pairs, respectively.

Induction of DNA-topoisomerase complex formation by (−)BO-2367 and (+)BO-2367 in intact cells When L1210 cells were exposed to (−)BO-2367 at concentrations from 0.23 to 69 μM for 1 h, the amounts of DNA-topoisomerase complex increased to 3–17 times that of the enzyme control (Fig. 4). In accordance with the result on topoisomerase II-mediated DNA cleavage in enzyme assay, (−)BO-2367 was at least two times more effective than VP-16 against the formation of cleavable complex and the concentration-response relation of (−)BO-2367 was steeper than that of VP-16. (+)BO-2367 produced little complex at 23 μM.

Induction of double-strand DNA breakage by (−)BO-2367 and (+)BO-2367 in intact cells To confirm that the DNA-topoisomerase complex induced by BO-compounds brought about double-stranded DNA breakage in cells, L1210 cells exposed to test compounds were submitted to pulse field gel electrophoresis. (−)BO-2367 fragmented the DNA dose-dependently to pieces of less than 10^6 base pairs (Fig. 5). Consistent with the formation of DNA-protein complex, the induction of DNA breakage by (−)BO-2367 (0.69–69 μM) was more potent than that by VP-16 (0.51–51 μM). (+)BO-2367 was completely ineffective at concentrations up to 69 μM.

Inhibitory effects of (−)BO-2367 and (+)BO-2367 on DNA gyrase (−)BO-2367 inhibited the DNA supercoiling activity of *E. coli* gyrase and *M. luteus* gyrase by 50% at 0.5 μM and 1.0 μM, respectively (Table I). (+)BO-2367 potently inhibited the supercoiling activity of *E. coli* gyrase, as did (−)BO-2367, ciprofloxacin and norfloxacin, while the effect of (+)BO-2367 on *M. luteus* gyrase was very weak, like that of ciprofloxacin and norfloxacin.

Cytotoxicity of (−)BO-2367 and (+)BO-2367 (−)BO-2367 inhibited the growth of murine leukemia P388 and L1210 cells in culture, giving IC_{50} values of 11 and 40 nM, respectively (Table II). The cytotoxic potency of (−)BO-2367 was comparable with that of VP-16, while the IC_{50} value of (+)BO-2367 was more than hundred-fold larger than that of (−)BO-2367.

Antitumor activity in mice (−)BO-2367 increased the life span of mice bearing ascitic L1210 or P388 leukemia and the effect was more marked in mice bearing L1210 (Table III). In mice implanted with L1210 i.p., although the mean survival time of control mice was longer than that usually observed, injection of (−)BO-2367 prolonged the survival period by more than 2 times compared with untreated tumor-bearing mice. The minimum effective dose was at most 0.02 mg/kg/day. Maximum tolerated dose was more than 0.313 mg/kg, but a dose of 1.25 mg/kg was toxic. In the case of P388-bearing mice, the effective dose range shifted one order magnitude: a dose of 0.02 mg/kg was ineffective, while a dose 1.25

Table I. Inhibitory Effect of (−)BO-2367 and (+)BO-2367 on DNA Supercoiling Activity of Gyrase

Compound	IC_{50} (μM)[a]	
	E. coli gyrase[b]	*M. luteus* gyrase
(−)BO-2367	0.5	1
(+)BO-2367	2.6	224
Ciprofloxacin	3.0	300
Norfloxacin	9.4	780

a) IC_{50} values were determined from the results of densitometric analysis.
b) Gyrase (1 unit) and relaxed pBR322 DNA were incubated with samples at 37°C for 30 min and subjected to gel electrophoresis.

Table II. Cytotoxicity of (−)BO-2367 and (+)BO-2367 toward L1210 and P388 Cell Lines

Compound	IC_{50} (μM)	
	L1210[a]	P388
(−)BO-2367	0.043	0.011
(+)BO-2367	4.3	1.3
VP-16	0.030	0.007

a) Cells plated at 10^4/well were cultured with samples for 3 days. Cell growth was monitored by using a Coulter counter.

Table III. Antitumor Activity of (−)BO-2367 on i.p.-Implanted Murine Leukemia

Cell line[a]	Compound	Treatment (i.p.)[b] (mg/kg/day)	Survival (days)	
			Mean±SD	T/C%
L1210	(−)BO-2367	0	13.7±2.58	(100)
		0.005	13.2±1.48	(96)
		0.020	31.0±2.92*	(226)
		0.078	32.4±4.93*	(236)
		0.313	21.2±4.32*	(155)
		1.25	9.4±1.34*	(69)
	VP-16	0.020	14.2±1.64	(104)
		0.078	22.8±8.20	(166)
		0.313	31.4±3.78*	(229)
P388	(−)BO-2367	0	11.2±0.42	(100)
		0.020	12.0±0.00	(107)
		0.078	14.0±0.71*	(125)
		0.313	16.4±0.55*	(146)
		1.25	15.2±1.48*	(136)
	VP-16	0.020	12.8±0.45*	(114)
		0.078	16.6±2.61*	(148)
		0.313	22.2±3.83*	(198)
		1.25	>29.0±2.24*	(>259)

a) Tumor: L1210, 1×10^6 cells/mouse; P388, 1×10^4 cells/mouse.
b) Treatment schedule: Q1D10.
* $P<0.05$ by Student's *t* test.

Table IV. Antitumor Activity of (−)BO-2367 on s.c.-Implanted Colon 26 Solid Tumor[a]

Compound	Dose[b] (mg/kg/day)	Tumor weight (g)	
		Mean±SD	% Inhibition
Control	0	3.46±0.50	0
(−)BO-2367	0.020	1.50±0.58*	57
	0.078	1.15±0.82*	67
	0.313	0.45±0.23*	87
	1.25	0.00±0.00**	100
VP-16	1.25	1.28±0.50*	63
	5.0	0.60±0.21*	83

a) Tumor: Colon 26, 1×10^6 cells/mouse.
b) Treatment schedule: Q1D20 (s.c.).
* $P<0.05$ by Student's *t* test.
** $P<0.05$ by the Mann-Whitney U-test.

mg/kg was still effective. The (+)BO-2367 did not exhibit any antitumor effect at up to 1.25 mg/kg on the P388 model (data not shown).

Antitumor effects on solid tumors were examined using colon 26 carcinoma (Table IV). I.p. treatment with (−)BO-2367 was ineffective at doses of 0.02 and 0.078 mg/kg/day (data not shown). When (−)BO-2367 was injected s.c. near the tumor, it inhibited the tumor growth by more than 50% even at 0.02 mg/kg. The inhibitory effect was dose-dependent, and at 1.25 mg/kg all mice (n=5) implanted with the tumor were cured. VP-16 needed higher doses to give comparable antitumor effects in mice.

DISCUSSION

Some quinolone compounds (CP-67,015,[11] -67,804, -115,953,[12] WIN58161[13] and S-116[14]) have recently been reported to inhibit mammalian topoisomerase II, but their inhibitory activity on topoisomerase II was less potent than that of VP-16, except for CP-115,953. It is noteworthy that the novel quinolone-based gyrase inhibitor (−)BO-2367 inhibited the DNA-relaxing activity of mammalian topoisomerase II and enhanced the topoisomerase II-mediated double-stranded cleavage more potently than VP-16. The effect of (−)BO-2367 on the formation of cleavable complex and the subsequent double-stranded DNA cleavage in intact cells was also stronger than that of VP-16.

(−)BO-2367 inhibited L1210 topoisomerase II by stabilizing DNA-topoisomerase II complex in the same way as VP-16. However, its profile of action on topoisomerase II was a little different from that of VP-16, as judged from their effects on DNA relaxation and DNA cleavage by topoisomerase II. (−)BO-2367 effectively stimulated the induction of DNA cleavage rather

than the inhibition of DNA relaxation, whereas VP-16 strongly inhibited DNA relaxation compared with the induction of DNA cleavage. This difference between (−)BO-2367 and VP-16 was more evident as their concentrations were increased. Osheroff *et al.* reported a difference in the mechanisms of inhibitory action on mammalian topoisomerase II between VP-16 and some CP-numbered quinolones.[12] Those quinolones stimulated the double-strand cleavage without significantly inhibiting the relaxing activity of mammalian topoisomerase II, which was considered to be due to the primary enhancement of cleavage rather than the inhibition of topoisomerase II-mediated religation of DNA.[12, 15] The character of (−)BO-2367 was consistent with their findings.

Various quinolone compounds have been reported to inhibit mammalian topoisomerase II, though there have been few papers on *in vivo* antitumor activity of quinolone compounds. Yamashita *et al.* recently reported the antitumor activity and the effect on topoisomerase II-mediated cleavage of some quinolone compounds, including S-116.[14] However, their activity on topoisomerase II-mediated cleavage *in vitro* and their maximal antitumor activity against P388 *in vivo* appeared to be lower than those of VP-16. Since (−)BO-2367 activated *in vitro* topoisomerase II-mediated cleavage more potently than did VP-16, (−)BO-2367 was expected to inhibit tumor growth *in vivo* more effectively. The minimum effective doses of (−)BO-2367 against P388, L1210 and colon 26 were lower than or comparable to those of VP-16. In contrast, the maximal antitumor activities of (−)BO-2367 were inferior to those of VP-16. Considering that (−)BO-2367 potently inhibited the growth of colon 26 when it was injected into a site near the tumor, the pharmacokinetic properties of quinolones may explain its lower antitumor effects compared to VP-16.[16]

BO-2367, CP-115,953 and S-116 are all C6, C8-difluoro, N1-cyclopropyl-substituted quinolones. This common structure is likely to play a role in inhibiting mammalian topoisomerase II. Although the inhibitory effect of (−)BO-2367 on *E. coli* gyrase was similar to that of (+)BO-2367, its effect on mammalian topoisomerase II was much stronger than that of (+)BO-2367. Therefore, the stereostructure of the C7 substituent of (−)BO-2367 contributed greatly to the specific inhibition of mammalian topoisomerase II. This evidence indicates that a fine difference of the structure of the C7 substituent, including stereoisomerism, greatly affects the interaction with not only gyrase but also mammalian topoisomerase II. Furthermore, (+)BO-2367, as well as ciprofloxacin and norfloxacin, exhibited only a slight inhibition of *M. luteus* gyrase and mammalian topoisomerase II, whereas they inhibited the *E. coli* gyrase as effectively as did (−)BO-2367. If the substituent at the C7 position is the drug-enzyme interaction domain, as proposed by Shen *et al.*,[17] mammalian topoisomerase II and *M. luteus* gyrase may share similar modes of drug interaction, while that of *E. coli* gyrase may be somewhat different.

In conclusion, enantiomers of BO-2367 are very helpful for analyzing the stereostructure of the drug-DNA-enzyme (gyrase or topoisomerase II) complex, as were enantiomeric ofloxacin derivatives.[18] An X-ray crystallographic analysis of (−)- and (+)BO-2367 is in progress. (−)BO-2367 may be a good lead compound for antineoplastic agents of a new type.

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