Journal of Enzyme Inhibition and Medicinal Chemistry, December 2003 Vol. 18 (6), pp. 497–503

Taylor & Francis
healthsciences

2070-DTI, A Topoisomerase Inhibitor Produced by *Streptomyces* sp. Strain No. 2070

KEITAROU SUZUKI, SATORU DOI, SHOJI YAHARA and MASARU UYEDA*

Department of Pharmaceutical Microbiology, Faculty of Medical and Pharmaceutical Sciences, Kumamoto University, Kumamoto 862-0973, Japan

(Received 26 June 2003; In final form 28 July 2003)

A novel inhibitor of topoisomerase II designated as 2070-DTI was isolated from the culture filtrate of *Streptomyces* sp. strain No. 2070. The structure was determined to be that of the known soyasaponin I on the basis of spectroscopic methods (NMR and MS). 2070-DTI strongly inhibited the decatenation activity of human placenta topoisomerase II in a noncompetitive manner, and weakly inhibited or was inert towards the relaxation activities of various topoisomerase I's and DNA-related enzymes. 2070-DTI is an inhibitor belonging to the cleavable complex-nonforming type without DNA intercalation.

Keywords: Topoisomerase inhibitor; *Streptomyces*; soyasaponin; 2070-DTI

INTRODUCTION

Topoisomerases I and II (topo I and topo II) are essential nuclear enzymes that catalyze the concerted breaking and rejoining of DNA strands, and the enzymes are involved in producing the necessary topological and conformational changes in DNA which are critical to many cellular processes such as replication, recombination and transcription.[1] In addition to their normal cellular functions, both enzymes are known as important cellular targets of antitumor drugs[2] and some drugs preventing the actions of the enzymes are used clinically in the treatment of a variety of cancers. Many topo inhibitors have been reported and these inhibitors are classified into three types; the cleavable complex-forming types with and without DNA intercalation, and the cleavable complex-nonforming type. The inhibitors of the first and second types inhibit the DNA rejoining reaction of topo by holding together the cleavable complex consisting of the enzyme and broken DNA. Doxorubicin[3] and amsacrine[4] are inhibitors of the first type, and camptothecin[5] and epipodophyllotoxin (etoposide)[6] are known as the inhibitors of second type. The inhibitors of the two types promote the accumulation of damaged DNA in the cells and therefore arrest cell cycle progression.[7,8] Inhibitors of the third type such as epigallocatechingallate[9] and the diketopiperazine family[10,11] inhibit the DNA breaking and rejoining reactions of topo by a direct action on the enzyme molecule without forming the cleavable complex. Inhibitors of this type, the cleavable complex-nonforming type, do not cause DNA damage. In the search for topo inhibitors, we have screened various microorganisms and isolated five kinds of inhibitors designated as 2280-DTI, 2890-DTI,[12] macrostatin,[13] topostatin[14–16] and isoaurostatin[17] all of which are inhibitors belonging to the cleavable complex-nonforming type. Here, we report a new topo inhibitor designated as 2070-DTI isolated from the culture filtrate of *Streptomyces* sp. strain No. 2070. This report describes the isolation, structure, inhibitory properties, effects on the cell cycle and DNA of 2070-DTI.

MATERIALS AND METHODS

Enzymes, Substrates and Inhibitors

Topo I from calf thymus gland (EC 5.99.1.2), T4 DNA ligase (EC 6.5.1.1), *Bam* HI (EC 3.1.23.6), *Eco* RI (EC 3.1.23.13), *Hin* dIII (EC 3.1.23.21), supercoiled

*Corresponding author. Tel./fax: +81-96-371-4323. E-mail: uyeda@gpo.kumamoto-u.ac.jp

ISSN 1475-6366 print/ISSN 1475-6374 online © 2003 Taylor & Francis Ltd
DOI: 10.1080/1475636031000161307 6

pBR322 and pUC19 DNA were obtained from MBI Fermentas (Vilnius). Topo II from human placenta (EC 5.99.1.3) and kinetoplast DNA were obtained from TopoGEN (Columbus). *Alu* I (EC 3.1.23.1), *Sca* I (EC 3.1.21.4) and *Pst* I (EC 3.1.23.31) were obtained from Gibco BRL (Tokyo). Na^+, K^+-ATPase (EC 3.6.1.3), DNase I (EC 3.1.21.1), DNase II (EC 3.1.22.1), RNase A (EC 3.1.27.5), yeast RNA and calf thymus DNA were obtained from Sigma (Tokyo). The method for preparation of topo I from COLO 201 (human colon carcinoma), HeLa (human cervix carcinoma), A549 (human lung carcinoma), Vero (african monkey kidney) and NIH3T3 (mouse embryo) cells has been described in a previous paper.[18] Camptothecin, etoposide and doxorubicin hydrochloride were obtained from Aldrich (Tokyo), Calbiochem (La Jolla) and Sigma, respectively.

Enzyme Reaction

Relaxation activities of topo I and II were measured by detecting the conversion of pBR322 DNA to its relaxed form.[19,20] Decatenation activity of topo II was measured by detecting the conversion of kinetoplast DNA (kDNA) to mini-circle monomers.[21] DNA cleavage activities of topo I and II were determined by measuring the increase of nicked and linearized pBR322 DNA induced by inhibitors, respectively.[5,21] Activities of restriction enzymes (*Bam* HI, *Eco* RI, *Hin* dIII, *Alu* I, *Sca* I and *Pst* I) and nucleases (DNase I, DNase II and RNase A) were determined by measuring the concentration of undigested supercoiled pBR322 DNA and RNA after enzyme reactions.[18] T4 DNA ligase activity was determined by measuring the ligation of linearized pUC19 DNA which was cleaved by *Hin* dIII.[18] Na^+, K^+-ATPase activity was determined by measuring the concentration of inorganic phosphate released from ATP by the enzyme.[22]

DNA Intercalation

DNA intercalation of the inhibitor was evaluated by the ethidium bromide (EtBr) competition assay and CD (circular dichroism) spectral change as described previously.[23] The intensity of fluorescence of EtBr and the CD spectrum of DNA were measured by a spectrofluorometer (Hitachi F-4010) and a spectropolarimeter (JASCO J-720), respectively. Thermal transition spectrum of DNA was measured in 10 mM phosphate buffer (2 ml) containing 100 mM NaCl, 40 μg calf thymus DNA by a spectrophotometer (Shimazu MaltiSpec-1500). The temperature increase rate was 1°C/min from 30°C to 95°C.

Production and Isolation of 2070-DTI

Streptomyces sp. strain No. 2070 was cultivated for 4 days at 28°C on a rotary shaker (180 rpm) in S medium composed of 2.0% glucose, 3.0% starch, 1.0% corn steep liquor, 1.0% soybean flour, 0.5% peptone, 0.3% NaCl and 0.5% $CaCO_3$ at pH 7.0. After cultivation, mycelia and cellular residues of the culture broth were removed by centrifugation at $7000 \times g$ at 5°C for 10 min. The supernatant was extracted with 2 volumes of 1-BuOH at pH 10 and the organic layer was concentrated *in vacuo*. The extract was applied to a column of Diaion HP-10. After washing with 40% MeOH, 2070-DTI was eluted with 70% MeOH and the eluate was applied to a column of Silica gel 60. 2070-DTI was eluted with $CHCl_3$–MeOH–H_2O (6:3:0.5) after washing with $CHCl_3$–MeOH (8:3). The eluate containing 2070-DTI was concentrated and applied to a column of ODS and eluted with 70% MeOH after washing with 40% MeOH. The eluate was dried *in vacuo* and termed purified 2070-DTI. Finally, 12 mg of 2070-DTI was obtained as an amorphous powder from 1000 ml of the culture filtrate.

Cell Cycle Analysis

HeLa cells were grown at 37°C in a humidified atmosphere containing 5% CO_2 in MEM medium, supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 unit/ml penicillin and 100 μg/ml streptomycin. For cell cycle analysis by flow cytometry, HeLa cells (1×10^6 cells) in exponential growth were treated with inhibitor at several concentrations for 24 hr. After treatment, the cells were washed with phosphate buffer saline (PBS, 8.4 mM $Na_2HPO_4 \cdot 12H_2O$, 1.5 mM KH_2PO_4, 136.9 mM NaCl, 2.7 mM KCl, pH 7.2) and fixed in cold 70% MeOH and stored at −20°C. The cell pellet was incubated with PBS containing 0.1% RNase for 40 min at 37°C and then 100 μl of propidium iodide at 500 μg/ml was added. The cells were analyzed with a flow cytometer (Becton Dickinson FACS Calibur) using the ModFit LT which is software for determining the percentage of cells in G_0/G_1, S and G_2/M phases.

RESULTS AND DISCUSSION

Structure Elucidation

2070-DTI gave a quasi molecular ion peak at *m/z* 986 $[M-H+2Na]^+$ in its positive ion FAB mass spectrum. As shown in Table I, the ^{1}H-NMR spectrum displayed signals for seven tertiary methyl groups (δ 0.83, 0.90, 0.91, 0.97, 1.01, 1.12 and 1.26), a secondary methyl group (δ 1.27), an oxygenated methylene (δ 3.21), two oxygenated

TABLE I ^{13}C and ^{1}H chemical shift assignments of 2070-DTI by HMQC, HMBC and H–H COSY spectra in pyridine d_5

Position	^{13}C value (δ)	^{1}H chemical shift
C-1	39.8 CH_2	1.00 1.65
C-2	27.3 CH_2	1.76 1.84 or 1.76 1.95
C-3	92.8 CH	3.40 (m)
C-4	44.8 tert-C	–
C-5	57.5 CH	0.94
C-6	19.4 CH_2	0.97 1.62
C-7	34.4 CH_2	1.40 1.57
C-8	40.8 tert-C	–
C-9	49.0 CH_2	1.57
C-10	37.5 tert-C	–
C-11	24.9 CH_2	1.85 1.87
C-12	123.7 CH	5.24 (br, s)
C-13	145.2 tert-C	–
C-14	43.4 tert-C	–
C-15	26.9 CH_2	1.76 1.95 or 1.76 1.84
C-16	29.9 CH_2	1.27 1.75
C-17	38.6 tert-C	–
C-18	46.8 CH	2.10
C-19	47.5 CH_2	–
C-20	31.4 tert-C	0.95 175
C-21	42.2 CH_2	1.32 1.46
C-22	76.7 CH	3.37 (m)
C-23	23.4 CH_3	1.26
C-24	64.3 CH_2	3.21 4.13
C-25	16.4 CH_3	0.90
C-26	17.6 CH_3	0.97
C-27	25.5 CH_3	1.12
C-28	29.1 CH_3	1.01
C-29	32.6 CH_3	0.91
C-30	20.5 CH_3	0.83
GlcUA-1	105.7 CH	4.49 (d, 7.3 Hz)
GlcUA-2	78.1 CH	3.58
GlcUA-3	76.4 CH	3.55
GlcUA-4	74.3 CH	3.41
GlcUA-5	76.5 CH	3.41
GlcUA-6	172.5 >C=O	–
Gal-1	102.2 CH	4.87 (d, 7.3 Hz)
Gal-2	78.0 CH	3.64
Gal-3	77.0 CH	3.76
Gal-4	77.1 CH	3.37
Gal-5	71.6 CH	3.72
Gal-6	62.4 CH_2	3.70 3.76
Rha-1	102.3 CH	5.14 (s)
Rha-2	72.2 CH	3.70
Rha-3	72.2 CH	3.92
Rha-4	73.7 CH	3.50
Rha-5	69.5 CH	4.08
Rha-6	18.4 CH_3	1.27 (d, 6.0 Hz)

methines (δ 3.40 and 3.37), an olefinic proton (δ 5.24), and three anomeric protons (δ 4.49, 4.87 and 5.14). The ^{13}C-NMR spectrum (Table I) of 2070-DTI exhibited 48 carbon signals attributable to a α-rhamnopyranosyl residue, a β-galactopyranosyl residue shifted at C-2 and a β-glucuronopyranosyl residue shifted at C-2 in the sugar moiety, two oxygenated methines (δ 92.8 and 76.7), an oxygenated methylene (δ 64.3), two olefinic carbon signals (δ 123.7 and 145.2), seven methyls, nine methylenes, three methines, and six quaternary carbons in aglycone moiety. These results suggest that 2070-DTI is a type of soyasaponin containing three sugars. By comparative studies on the H–H COSY, HMQC and HMBC spectra of 2070-DTI with those of soyasaponins, the structure of 2070-DTI was determined to be soyasapogenol B-3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1 → 2)-β-D-glucuronopyranoside (soyasaponin I)[24,25] as shown in Figure 1.

Inhibition of Topoisomerase Relaxation and Decatenation Activities by 2070-DTI

Inhibitory activities of 2070-DTI against topo I and II were measured by relaxation and decatenation assays. As shown in Figure 2, relaxation and decatenation activities of topo II were strongly inhibited in the presence of increasing 2070-DTI and 50% inhibitory concentrations (IC_{50}) were 24 and 18 μM, respectively. Alternatively, topo I was weakly inhibited by 2070-DTI with an IC_{50} of 123 μM.

Inhibitory Spectrum of 2070-DTI

The inhibitory effects of 2070-DTI on various topo I's and DNA-related enzymes were examined and are summarized in Table II. For comparison, camptothecin, etoposide and doxorubicin were also examined as selective inhibitors against topo I and II, respectively. 2070-DTI showed strong inhibition against topo II, and weak inhibition against topo I from Vero cells. Coumarin compounds such as novobiocin and coumermycin are known as topo II inhibitors which inhibit the ATPase activity of topo II.[26] Novobiocin strongly inhibited Na^+, K^+-ATPase ($IC_{50} = 19\,\mu M$) as well as topo II ($IC_{50} = 27\,\mu M$) in our assay systems. 2070-DTI showed no inhibition against Na^+, K^+-ATPase, therefore the inhibition of 2070-DTI against topo II may not be related to the ATPase site in the topo II molecule. Among the DNA-related enzymes tested, *Bam* HI and *Pst* I were weakly inhibited by 2070-DTI, whereas the other enzymes such as some restriction endonucleases, RNase, DNase and DNA ligase were unaffected. These results suggest that 2070-DTI is a selective inhibitor against topo II and that the inhibitory mechanism may differ from those of other topo inhibitors because the inhibitor showed a different inhibitory spectrum.

Ki Values of 2070-DTI against Topoisomerases I and II

The types of inhibitions were determined by Lineweaver–Burk plots[27] of substrate concentrations against the rate of relaxation of pBR322 DNA or decatenation of kinetoplast DNA by topo in the presence and absence of 2070-DTI. As shown in Figure 3[A] and [B], relaxation of pBR322

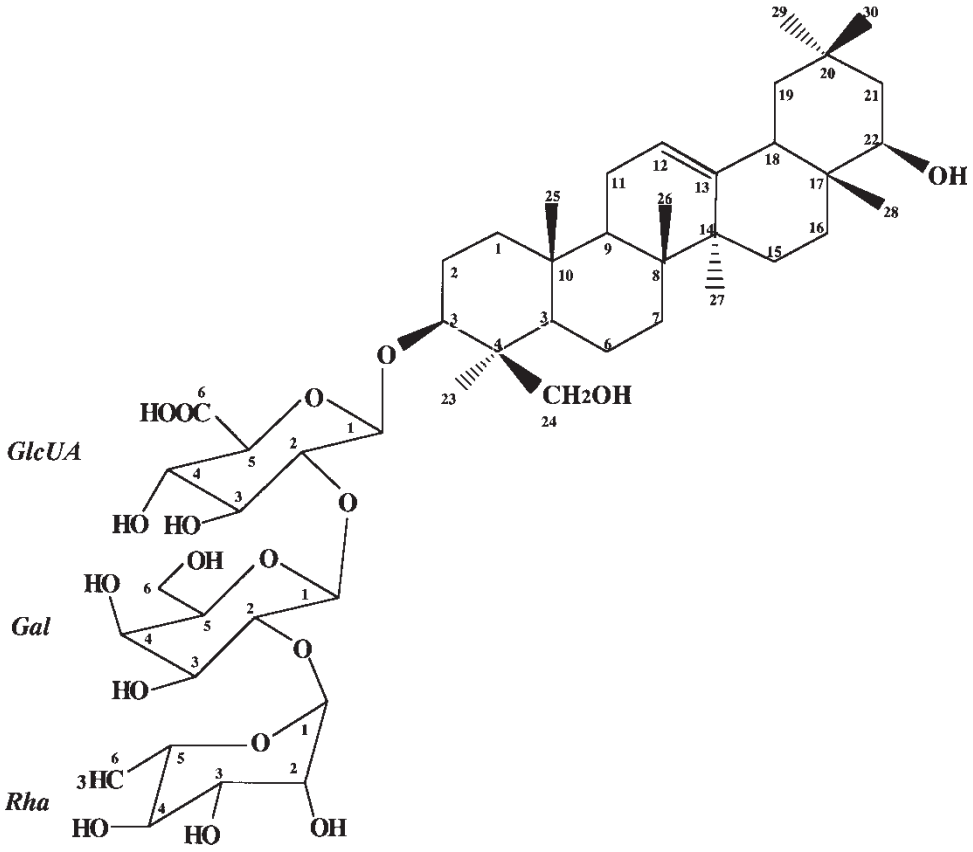

FIGURE 1 Structure of 2070-DTI.

DNA by topo I and II were noncompetitively inhibited by 2070-DTI, and the *Ki* values were 57.0 μM and 39.0 μM, respectively. The *Km* values of relaxation by topo I and II were 6.7 nM and 7.7 nM, respectively. Decatenation of kDNA by topo II was also noncompetitively inhibited by 2070-DTI as shown in Figure 3[C], and the *Ki* and *Km* values were 5.9 μM and 22.2 nM, respectively. From the manner of the inhibition, 2070-DTI was considered to bind to a different site from the binding site of the substrate on the enzyme molecule. In view of the inhibitory potency (*Ki*/*Km*), 2070-DTI showed inhibitory potency in the following order: Topo II decatenation (0.3×10^{-3}) > Topo II relaxation (5.1×10^{-3}) > Topo I relaxation (8.5×10^{-3}). The inhibition by 2070-DTI of topo II decatenation was 28-fold higher than that for topo I relaxation.

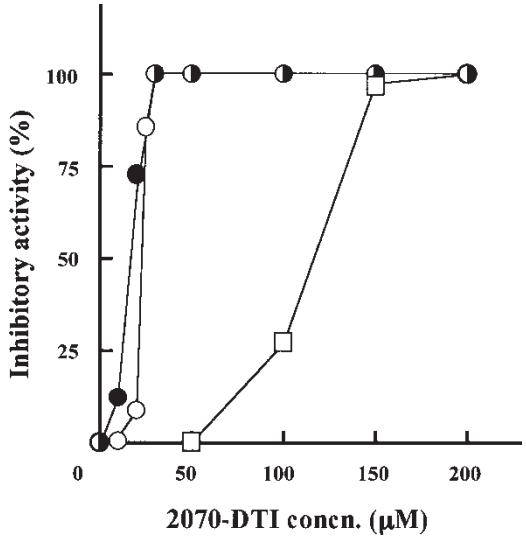

FIGURE 2 Inhibitory activities of 2070-DTI against topo I relaxation (□), topo II relaxation (○) and topo II decatenation (●).

Stabilization of the Topoisomerase–Cleavable Complex by 2070-DTI

Topo inhibitors of the cleavable complex-forming type, such as camptothecin and etoposide, stabilize the cleavable complex (topo-DNA reaction intermediate) and inhibit the DNA rejoining reaction of topo; this inhibitory mechanism for the inhibitors induces nicked or linearized DNA in the cleavage assay. To determine whether 2070-DTI is an inhibitor of the cleavable complex-forming type or not, cleavage assays were conducted. Camptothecin and etoposide were used as the controls for cleavable

TABLE II Inhibitory spectra of topisomerase inhibitors

Enzyme (*origin*)		Inhibitory activity (IC_{50}, μM)			
		2070-DTI	Camptothecin	Etoposide	Doxorubicin
Topoisomerase I*	(*Calf thymus glands*)	123	17	>200	>200
	(*NIH3T3*)	124	12	>200	>200
	(*Vero*)	82	27	>200	>200
	(*COLO201*)	159	17	>200	>200
	(*HeLa*)	170	9	>200	>200
	(*A549*)	159	4	>200	>200
Topoisomerase II*	(*Human placenta*)	24	>200	87	1
Topoisomerase II†	(*Human placenta*)	18	>200	35	1
Alu I	(*Arthrobacter luteus*)	>200	>200	>200	24
Bam HI	(*Bacillus amyloliquefacines*)	79	>200	>200	>200
Eco RI	(*Escherichia coli*)	>200	>200	>200	>200
Hin dIII	(*Haemophilus influenzae*)	>200	>200	>200	96
Pst I	(*Providencia stuartii*)	82	>200	>200	>200
Sca I	(*Streptomyces caespitosus*)	135	>200	>200	25
RNase A	(*Bovine pancreas*)	>200	>200	>200	>200
DNase I	(*Bovine pancreas*)	>200	>200	>200	>200
DNase II	(*Porcine spleen*)	>200	>200	>200	>200
T4 DNA ligase	(*Escherichia coli*)	>200	>200	>200	73
Na^+, K^+-ATPase	(*Porcine cerebral cortex*)	>200	>200	>200	>200

* Relaxation activity. † Decatenation activity.

complex-forming inhibitors against topo I and II, respectively. As shown in Figure 4[A], camptothecin induced nicked DNA with increasing concentrations. Unlike camptothecin, 2070-DTI could not induce nicked DNA even at 200 μM. The results for the stabilization of topo II-cleavable complex are shown in Figure 4[B]. Etoposide induced the linearized DNA, but 2070-DTI failed to linearize DNA even at 1000 μM. These results suggest that 2070-DTI is an inhibitor of the cleavable-nonforming type. 2070-DTI may directly act on the topo molecule in an earlier step than the formation of the topo-DNA complex and inhibit the DNA breaking and rejoining reactions by the enzyme.

Effect of 2070-DTI on the Cell Cycle of HeLa Cells

Camptothecin and etoposide promote the accumulation of damaged DNA by stabilization of the cleavable complex in the cells, thereby arresting cell cycle progression. As shown in Figure 5, HeLa cells were arrested at the S phase and G_2/M phase when cultured with 0.1 μM camptothecin and 2 μM etoposide, respectively. On the other

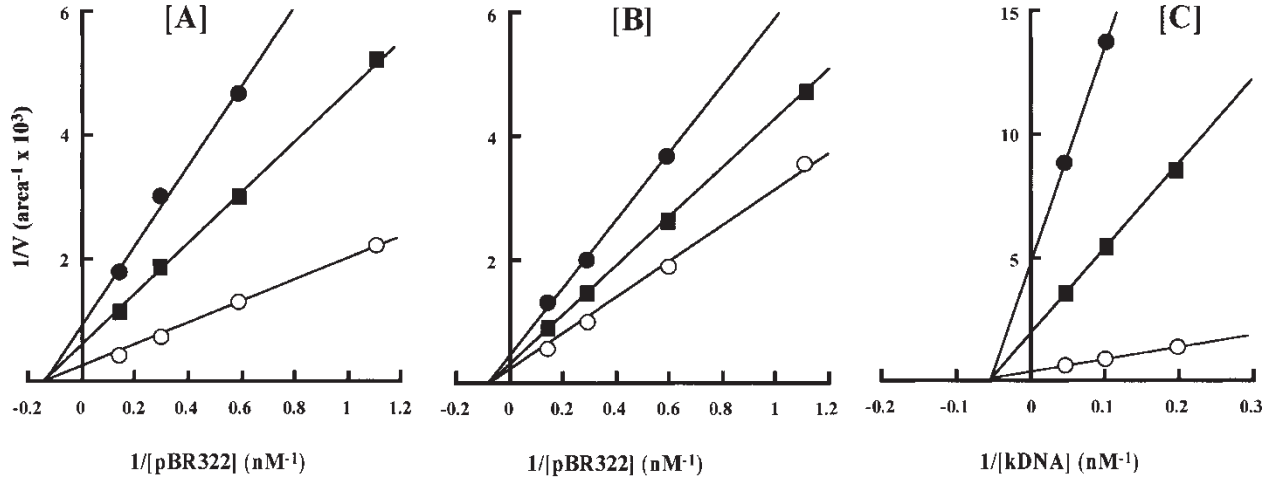

FIGURE 3 Lineweaver–Burk plots of pBR322 DNA concentrations against rate of relaxation by topo I and II, and kDNA concentrations against rate of decatenation by topo II with and without 2070-DTI. The concentrations of 2070-DTI were 150 μM (●) and 123 μM (■) in topo I relaxation [**A**], 30 μM (●) and 24 μM (■) in topo II relaxation [**B**], and 30 μM (●) and 18 μM (■) in topo II decatenation [**C**]. The value of control without 2070-DTI is expressed as an open circle (○). Inhibitory potencies (IC_{50}) of 2070-DTI against topo I and II relaxations were 123 μM and 24 μM, respectively. The IC_{50} against topo II decatenation was 18 μM. The *Ki* values of 2070-DTI against topo I and II relaxations were 57 μM and 39 μM. The *Km* values of topo I and II against pBR322 DNA were 6.7 nM and 7.7 nM. The *Ki* and *Km* values against topo II decatenation were 5.9 μM and 22.2 nM, respectively.

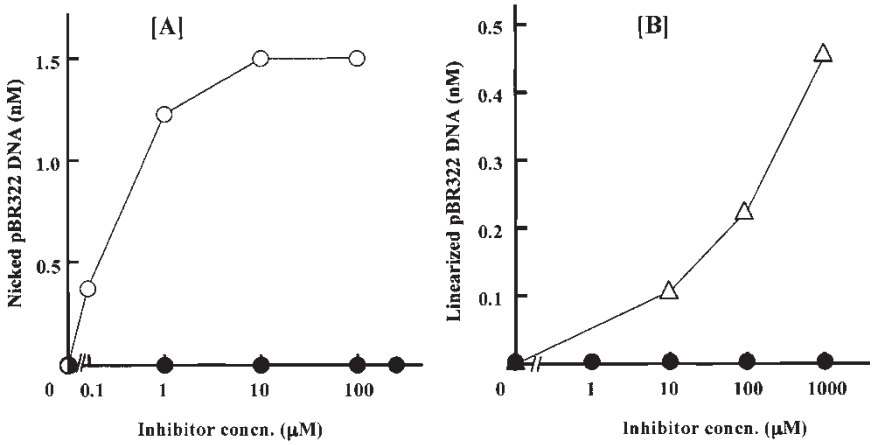

FIGURE 4 Stabilization of topo I [**A**] and topo II [**B**]-cleavable complexes by 2070-DTI (●), camptothecin (○) and etoposide (Δ).

hand, 2070-DTI had not affect on the cell cycle even at an extremely high concentration (200 μM). These finding are in good agreement with the results of the cleavage assay of 2070-DTI. The results suggest that 2070-DTI do not cause damage of DNA by cleavable complex formation in the cells.

DNA Interaction with 2070-DTI

Some topo inhibitors such as doxorubicin and amsacrine are DNA intercalators. To determine whether 2070-DTI has the ability to intercalate into DNA strands, the EtBr competition assay was carried out using calf thymus DNA. Doxorubicin was used as a control for intercalation at the same concentration. As shown in Figure 6, doxorubicin competed with EtBr for DNA and decreased the intensity of fluorescence of EtBr. Alternatively, 2070-DTI did not decrease the intensity of fluorescence and therefore it is considered that 2070-DTI is not a DNA intercalator. In order to confirm this result, changes in the CD and thermal transition spectra of DNA by addition of 2070-DTI were measured since both spectra are sensitive to the conformation changes induced in DNA by intercalators.[28,29] As shown in Figure 7, the both spectra of DNA were in changed with increasing concentrations of 2070-DTI. These results make it clear that 2070-DTI has no ability to intercalate into DNA.

Thus, 2070-DTI is a selective inhibitor against topo II and is different from inhibitors causing DNA damage such as cleavable complex-forming inhibitors and DNA intercalators.

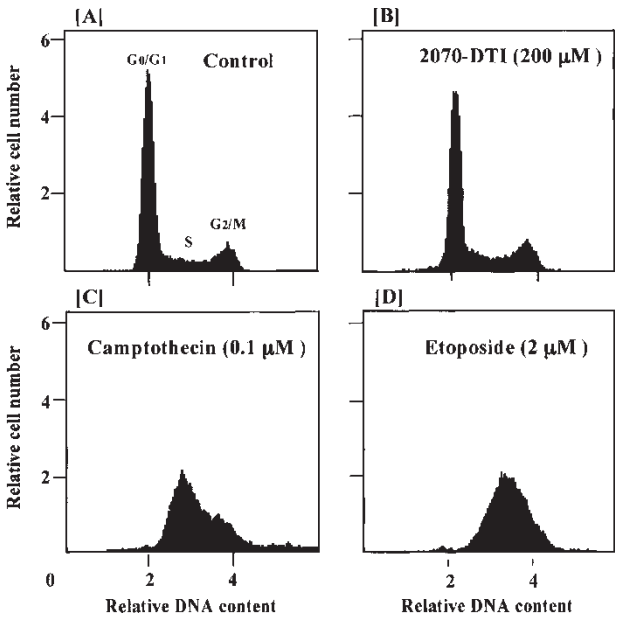

FIGURE 5 Effects of 2070-DTI, camptothecin and etoposide on the cell cycle progression of HeLa cells. [**A**]: control, [**B**]: 2070-DTI (200 μM), [**C**]: camptothecin (0.1 μM), [**D**]: etoposide (2 μM).

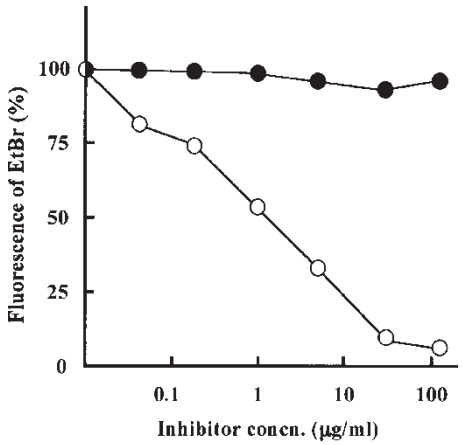

FIGURE 6 Effects of 2070-DTI (●) and doxorubicin (○) on calf thymus DNA binding competition with EtBr.

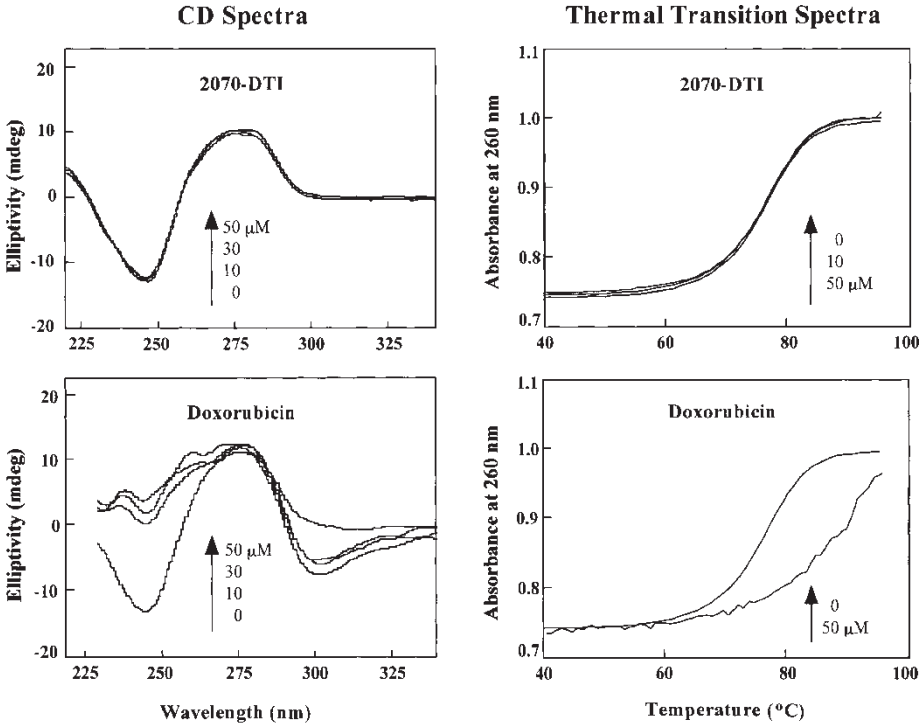

FIGURE 7 Changes of CD and thermal transition spectra of calf thymus DNA by addition of 2070-DTI. The concentrations of inhibitors were 0, 10, 30 and 50 μM.

References

[1] Wang, J.C. (1985) *Ann. Rev. Biochem.* **54**, 655–697.
[2] D'arpa, P. and Liu, L.F. (1989) *Biochem. Biophys. Acta* **989**, 163–177.
[3] Tewey, K.M., Rowe, T.C., Yang, L., Halligan, B.D. and Liu, L.F. (1984) *Science* **226**, 466–468.
[4] Nelson, E.M., Tewey, K.M. and Liu, L.F. (1984) *Proc. Natl Acad. Sci. USA* **81**, 1361–1365.
[5] Hsiang, Y.H., Hertzberg, R., Hecht, S. and Liu, L.F. (1985) *J. Biol. Chem.* **260**, 14873–14878.
[6] Chow, K.C., MacDonald, T.L. and Ross, W.E. (1988) *Mol. Pharmacol.* **34**, 467–473.
[7] Chino, M., Nishikawa, K., Yamada, A., Ohsono, M., Sawa, T., Hanaoka, F., Ishizuka, M. and Takeuchi, T. (1998) *J. Antibiot.* **51**, 480–486.
[8] Ogiso, Y., Tomida, A., Lei, S., Omura, S. and Tsuruo, T. (2000) *Cancer Res.* **60**, 2429–2434.
[9] Suzuki, K., Yahara, Y., Hashimoto, F. and Uyeda, M. (2001) *Biol. Pharm. Bull.* **24**, 1088–1090.
[10] Funabashi, Y., Horiguchi, T., Iinuma, S., Tanida, S. and Harada, S. (1994) *J. Antibiot.* **47**, 1202–1218.
[11] Ishida, R., Miki, T., Narita, T., Yui, R., Sato, M., Utsumi, K.R., Tanabe, K. and Andoh, T. (1991) *Cancer Res.* **51**, 4909–4916.
[12] Suzuki, K., Siddique, T., Nishimura, H., Sekimoto, J. and Uyeda, M. (1998) *J. Enz. Inhib.* **13**, 41–55.
[13] Suzuki, K., Sekimoto, J., Siddique, T., Kamiya, A. and Uyeda, M. (1998) *J. Enz. Inhib.* **14**, 69–83.
[14] Suzuki, K., Nagao, K., Monnai, Y., Yagi, A. and Uyeda, M. (1998) *J. Antibiot.* **51**, 991–998.
[15] Suzuki, K., Yahara, S., Kido, Y., Nagao, K., Hatano, Y. and Uyeda, M. (1998) *J. Antibiot.* **51**, 999–1003.
[16] Suzuki, K., Yamaizumi, M., Tateishi, S., Monnai, Y. and Uyeda, M. (1998) *J. Antibiot.* **52**, 460–465.
[17] Suzuki, K., Yahara, S., Maehata, K. and Uyeda, M. (2001) *J. Nat. Prod.* **64**, 204–207.
[18] Suzuki, K. and Uyeda, M. (2002) *Biosci. Biothechnol. Biochem.* **66**, 1706–1712.
[19] Muller, M.T., Spitzner, J.R., DiDanato, J.A., Metha, V.B., Tsutsui, K. and Tsutsui, K. (1988) *Biochemistry* **27**, 8369–8379.
[20] Spitzner, J.R. and Muller, M.T. (1988) *Nucleic Acids Res.* **16**, 5533–5556.
[21] Yamashita, Y., Kawada, S., Fujii, N. and Nakano, H. (1990) *Cancer Res.* **50**, 5841–5844.
[22] Chan, K-M., Delfert, D. and Junger, D.K. (1986) *Anal. Biochem.* **157**, 375–380.
[23] Baguley, B.C., Denny, W.A., Atwell, G.J. and Chain, B.F. (1981) *J. Med. Chem.* **24**, 170–177.
[24] Georges, M., Catherine, L., Mohamed, B. and Louisette, L.M. (1992) *J. Nat. Prod.* **55**, 1339–1342.
[25] Corinna, M., Sattler, I., Marion, Z., Hinze, C., Koch, C., Maier, A., Grabley, S. and Thiericke, R. (1999) *J. Antibiot.* **52**, 1124–1134.
[26] Maxwell, A. (1993) *Mol. Microbiol.* **9**, 681–686.
[27] Lineweaver, H. and Burk, D. (1934) *J. Am. Chem. Soc.* **56**, 658–666.
[28] Maier, A., Maul, C., Zerlin, M., Sattler, I., Grabley, S. and Thiericke, R. (1999) *J. Antibiot.* **52**, 945–951.
[29] Uno, T., Hamasaki, K., Tanigawa, M. and Shimabayashi, S. (1997) *Inorg. Chem.* **36**, 1676–1683.