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ED-110, a Novel Indolocarbazole, Prevents the Growth of Experimental Tumors in Mice

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A new indolocarbazole compound, ED-110, which was obtained by glucosylating a microbial product (BE-13793C) and is a potent topoisomerase I inhibitor, showed characteristic inhibitory effects on the growth of 12 human tumor cell lines tested. The IC_{50} values of ED-110 against 9 of the 12 lines ranged from 11.5 μg/ml to 0.07 μg/ml, while the remaining 3 lines were quite resistant (IC_{50}, >100 μg/ml). In *in vivo* experiments, i.p. treatment with ED-110 increased the survival period by more than two-fold in mice implanted i.p. with P388, L1210, L5178Y or EL4 murine leukemic cells. The minimum effective dose increasing the life-span of mice bearing P388 leukemia by 25% was <2.5 mg/kg/day×10 and the maximum tolerated dose was >160 mg/kg/day×10. ED-110 was also effective against the spontaneous metastasis of mouse Meth A fibrosarcoma cells and the growth of xenografted MKN-45 human stomach cancer cells as well as s.c. implanted mouse colon 26 and IMC carcinoma cells. These results indicated that ED-110 may have potential as a new antineoplastic agent with a large chemotherapeutic index and a wide range of effective doses.

Key words: Topoisomerase I — Antitumor agent — ED-110 — Indolocarbazole

In the field of cancer chemotherapy, a variety of anticancer drugs have been used. Most of them are antimetabolites and derivatives of various antibiotics and plant extracts. Although their modes of action are extremely diverse, they generally interfere with essential growth processes such as DNA, RNA or protein synthesis in cells. Recently, various new anticancer-drug-screening systems (which target the function of oncogene products or signal transduction pathways in tumor cells) have been developed through the application of new techniques in molecular biology.[1–5] Among the intracellular target enzymes for anticancer agents, DNA topoisomerases control the topology and superhelical density of DNA by transiently breaking and rejoining DNA strands. They play important roles in DNA replication, RNA transcription, chromosomal segregation and, consequently, the proliferation of mammalian cells.[6–9] Therefore DNA topoisomerases are very attractive targets for cancer therapy.[10–12] Several anticancer agents targeting topoisomerases have been developed.

Anthracyclines and epipodophyllotoxins (etoposide, teniposide) interfere with topoisomerase II.[13,14] Amsacrine (mAMSA) is a topoisomerase II inhibitor with DNA-intercalating activity,[15] and genistein also inhibits topoisomerase II.[16] On the other hand, camptothecin and its derivatives (CPT-11 and topotecan) prevent the action of topoisomerase I, thereby inhibiting the growth of experimental tumor cell lines.[17–19] Although fewer topoisomerase I inhibitors than topoisomerase II inhibitors have been discovered to date, the clinical potential of topoisomerase I inhibitors seems to be as substantial as that of topoisomerase II inhibitors. Therefore, we decided to search for new anticancer agents which target topoisomerase I.

By screening for topoisomerase I inhibitors in fermented broth of microorganisms, an indolocarbazole antibiotic, BE-13793C, was found as a new topoisomerase inhibitor.[20] BE-13793C could effectively suppress the growth of Ehrlich ascitic carcinoma cells in mice. Therefore, it was further modified in order to obtain more potent agents. ED-110, a glucosyl derivative of BE-13793C, was obtained by derivatization[21] and found to induce selectively the single-strand DNA cleavage by topoisomerase I (but not the double-strand DNA cleavage by topoisomerase II) at lower concentrations than the parent compound.[22] The IC_{50} value of ED-110 for the *in vitro* growth of P388 cells was 44 n*M*.[22]

This communication deals with the inhibitory effects of ED-110 on various experimental tumor cell lines *in vitro* as well as *in vivo*.

MATERIALS AND METHODS

Mice Female CDF_1 (BALB/c×DBA/2) mice were purchased from Charles River Japan. They were 5 or 6 weeks old at the start of each experiment. BALB/c nu/nu female mice were from Japan CLEA and were 12 weeks old at the start of the experiment.

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Tumors *In vitro* cytotoxicity tests were performed with 12 human and 7 murine tumor cell lines. Human MKN-28, MKN-45, MKN-74 and PC-13 cells were purchased from Immuno Biological Laboratories (Gunma) and maintained with RPMI 1640 medium supplemented with 10% fetal calf serum (FCS). KB and A 431 cells were purchased from Dainippon Pharmaceutical Co. (Osaka) and were maintained in Dulbecco's modified Eagle's medium (D-MEM) containing 10% FCS. MCF7 cells were provided by ATCC (ATCC, HTB 22). HCT116 and PSN 1 cells were provided by Dr. N. Shindo-Okada and Dr. M. Terada of the National Cancer Center Research Institute, Tokyo, respectively, and were cultured in D-MEM and RPMI 1640 medium, respectively (with 10% FCS). DLD-1, LS 180 and WiDr cells were gifts from Dr. H. Fukazawa of the National Institute of Health, Tokyo. DLD-1 cells were maintained in RPMI 1640 medium with 10% FCS. LS 180 and WiDr cells were cultured in Eagle's MEM plus 10% FCS. Murine tumor cell lines used in *in vitro* studies were P388, L1210, L5178Y and EL4 leukemia, colon 26 carcinoma, Meth A fibrosarcoma and IMC carcinoma. P388 and colon 26 cells were provided by Dr. T. Tsuruo of the Institute of Applied Microbiology, University of Tokyo. L1210, EL4 and Meth A cells were from Dr. T. Ikegawa of the National Cancer Center Research Institute, Tokyo. L5178Y and IMC carcinoma cells were from Dr. H. Suzuki of Toagosei Chemical Industry Co., Tsukuba, and Dr. M. Ishizuka of the Institute of Microbial Chemistry, Tokyo, respectively. These murine cell lines were maintained with RPMI 1640 medium containing 10% FCS, and in the case of P388 cells 20 μM β-mercaptoethanol was further added.

In vivo antitumor tests were carried out using MKN-45 human cancer cells and the 7 murine tumor cell lines which were used in the *in vitro* tests. Mice were inoculated with these cells propagated *in vitro*. Etoposide was used as a positive control to verify the conditioning of each experiment.

ED-110 and the other chemicals ED-110, of which the structure is shown in Fig. 1, was synthesized at our institute by glucosylating BE-13793C according to well-documented techniques.[21] ED-110 was dissolved in dimethyl sulfoxide (DMSO) and serially diluted to appropriate concentrations with phosphate-buffered saline before use. Etoposide and cisplatin were purchased from Nippon Kayaku Co., Tokyo. Adriamycin was obtained from Sigma, St. Louis.

Cytotoxicity test The tumor cells were suspended in the respective medium at 10^5 cells/ml and 0.1 ml of the suspension was dispensed into each well of 96-well microtest plates. After subculture for 24 h at 37°C, 0.1 ml of the medium containing serially diluted test compound was added to each well and the incubation was continued for 72 h. The final concentration of DMSO did not exceed 0.5%. Cell growth was measured by means of the colorimetric tetrazolium/formazan (MTT) assay[23] for the nonadherent leukemic cells or the sulforhodamine B (SRB) method[24] for the other adherent cells.

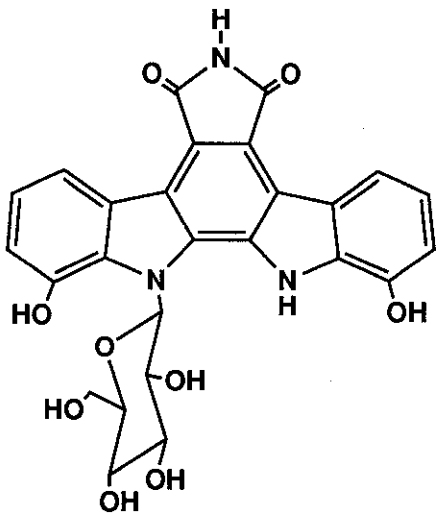

Fig. 1. Structure of ED-110.

Antitumor test in mice Life span-increasing effects in mice bearing leukemia were examined using CDF_1 mice implanted i.p. or i.v. with 1×10^6 P388, L1210, L5178Y or EL4 leukemic cells. ED-110 solution was injected i.p. at 0.1 ml/10 g body weight once daily for 10 consecutive days starting one day after tumor implantation. The control mice were injected with vehicle. During the treatment and observation periods, body weight and appearance were recorded periodically. At the end of the observation period, all survivors were killed for gross examination of their peritoneal fluid and organ damage. Colon 26 and IMC carcinoma cells were inoculated s.c. into a flank of CDF_1 mice at 10^6 cells/mouse on day 0, and the tumor-bearing mice were treated by i.p. injection once daily for 20 consecutive days from day 1. Antitumor effects on colon 26 and IMC carcinoma were recorded by weighing the tumors on day 28. CDF_1 mice injected s.c. with 10^4 Meth A cells were treated using the same schedule as that used in the tests for colon 26 and IMC carcinoma. In this case, the antitumor activity of ED-110 was judged by measuring the survival period but not the tumor weight, because the Meth A cells metastasized systemically[25, 26] and killed all untreated mice within 20 days. Spontaneous regression of primary and metastasized tumors was not observed. The evaluation of the efficacy of ED-110 against human cancer cell lines was carried out using MKN-45 human stomach cancer cells

and BALB/c nu/nu mice. The mice were s.c. implanted with 10^6 MKN-45 cells and treated i.p. with ED-110 from 5 days after tumor inoculation. To evaluate the inhibitory effect on tumor growth, tumor volume was calculated on day 21 according to the following formula; $(L \times W^2)/2$, where L = length (cm) and W = width (cm).

Statistical analysis All animal data were analyzed by using the Mann-Whitney U-test and were also analyzed by using the Student *t* test in cases where the results of the F-test were insignificant.

RESULTS

***In vitro* cytotoxicity** IC_{50} values of ED-110 against experimental human tumor cell lines ranged from 0.07 to 11.5 μg/ml (except those for DLD-1, WiDr and PSN 1 cells). DLD-1, WiDr and PSN 1 cells were resistant to ED-110 and their IC_{50}s were larger than 100 μg/ml (Table I). Such significant differences in IC_{50}s for various cell-lines were not observed with adriamycin, etoposide, cisplatin and camptothecin. The cytotoxicity of ED-110 was also examined on murine tumor cell lines. The IC_{50}s for murine P388, L1210, L5178Y, EL4, colon 26 and IMC carcinoma cells were 0.022, 3.9, 0.048, 0.24, 0.31 and 0.022 μg/ml, respectively, again showing a difference of more than 100 fold in cytotoxicity depending upon the cell-line used.

Life-span-prolonging effects on mice bearing leukemia cells I.p. injections of ED-110 were effective in increasing the survival periods of mice implanted i.p. with P388 leukemia cells at doses from 0.625 to 160 mg/kg (Table II). Survival was recorded for 30 days, which was about 3 times longer than the mean survival days of the tumor-bearing mice given vehicle only. In the case of mice treated with 160 mg/kg of ED-110, all mice survived for 30 days and 2 out of 5 mice given 40 mg/kg of ED-110 were alive. These surviving mice appeared to be tumor-free on the basis of body weight change, appearance and autopsy findings. Moreover, the treatment with i.p. injections of ED-110 was effective in increasing the survival period of mice implanted i.v. with P388 cells at doses of 40 mg/kg and more.

ED-110 was effective in increasing the life-span of mice implanted i.p. with not only P388 but also L1210, L5178Y and EL4 cells (Table III). The minimum effective dose for mice implanted with L1210 seemed to be 0.156 mg/kg, though a statistically significant effect was obtained at a dose of 0.625 mg/kg, but not 0.156 mg/kg. The 160 mg/kg dose was tolerated, while the T/C% value and the number of mice surviving on day 60 suggested that the 40 mg/kg dose was the most effective. The effect on L5178Y was very similar to that on L1210; ED-110 was effective over a wide range of doses (from 2.5 to 160 mg/kg). The most effective dose for this tumor was also 40 mg/kg. The effective range of doses for EL4 was slightly higher than that for L1210 and L5178Y. The effect of the 2.5 mg/kg dose of ED-110 was moderate and the effect of the 160 mg/kg dose seemed to be the same as (or slightly better than) that of the 40 mg/kg dose, based on the number of surviving mice.

Growth inhibitory effects on mouse solid tumors ED-110 inhibited the growth of colon 26 and IMC carcinoma by 83% and 91%, respectively, when injected i.p. at 160 mg/kg (Table IV). However, it reduced the body weight of mice implanted s.c. with IMC carcinoma cells at a dose of 160 mg/kg, and the 40 mg/kg dose (which was highly effective in the leukemia model) failed to suppress the growth of these tumors.

Table I. Comparison of the Cytotoxicity of ED-110 with Other Anticancer Agents in Human Tumor Cell Lines

Cell line	Origin	IC_{50} (μg/ml)				
		ED-110	ADM[a)]	VP-16	CDDP	CPT
MKN-28	Stomach	11.5	0.12	3.18	1.59	
MKN-45	〃	0.07	0.29	1.53	0.87	0.005
MKN-74	〃	2.0	0.99	5.80	3.30	0.016
DLD-1	Colon	>100	0.56	0.88	3.90	0.016
LS 180	〃	1.0	0.04	1.18	1.14	0.004
WiDr	〃	>100	0.27	5.65	5.40	
HCT116	〃	0.42	0.30	5.89	6.30	
A 431	Epidermoid	2.0	0.10	0.35	0.99	0.003
KB	Oral	3.0	0.04	0.27	0.23	
PC-13	Lung	0.5	0.10	0.59	2.85	
PSN 1	Pancreas	>100	0.16	1.30	0.36	0.007
MCF7	Breast	3.6	0.06	0.39	1.71	

a) ADM, adriamycin; VP-16, etoposide; CDDP, cisplatin; CPT, camptothecin. Cells were cultured with drugs at 37°C for 72 h, and the growth was recorded colorimetrically using sulforhodamine B.[23)]

Table II. Life-span-prolonging Effect of ED-110 in Mice Bearing P388 Leukemic Cells

Compound	Dose (mg/kg×day)	Tumor	Survival days		Survivor[a]
			Median	Mean±SD	
Control	0×10	i.p.	10	9.6±0.5	
ED-110	0.039×10	i.p.	10	10.2±0.5	
	0.156×10	i.p.	11	11.2±0.5[b]	
	0.625×10	i.p.	13	12.6±0.6[b]	
	2.5×10	i.p.	15	15.0±0.7[b]	
	10.0×10	i.p.	16	16.0±1.2[b]	
	40.0×10	i.p.	29	25.8±5.5[b]	2/5
	160.0×10	i.p.	>30	30.0±0.0[b]	5/5
Control	0×10	i.v.	8	8.0±0.0	
ED-110	2.5×10	i.v.	8	8.0±0.0	
	10.0×10	i.v.	8	8.2±0.4	
	40.0×10	i.v.	11	11.0±0.7[b]	
	160.0×10	i.v.	16	16.2±2.3[b]	

a) No. of mice surviving on day 30/No. of mice tested.
b) $P<0.05$ by Mann-Whitney U-test.
CDF_1 mice (cont, n=10; test, n=5) were inoculated i.p. or i.v. with 10^6 P388 cells on day 0 and treated i.p. with test compound once daily for 10 consecutive days beginning on day 1. Survival was recorded for 30 days (the survival time of mice alive on day 30 was taken as 30 days).

Table III. Antitumor Effects of ED-110 in Mice Bearing L1210, L5178Y or EL4 Leukemia Cells

Tumor	Compound	Dose (mg/kg×day)	Survival days		Survivor[a]
			Median	Mean±SD	
L1210	Control	0×10	15	16.0±2.4	
	ED-110	0.039×10	15	17.0±4.7	
		0.156×10	27	25.2±8.8	
		0.625×10	33	24.0±3.2[b]	
		2.5×10	41	31.6±2.8[b]	
		10.0×10	>60	41.8±12.2[b]	1/5
		40.0×10	>60	57.8±5.4[b]	4/5
		160.0×10	>60	40.8±26.9	3/5
	Etoposide	2.5×10	>60	55.6±6.0[b]	3/5
L5178Y	Control	0×10	19	19.9±4.0	
	ED-110	2.5×10	25	31.2±16.4	1/5
		10.0×10	39	42.2±17.1[b]	2/5
		40.0×10	>60	54.4±12.5[b]	4/5
		160.0×10	>60	45.6±24.7	3/5
	Etoposide	0.63×10	27	33.8±16.3[b]	1/5
EL4	Control	0×10	17.5	17.6±0.7	
	ED-110	2.5×10	24	23.4±2.2	
		10.0×10	27	27.0±3.1[b]	
		40.0×10	>60	48.4±15.8[b]	3/5
		160.0×10	>60	48.8±25.0[b]	4/5
	Etoposide	0.63×10	32	36.0±13.8[b]	1/5

a) No. of mice surviving on day 60/No. of mice tested.
b) $P<0.05$ by Mann-Whitney U-test.
CDF_1 mice (cont, n=10; test, n=5) inoculated i.p. with 10^6 L1210, L5178Y or EL4 cells on day 0 were treated i.p. with test compounds once daily for 10 consecutive days from day 1. Survival was recorded for 60 days (the survival time of mice alive on day 60 was taken as 60 days).

Prevention of spontaneous metastasis of Meth A fibrosarcoma Meth A cells implanted s.c. into a side flank of mice produced solid tumors at the implanted sites and the tumor cells metastasized systemically. Metastases were found in various organs, such as the liver, spleen and lungs (data not shown). The mice implanted with

Table IV. Inhibitory Effect of ED-110 on the Growth of Murine IMC Carcinoma and Colon 26 Cells Implanted s.c. in Mice

Tumor	Compound	Dose (mg/kg×day)	Tumor weight		Body weight change (g)[a]
			g±SD	Inhibition%	
IMC carcinoma	Control	0×20	0.93±0.70	0	0.7
	ED-110	2.5×20	1.23±1.14	−33	1.5
		10.0×20	0.81±0.81	12	2.9
		40.0×20	0.73±1.29	21	0.7
		160.0×20	0.09±0.04[b]	91	−1.9
	Etoposide	0.6×20	0.66±0.47	29	0.7
		2.5×20	0.17±0.10[b]	81	1.9
Colon 26	Control	0×20	2.10±0.65	0	0
	ED-110	2.5×20	1.91±0.37	9	0.9
		10.0×20	1.99±0.20	5	2.2
		40.0×20	1.93±0.50	8	1.6
		160.0×20	0.35±0.61[b]	83	2.3
	Etoposide	0.6×20	1.99±0.27	5	0.7
		2.5×20	1.20±0.40[b]	43	1.2

a) Body weight change except tumor weight in the period from day 1 to 28.
b) $P<0.05$ by Mann-Whitney U-test.
CDF_1 mice (cont, n=10; test, n=5) implanted s.c. with 10^6 IMC carcinoma or colon 26 cells on day 0 were treated i.p. with compounds once daily for 20 consecutive days beginning on day 1. The mice were killed on day 28 and the tumor was weighed.

Table V. Life-span-prolonging Effect of ED-110 in Mice Implanted s.c. with Meth A Cells

Compound	Dose (mg/kg×day)	Mean survival days±SD	Survivor[a]	Body weight change (g)[b]
Control	0×20	18.4±4.1		2.5
ED-110	2.5×20	18.8±1.3		2.4
	10.0×20	22.0±4.6		2.4
	40.0×20	27.0±6.3[c]		2.0
	160.0×20	26.4±30.7	2/5	−1.0
Etoposide	0.63×20	24.2±3.4[c]		0.9
	2.5×20	33.0±1.6[c]		0.8

a) No. of mice surviving on day 60/No. of mice tested.
b) Body weight change in the period from day 1 to 17.
c) $P<0.05$ by Mann-Whitney U-test.
CDF_1 mice (cont, n=10; test, n=5) were i.p. inoculated with 10^4 Meth A cells on day 0 and i.p. treated with test compounds once daily for 20 consecutive days beginning on day 1. Survival was recorded for 60 days (the survival time of mice alive on day 60 was taken as 60 days).

Meth A cells died of metastasis and the mean survival time of the controls was 18.4 days (Table V). When the mice were treated with the 40 mg/kg dose of ED-110, their survival time was prolonged by 47%. Moreover, 2 out of 5 mice given the 160 mg/kg dose of ED-110 escaped metastatic death.

Table VI. Growth-suppressive Effect of ED-110 on MKN-45 Human Stomach Cancer Cells in Nude Mice

Compound	Dose (mg/kg)	Schedule	Tumor growth	
			mm^3±SD	Inhibition%
Control			186±86	0
ED-110	2.5	Q1D×16	132±37	29
	10.0	〃	86±32[a]	54
	2.5	Q3D×6	129±59	31
	10.0	〃	71±32[a]	62
5-Fluorouracil	10.0	〃	198±159	−6

a) $P<0.05$ by Mann-Whitney U-test.
BALB/c nu/nu mice (female, 12 weeks: cont. n=12; test n=6) implanted s.c. with 10^6 MKN-45 cells on day 0 were treated i.p. with drugs once daily for 16 consecutive days or once every 3 days (6 times) from day 5.

Efficacy against xenografted MKN-45 human stomach cancer in nude mice MKN-45 cells inoculated s.c. into a flank of BALB/c nude mice produced palpable tumor nodules within 5 days after the implantation. The mice were randomized on day 5 and treated i.p. with drugs consecutively or intermittently from day 5. As shown in Table VI, ED-110 tended to suppress the growth of MKN-45 cells at a dose of 2.5 mg/kg, and at a dose of 10 mg/kg both consecutive and intermittent injections of ED-110 significantly suppressed the tumor growth.

DISCUSSION

ED-110 seems to be a very attractive antitumor agent because it is a semi-synthetic compound having a novel structure.[21] In addition, the drug inhibits topoisomerase I but not topoisomerase II,[22] shows cytotoxicity against certain tumor cell lines, and strongly inhibits the growth of some experimental tumors in mice.

The mechanism responsible for this selective toxicity is not understood yet. It is noteworthy that ED-110 effectively prevented the growth of multidrug-resistant P388 cells as well as the parental drug-sensitive cells.[22] The multidrug-resistant P388/VCR cells are known to overproduce *mdr*1 gene product, p-glycoprotein.[27] DLD-1, WiDr and PSN1 cells (which were apparently resistant to ED-110 in terms of cytotoxicity) are not multidrug-resistant cell lines, indicating that the difference in susceptibility of the tumor cells to ED-110 is not due to *mdr*. Moreover, the mutation of topoisomerase I gene in these cell lines has not been reported, and camptothecin, a potent topoisomerase I inhibitor, inhibited the growth of these cell lines as well as the others. Although the clinical potential of a new cytotoxic agent cannot necessarily be predicted from its cytotoxicity toward experimental tumor cell lines, it is possible that ED-110 will inhibit the growth of certain tumors in humans. Since ED-110 is a topoisomerase I inhibitor, studies on the relationship between the cytotoxicity and inhibition of topoisomerase I in different tumor cells would be interesting. Studies on the metabolism of ED-110 in cell lines (both sensitive and insensitive to ED-110) have also been initiated.

Antitumor effects of ED-110 on P388, L1210, L5178Y and EL4 leukemia in mice were quite satisfactory. The compound was effective over a very wide range of doses; against ascitic P388 murine leukemia, the minimum dose increasing the life span of mice 30% was 0.625 mg/kg, and all the mice which were treated with 160 mg/kg dose of ED-110 survived during the experimental period and appeared to be completely cured. This marked efficacy against leukemia cells was also apparent in other leukemia models. Furthermore, it was noteworthy that the i.p. injection of ED-110 prolonged the life span of mice implanted i.v. with P388 cells. We have often observed that substances found in anticancer screening to have potent antitumor effects on ascitic leukemia cells by i.p. injection are not effective against leukemia cells implanted i.v.

Various anticancer drugs developed thus far are effective against leukemia in mice, but their antiproliferative effects on solid tumors are poor in many cases. Therefore it is interesting to note that ED-110 was effective in inhibiting the growth of solid tumors and the spontaneous metastasis. For the metastases from Meth A fibrosarcoma implanted into a flank of mice, the effective dose of ED-110 by i.p. injection was 40 mg/kg, and the survival period of the tumor-bearing mice was increased at a dose of 10 mg/kg or more. ED-110 might be effective against the vascular metastasis of tumors sensitive to ED-110. Moreover, ED-110 was effective in inhibiting the growth of MKN-45 stomach cancer cells in mice at a dose of 10 mg/kg. Treatment with higher doses of ED-110 may have a much stronger antitumor effect on MKN-45 tumor because at a dose of 160 mg/kg, ED-110 suppressed the growth of colon 26 and IMC solid carcinomas by 83% and 91%, respectively, and the IC_{50} values of ED-110 for the *in vitro* growth of MKN-45, colon 26 and IMC carcinoma cells were all in the range from 0.02 to 0.31 μg/ml. Throughout these studies, it was found that the lethal toxicity of ED-110 was low, and in preliminary safety studies, CDF_1 mice injected i.p. with 500 mg/kg of ED-110 all remained alive during the observation period (10 days).

ED-110 structurally resembles rebeccamycin,[28] staurosporine[29] and K252a,[30] all of which are indolocarbazole compounds. Rebeccamycin was reported to cause single-strand DNA breakage in A549 cells and to prolong the survival period of mice bearing P388 and L1210 leukemia by 25 to 75% at doses ranging from 8 to 256 mg/kg.[31] The mode of action of rebeccamycin should be similar to that of ED-110. Both staurosporine and K252a are potent protein kinase C inhibitors showing IC_{50} values of 2.7 and 32.9 nM, respectively.[29, 30] ED-110 showed a much weaker inhibitory effect on protein kinase C than those compounds; the IC_{50} value of ED-110 was 11.4 μM (data not shown). The pharmacological effect of ED-110 on protein kinase C does not appear significant.

In conclusion, ED-110 is an attractive topoisomerase I inhibitor with a chemical structure that is quite different from that of camptothecin. Studies on other intracellular targets of ED-110 are ongoing. The cytotoxicity of ED-110 was selective for several tumor cell-lines examined (but not all), and ED-110 inhibited the growth of solid tumors in mice. These results suggest that it may be useful clinically for the treatment of certain types of human cancer. ED-110 is, at the very least, a useful lead compound to develop agents for clinical application. Development of a compound more effective than ED-110, by the improvement of its water solubility and anticancer efficacy, is also currently under investigation.

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