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Antitumor activity and cytotoxicity of a new ankinomycin derivative, 3′-,11-dibutyryl ankinomycin

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Abstract. Ankinomycin is a new antitumor antibiotic found in the culture broth of *Streptomyces* sp. SF2587. Ankinomycin showed marked cytotoxicity and antitumor activity against some murine leukemias, but the activity against murine solid tumors was rather weak because of its strong acute toxicity. We synthesized ankinomycin acyl derivatives and examined their antitumor activity. Among the derivatives, 3′,11-dibutyryl ankinomycin (AN1006) exhibited the highest antitumor activity. The antitumor activity of AN1006 was dependent on the administration schedule, and on the most effective schedule, AN1006 showed activity comparable with that of Adriamycin (ADM) against murine solid tumors and leukemias. AN1006 showed a cytotoxic spectrum different from that of ADM, exhibiting cytotoxicity stronger than that of ADM against colon carcinoma, stomach carcinoma, and some leukemia cell lines. According to these in vitro effects, AN1006 showed antitumor activity superior to and equal to that of ADM against human colon xenografts and stomach carcinoma xenografts in athymic nude mice, respectively. AN1006 was effective against multidrug-resistant tumors in vitro and in vivo. AN1006 is an interesting candidate for further evaluation.

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Abbreviations: ANK, ankinomycin; AN1006, 3′-,11-dibutyryl ankinomycin; ADM, Adriamycin; ILS, increase in life span; ILS_{max}, the maximal value of increase in life span; IC_{50}, concentration required for 50% inhibition of cell growth; P388/ADM, Adriamycin-resistant P388 cells; LLC, Lewis lung carcinoma; CEM/VLB_{100}, vinblastine-resistant CCRF-CEM cells; 2780AD, Adriamycin-resistant A2780 cells; KBC-4, colchicine-resistant KB3-1 cells; K562/ADM, Adriamycin-resistant K562 cells; MCF-7/ADM, Adriamycin-resistant MCF-7 cells

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Introduction

Ankinomycin (ANK) is a new antitumor antibiotic found in the culture broth of *Streptomyces* sp. SF2587. ANK belongs to the oxabenzanthraquinone antibiotic group called pluramycins (reviewed in [13]). Although some of the pluramycins, such as hedamycin, pluramycin, and neopluramycin, show strong cytotoxicity and antitumor activity, their clinical usefulness has not been examined, mainly due to their chemical instability. The compounds rapidly lose their biological activity in solution because of the photolability of the angolosamine [2,3,6-trideoxy-3(dimethylamino)-D-arabino-hexose] residue [12]. ANK lacks the angolosamine in its chemical structure and is expected to be more stable than other pluramycins. ANK shows marked cytotoxicity to tumor cell lines, including multidrug-resistant tumor cells. The compound possesses marked antitumor activity against such murine leukemias as P388 and L1210; however, it exerts rather weak antitumor effects against murine solid tumors, probably because of its strong acute toxicity [4].

Since acetylation of pluramycins lowers their acute toxicity [5], we synthesized acyl derivatives of ankinomycin and examined their antitumor activity. All of the synthesized compounds possessed good antitumor activity against P388 murine leukemia. Among the derivatives, 3′,11-dibutyryl ankinomycin (AN1006) exhibited the highest antitumor activity. In this report, we describe the cytotoxicity and antitumor activity of AN1006 against several tumor models, including multidrug-resistant tumors.

Materials and methods

Animals and tumor cells. Adult female BALB/c × DBA/2Cr F_1 (called $CD2F_1$) mice and C57BL/6 × DBA/2Cr F_1 (called $BD2F_1$) mice were obtained from Charles River Japan, Inc. (Tokyo). Female BALB/c-nu/nu athymic mice were obtained from Nihon Clea Inc. (Tokyo).

P388 leukemia, a subline of P388 resistant to Adriamycin (P388/ADM), L1210 leukemia, B16 melanoma, colon adenocarcinomas 26 (Colon 26) and 38 (Colon 38), Lewis lung carcinoma (LLC), M5076 sarcoma, and human colon xenografts HT-29, DLD-1, WiDr, KM-12,

and KM2012 were supplied by the National Cancer Institute (Bethesda, Md.). Other cell lines were kindly provided as follows: human myelogenous leukemia K562 [6], by Dr. K. Ezaki of the Cancer Chemotherapy Center; acute lymphoblastic leukemia CCRF-CEM and its vinblastine-resistant subline CEM/VLB$_{100}$ [2], by Dr. W. T. Beck, St. Jude Children's Hospital (Memphis, Tenn.); human ovarian cancer A2780 and its Adriamycin-resistant variant 2780AD [10], by Drs. R. F. Ozols and T. C. Hamilton, National Cancer Institute; the KB3-1 subclone of human nasopharynx carcinoma KB and its colchicine-resistant variant KBC-4 [1], by Dr. I. Pastan, National Cancer Institute; human acute promyelocytic leukemia HL-60 [3], by Dr. M. Shimoyama, National Cancer Center Hospital; and human acute monocytic leukemia THP-1 [15], erythroleukemia HEL [7], and diffuse histiocytic lymphoma U937 [14], by the Japanese Cancer Research Resource Bank. St-4, 4-1St, Adriamycin-resistant sublines of K562 (K562/ADM) [17] and MCF-7 (MCF-7/ADM) were established in our laboratory.

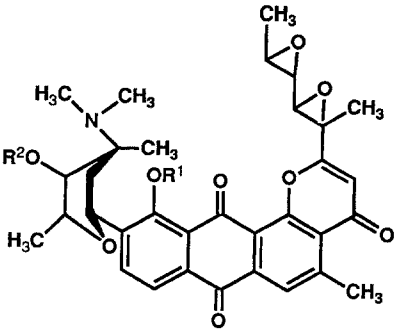
Evaluation of antitumor activity against murine tumors. The implantation of P388, P388/ADM, L1210, B16, Colon 26, Colon 38, LLC, and M5076 were carried out as previously described [16]. Briefly, 0.1 ml of a cell suspension containing 10^6 cells of P388 or P388/ADM or 10^5 cells of L1210 was inoculated i. p. into CD2F$_1$ mice. Cell suspensions of LLC, B16, Colon 26 and 38, and M5076 were prepared from surgically removed tumors as mushes. The cell suspensions were passed through 40-mesh sieves, and the cells were counted by the trypan blue dye-exclusion method and inoculated s. c.. A total of 100,000 LLC cells or Colon 26 cells and 10^6 cells of M5076 were inoculated in a volume of 0.2 ml into the right flank of B6D2F$_1$, CD2F$_1$, and B6D2F$_1$ mice, respectively. A tumor cell suspension of B16 in a volume of 0.25 ml was inoculated s. c. into the right flank of B6D2F$_1$ mice. A tumor brei of Colon 38 was diluted three times with Hanks' balanced salt solution (HBSS), and 0.2 ml of the suspension was inoculated s. c. into the right flank of B6D2F$_1$ mice. Mice were given drug solutions at a costant rate of 0.01 ml/g body weight. Antitumor activity was determined by the increase in life span (ILS) calculated from the mean duration of survival of the drug-treated group and of the control group or by the tumor-mass inhibition calculated from the mean tumor volume of the treated group and of the control group. Tumor volume was calculated using the following formula according to the method of the National Cancer Institute:

$$\text{Tumor volume (mm}^3\text{)} = (L \times W^2)/2,$$

where L (in millimeters) and W (in millimeters) are the long and short diameters of the tumor, respectively. Statistical significance was determined by Student's t-test. A probability value of less than 5% was considered to be statistically significant.

Cell culture and drug treatment. Tumor cells were maintained in RPMI 1640 medium (Nissui, Tokyo) supplemented with 5% fetal bovine serum (Gibco, Grand Island, N. Y.) and 100 μg kanamycin/ml (Meiji Seika, Tokyo), hereafter called growth medium. For the cytotoxicity experiment, 4×10^4 cells in 2 ml growth medium were incubated for 6 or 24 h after the cell seeding for suspension culture and for adherent cell culture, respectively. Then, drugs were added and the cells were further incubated for 72 h in the presence of drugs. After the incubation, the number of cells were counted with a model ZBI Coulter counter (Coulter Electronics, Ltd., Luton, England). Three samples were used for each drug concentration. The concentration required for 50% inhibition of cell growth (IC$_{50}$ value) was determined by plotting the logarithm of the drug concentration against the relative numbers of treated and untreated cells.

Antitumor activity against the human tumor xenograft model. The human tumor xenografts used in this study were maintained in female Balb/c-nu/nu athymic mice. Chemotherapeutic experiments have been described elsewhere [18]. Briefly, fragments of xenografts were implanted s. c. into the right subaxillary region of athymic mice. When the tumors became palpable (100–300 mm^3), the mice were randomized to several groups of six mice each. Mice were given drug solutions i. v. every 7 days for a total of four administrations. Each tumor volume was calculated using the formula described above and was expressed as the relative tumor volume (RV), RV = V_n/V_o, where V_n is the tumor volume on day n and



	^{1}R	^{2}R
Ankinomycin	H	H
AN1002	$COCH_3$	$COCH_3$
AN1004	COC_2H_5	COC_2H_5
AN1005	H	COC_3H_7
AN1006	COC_3H_7	COC_3H_7
AN1018	COC_3H7	H

Fig. 1. Structure of ankinomycin derivatives

V_o is the initial tumor volume on day 0. The effectiveness of each drug was evaluated on day 35 by the following formula:

$$T/C(\%) = (\text{mean RV of treated mice/mean RV of control mice}) \times 100.$$

Evaluation of a drug as effective was based on a T/C(%) value of less than 50% with statistical significance as determined by Mann-Whitney's U-test ($P < 0.01$, one-sided) [8].

Results

Antitumor activity of ankinomycin acyl derivatives

Among the synthesized derivatives, we chose the compounds that had good solubility in water (about 20 mg/ml; Fig. 1) and tested their antitumor activity against i. p.-implanted P388 leukemia cells (Table 1). The acute toxicity of the derivatives was lower than that of ANK; therefore, the former were given at higher doses. The antitumor activity of the derivatives was significantly higher than that of ANK (significant at $P < 0.001$). Among the derivatives, AN1006 exhibited the most prominent effect against P388 ascites leukemia, and this compound was selected for further evaluation of its antitumor activity.

Antitumor activity against murine leukemia

The antitumor activity of AN1006 against murine leukemias P388 and L1210 was examined. The activity against P388 was examined after i. v. administration on day 1 (one-shot) or days 1, 5, and 9 (intermittent schedule; Table 2). The maximal ILS value (ILS$_{max}$) for AN1006 was 102% and 157% on the one-shot and intermittent schedule, respectively, and that for ADM was 82% and 106%, respectively. These results indicate that the activity

Table 1. Antitumor activity of ankinomycin derivatives against P388 leukemia

Drugs	% ILS_{max}
Ankinomycin	47.1 (1.5)[a]
AN1002	68.0 (12)
AN1004	60.0 (12)
AN1005	70.8 (6)
AN1006	106 (48)
AN1018	64.7 (24)
ADM	103 (12)

P388 cells (10^6 cells/mouse) were implanted i.p. into CD2F1 mice on day 0. Each agent was given i.v. on day 1. Six mice were used in each group. Experimental details are described in Materials and methods. The structure of each ankinomycin derivative is shown in Fig. 1. The SD of the increase in life span was within 10% of each value

[a] Numbers in parentheses represent the administration dose (mg/kg)

Table 2. Antitumor activity of AN1006 and ADM against P388 leukemia

Schedule	Drugs	Dose (mg/kg daily)	ILS (%)	Body weight loss at each nadir (g)
Day 1[a]:				
	AN1006	64	27	−5.0[c]
		48	102	−1.8
		32	70	−0.3
		16	42	0.7
		8	20	1.0
		4	10	0.5
	ADM	32	−15	−4.6[c]
		16	82	−1.2
		8	50	0.5
		4	20	0.7
		2	7	0.7
Days 1, 5, and 9[b]:				
	AN1006	48	41	−3.0[c]
		32	157	−0.6
		16	84	0.3
		8	41	−0.3
		4	12	0.8
		2	4.1	0.4
	ADM	16	40	−2.4[c]
		8	106	−0.4
		4	53	0.1
		2	12	0.9
		1	7.1	1.2

P388 cells (10^6 cells/mouse) were implanted i.p. on day 0. AN1006 or ADM was given i.v. on day 1 or days 1, 5, and 9. Six mice were used in each group. Experimental details are described in Materials and methods. The SD of the increase in life span was within 10% of each value

[a] The mean duration of survival for control animals was 10.0 ± 1.1 days

[b] The mean duration of survival for control animals was 9.8 ± 0.4 days

[c] Toxic

of AN1006 was more dependent on the treatment schedule than that of ADM. This schedule dependency was also shown in L1210. The ILS_{max} for AN1006 was 59% and 101% on the one-shot and intermittent schedule, respectively, and that for ADM was 138% and 102%, respectively (Table 3).

Table 3. Antitumor activity of AN1006 and ADM against L1210 leukemia

Schedule	Drugs	Dose (mg/kg daily)	ILS (%)	Body weight loss at each nadir (g)
Day 1[a]:				
	AN1006	64	−19	−5.0[b]
		48	59	−1.3
		32	38	−0.3
		16	26	0.2
		8	−2	0.8
		4	−2	1.0
	ADM	32	102	−2.8[b]
		16	138	0
		8	28	0.7
		4	10	0.6
Days 1, 5, and 9[a]:				
	AN1006	48	42	−2.5[b]
		32	101	−0.5
		16	65	0.7
		8	18	0.8
		4	10	1.8
		2	−2	1.2
	ADM	16	77	−2.8[b]
		8	102	1.0
		4	40	1.2
		2	2	0.9

L1210 cells (10^5 cells/mouse) were implanted i.p. on day 0. AN1006 or ADM was given i.v. on day 1 or days 1, 5, and 9. Six mice were used in each group. Experimental details are described in Materials and methods. The SD of the increase in life span was within 10% of each value

[a] The mean duration of survival for control animals was 8.3 ± 0.7 days

[b] Toxic

Antitumor activity against murine solid tumors

Since the antitumor activity of AN1006 on the intermittent schedule was superior to that on the one-shot schedule in leukemia models, the drugs were given i.v. once a week starting on the day after tumor implantation against murine solid tumors. The tumor-inhibitory effects of AN1006 and ADM are shown in Table 4. Both AN1006 and ADM exhibited significant antitumor activity against all tumors examined in this study. As no significant difference was observed between the T/C(%) values for AN1006 and ADM according to Student's *t*-test, we concluded that the activity of AN1006 was comparable with that of ADM.

Cytotoxicity of AN1006

The cytotoxicity of AN1006 was compared with that of ADM against 13 human tumor cell lines (Table 5). AN1006 inhibited the cell growth at lower concentrations than did ADM against seven cell lines, including stomach carcinomas St-4 and 4-1St, colon carcinoma HT-29, and leukemia cell lines HEL, HL-60, and U937. AN1006 showed almost the same IC_{50} values as ADM toward other cell lines. These results indicate that the cytotoxic spectrum of AN1006 is different from that of ADM, with the former showing cytotoxicity stronger than that of ADM against

Table 4. Antitumor activity of AN1006 and ADM against murine solid tumors

Tumors	Drugs	Dose (mg/kg daily)	Tumor volume[a] (mm^3)	T/C (%)	Day
B16	Control	0	12,900 ±6,410	100	28
	AN1006	32	2,510 ±1,120*	19.5	28
		16	9,680 ±3,090	75.0	28
		8	13,600 ±4,632	105	28
	ADM	8	5,410 ±2,690*	42.0	28
		4	11,000 ±6,060	85.3	28
		2	10,700 ±6,710	82.9	28
Colon 26	Control	0	4,050 ±1,400	100	29
	AN1006	32	1,860 ± 576**	45.8	29
		16	3,640 ±1,240	89.9	29
		8	4,090 ±1,080	101	29
	ADM	8	1,150 ± 898**	28.3	29
		4	3,123 ±2,141	77.1	29
		2	3,210 ± 672	79.3	29
Colon 38	Control	0	2,280 ± 652	100	29
	AN1006	32	446 ± 536***	19.6	29
		16	1,290 ± 809	56.6	29
		8	1,460 ± 943	64.0	29
	ADM	8	49.3 ± 121***	2.2	29
		4	1,240 ± 770	54.4	29
		2	1,330 ± 670	58.3	29
LLC	Control	0	10,300 ±2,320	100	22
	AN1006	48	3,150 ±1,320***	30.6	22
		32	6,270 ± 252	60.9	22
		16	8,220 ±1,330	79.8	22
	ADM	8	3,180 ±1,100***	30.8	22
		4	5,100 ±1,030*	49.5	22
		2	8,910 ±1,350	86.5	22
M5076	Control	0	5,980 ±1,710	100	29
	AN1006	32	1,510 ± 218**	25.4	29
		16	3,750 ±1,100	62.7	29
		8	6,230 ±1,750	104	29
	ADM	8	1,060 ± 478***	17.7	29
		4	4,230 ±1,510	70.7	29
		2	7,250 ± 898	121	29

Each tumor cell line was inoculated s.c. on day 0 and each drug was given i.v. on days 1, 8, 15 and 22. Six mice were used in each group. Experimental details are described in Materials and methods. The mean body weight loss of each treatment group was less than 2.0 g at each nadir

[a] Mean value ± SD for six determinations

Significance: * $P<0.05$; ** $P<0.01$; *** $P<0.001$

Table 5. Cytotoxicity of AN1006 and ADM against human tumor cell lines

Origin	Cell line	IC_{50} value (n*M*)	
		AN1006	ADM
Nasopharynx	KB 3-1	15.4±0.3	11.2± 1.3
Breast	MCF-7	17.0±1.3	10.2± 0.5
Lung	A549	16.1±0.6	14.9± 2.0
Stomach	St-4	8.9±2.8	31.7± 4.2
	4-1St	8.1±1.4	810 ±67
Colon	HT-29	9.9±0.7	38.7± 1.4
Ovary	A2780	9.2±0.8	8.2± 0.1
Blood	K562	18.2±0.6	21.6± 1.3
	HEL	1.0±0.02	16.2± 0.3
	HL-60	3.3±0.5	18.2± 0.8
	THP-1	13.6±1.5	11.7± 0.5
	U937	11.7±1.1	23.1± 2.6
	CCRF-CEM	14.9±0.3	25.3± 1.1

Table 6. Antitumor activity of AN1006 and ADM against human xenograft tumors

Origin	Tumor cells	T/C (%)	
		AN1006	ADM
Colon	HT-29	38[a]	50
	DLD-1	60	62
	WiDr	46[a]	76
	KM-12	27[a]	28[a]
	KM20L2	37[a]	55
Stomach	St-4	52	23[a]
	St-40	7[a]	42[a]
	Sc-6	31[a]	42[b]

Each tumor cell line was inoculated s.c. on day 0, and each drug was given i.v. on days 1, 8, and 15. Six mice were used in each group. Experimental details are described in Materials and methods. The mean body weight loss of each treatment group was less than 2.0 g at each nadir

[a] Determined as effective according to both the T/C (%) value and Mann-Whitney's *U*-test

[b] Nonsignificant according to Mann-Whitney's *U*-test ($P>0.01$, one-sided)

colon carcinoma, stomach carcinoma, and some leukemia cell lines.

Antitumor activity against human tumor xenografts

Since AN1006 displayed better cytotoxicity than did ADM against colon and stomach carcinoma cells, we examined the antitumor activity of AN1006 against five human colon carcinoma xenografts and three human stomach carcinoma xenografts. AN1006 or ADM was given i.v. once a week four times at the maximum tolerated daily dose of 32 and 8.2 mg/kg, respectively (Table 6). AN1006 was effective against four of five colon carcinomas and two of three stomach carcinomas, whereas ADM was effective against one colon carcinoma and two stomach carcinomas. AN1006 showed an especially marked therapeutic effect against St-40 stomach carcinoma.

Cytotoxicity against multidrug-resistant human tumor cells

Among the cell lines tested above, 4-1St was rather resistant to ADM (Table 5). Actually, 4-1St showed a multidrug-resistant phenotype spontaneously and expressed the P-glycoprotein (unpublished data). 4-1St cells, however,

Table 7. Cytotoxicity of AN1006 and ADM against multidrug-resistant human tumor cell lines

Cell line	IC_{50} value (n*M*)	
	AN1006	ADM
KB3-1	15.4±0.3	11.2± 1.3
KBC-4	16.2±0.3 (1.1)[a]	972 ± 94 (87)
MCF-7	17.0±1.3	10.2± 0.5
MCF-7/ADM	17.5±0.8 (1.0)	1,390 ±170 (137)
A2780	9.2±0.8	8.2± 0.1
2780AD	11.6±0.3 (1.3)	806 ± 39 (98)
K562	18.2±0.6	21.6± 1.3
K562/ADM	27.5±0.9 (1.5)	2,070 ± 79 (96)
CCRF-CEM	14.9±0.3	25.3± 1.1
CEM/VLB100	19.4±1.8 (1.3)	747 ±100 (30)

[a] Numbers in parentheses represent the degree of resistance (*x*-fold) as compared with parent cells

Table 8. Antitumor activity of AN1006 and ADM against multidrug-resistant P388/ADM leukemia

Drugs	Tumors	Dose (mg/kg daily)	ILS (%)	Body weight loss at each nadir (g)
AN1006:				
	P388[a]	48	15	–2.7[c]
		32	158	–0.7
		16	94	–0.2
		8	46	0.6
		4	9	1.1
		2	6	2.0
	P388/ADM[b]	48	0	–2.2[c]
		32	57	–0.3
		16	15	0.7
		8	12	1.0
		4	1	0.5
		2	0	0.8
ADM:				
	P388[a]	16	89	–2.5[c]
		8	145	0.5
		4	75	–0.3
		2	15	0.8
	P388/ADM[b]	16	–3	–2.8[c]
		8	–3	–0.5
		4	1	0.7
		2	3	0.7

P388 or P388/ADM cells (10^6 cells/mouse) were implanted i.p. on day 0. AN1006 or ADM was given i.v. on days 1, 5, and 9. Six mice were used in each group. Experimental details are described in Materials and methods. The SD of the increase in life span was within 10% of each value

[a] The mean duration of survival for control animals was 10.0± 1.1 days

[b] The mean duration of survival for control animals was 9.8±0.4 days

[c] Toxic

did not show cross-resistance to AN1006. Therefore, we examined the effectiveness of AN1006 against other multidrug-resistant cell lines expressing P-glycoprotein (Table 7). Human multidrug-resistant cell lines KBC-4, MCF-7/ADM, 2780AD, K562/ADM, and CEM/VLB100 showed 87-, 137-, 98-, 96-, and 30-fold resistance to ADM, respectively, as compared with the respective parental cell line. These cell lines were sensitive to AN1006 and showed 1.1-, 1.0-, 1.3-, 1.5-, and 1.3-fold resistance to AN1006, respectively. These observations suggest that AN1006 would be effective against multidrug-resistant tumors in vivo.

Antitumor activity against multidrug-resistant P388/ADM

To confirm the above speculation, the antitumor effect of AN1006 against the murine multidrug-resistant tumor P388/ADM was examined (Table 8). A total of 1 million P388/ADM cells were implanted i.p. into $CD2F_1$ mice, and AN1006 or ADM was given i.v. on days 1, 5, and 9. AN1006 exhibited antitumor activity, and its ILS_{max} was 57%. ADM did not exhibit any antitumor activity.

Discussion

In the present study, we evaluated the antitumor activity and cytotoxicity of AN1006, a dibutyryl derivative of ankinomycin. Among the synthesized acyl derivatives, AN1006 was chosen because of its superior antitumor activity against P388 leukemia cells and its low acute toxicity. Indeed, the LD_{50} values for AN1006 and ANK following i.v. administration were 98 and 2 mg/kg, respectively.

The antitumor activity of AN1006 depended on its administration schedule, and intermittent treatment was superior to the one-shot schedule. On an intermittent schedule, AN1006 showed superior (against P388 leukemia) and equal (against L1210 leukemia) antitumor activity as compared with ADM. AN1006 also showed antitumor activity against various s.c. implanted solid tumors such as B16, Colon 26, Colon 38, LLC, and M5076. As the antitumor activity of AN1006 was statistically equivalent to that of ADM, we concluded that the antitumor activity of AN1006 against murine solid tumors and leukemias was comparable with that of ADM.

The cytotoxic spectrum of AN1006 was different from that of ADM. AN1006 showed cytotoxicity stronger than that of ADM against colon carcinoma, stomach carcinoma, and some leukemia cell lines. Similarly to these results, AN1006 was effective against four of five human colon carcinoma xenografts and two of three stomach carcinoma xenografts tested, whereas ADM was effective against one of five colon carcinomas and two of three stomach carcinomas. The antitumor activity of AN1006 seems to be superior to and equal to that of ADM against human colon and human stomach carcinomas, respectively.

AN1006 was effective against various multidrug-resistant human tumor cells in vitro and was effective against P388/ADM in vivo at one dose level (in repeated experiments). As AN1006 was effective against multidrug-resistant tumor cells in vitro and (partially) in vivo, we initially hypothesized that AN1006 did not interact with the P-glycoprotein. However, AN1006 has been shown to inhibit the incorporation of vincristine into plasma membrane vesicles of K562/ADM cells [9] and to inhibit the photoaffinity labeling of azidopine into the P-glycoprotein of K562/ADM cells ([11], data not shown). Therefore,

AN1006 can interact with the P-glycoprotein of multidrug-resistant tumor cells. The reason why AN1006 is effective against multidrug-resistant tumors remains to be clarified.

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