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Antitumor activity on murine tumors of a novel antitumor benzoylphenylurea derivative, HO-221

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Summary. A novel antitumor compound, *N*-[4-(5-bromo-2-pyrimidinyloxy)-3-chlorophenyl]-*N'*-(2-nitrobenzoyl) urea (HO-221) was evaluated for its antitumor activity in experimental tumor models. HO-221 preparation was given orally to tumor-bearing animals. The compound exhibited significant effects against various tumors such as P388 and L1210 leukemias; M5076 reticulum-cell sarcoma; colon 38 carcinoma; human xenografts MX-1, LX-1, GA-1, and Co-1; Lewis lung carcinoma; sarcoma 180; and Walker 256 carcinosarcoma and was especially effective against solid tumors. However, its effect on murine B16 melanoma was moderate. Intermittent administration of HO-221 produced better results. The effects of HO-221 on human tumor xenografts were compared with those of other antitumor agents. HO-221 showed activity against LX-1 lung and Co-1 gastrointestinal tumor and was also effective against advanced-stage L1210 leukemia and Lewis lung carcinoma. Furthermore, the effect of HO-221 on drug-resistant tumors was examined using murine leukemias L1210 and P388. It showed no cross-resistance with the known antitumor agents Adriamycin (ADM), daunomycin (DM), vincristine (VCR), mitomycin C (MMC), cisplatin (CDDP), 5-fluorouracil (5-FU), cytosine arabinoside (Ara-C), methotrexate (MTX), cyclophosphamide (CPA), or carboquone (CQ), and collateral sensitivity to HO-221 was found in MMC-, CDDP-, and CPA-resistant sublines. HO-221 exhibits significant reproducible, broad-spectrum antitumor activity against experimental tumors as well as human neoplasms.

Introduction

HO-221 is a new benzoylphenylurea derivative. Related compounds have been found to display insecticidal activity [9] due to the inhibition of chitin synthesis in insect tissue [7]. As had been interested in the various pharmacological actions of benzoylphenylurea compounds, we synthesized and tested many related compounds in various screening systems. We found that a few compounds, including HO-221, showed excellent antitumor activity in vitro or in vivo. HO-221 was chosen for further development because it had a significant antitumor effect on many animal tumor models and produced no particular toxicities in rats or beagles and its mechanism of action was different from that of known antitumor agents [3]. In this report, we describe the antitumor activity of HO-221 against various experimental tumor models.

Materials and methods

Chemicals. *N*-[4-(5-Bromo-2-pyrimidinyloxy)-3-chlorophenyl]-*N'*-(2-nitrobenzoyl)urea (HO-221; molecular weight, 492.67 Da) was synthethized (Fig. 1). A report on the synthesis and the structure-activity relationships of this and related compounds is in preparation. For in vivo study of its p.o. administration, HO-221 was pulverized by being shaken together with glass beads in a 5% (w/v) HCO60 (polyoxyethylene-hydrogenated caster oil 60) solution supplemented with Dynomill (Willy A. Bachofen Co.). For in vitro study, it was dissolved in fetal calf serum containing 1% dimethylsulfoxide (DMSO). Mitomycin C (MMC), Adria mycin (ADM) and 5-fluorouracil (5-FU) were purchased from Kyowa Hakko Kogyo Co., Ltd. (Osaka); cyclophosphamide (CPA) and vincristine (VCR) were obtained from Shionogi Pharmaceutical Co., Ltd. (Osaka); nimustine (ACNU) and carboquone (CQ) were supplied by Sankyo Co., Ltd. (Tokyo); tegafur (TGF) was purchased from Taiho Pharmaceutical Co., Ltd. (Tokyo); methotrexate (MTX) was obtained from Lederle Japan, Ltd. (Tokyo); cytosine arabinoside (Ara-C) was purchased from Nippon Shinyaku, Ltd. (Tokyo); daunomycin (DM) was supplied by Meiji Seika Kaisya, Ltd. (Tokyo); 6-mercaptopurine (6-MP) was obtained from Takeda Chemical Industries, Ltd. (Osaka); neocarzinostatin (NCS) was purchased from Yamanouchi Pharmaceutical Co.,

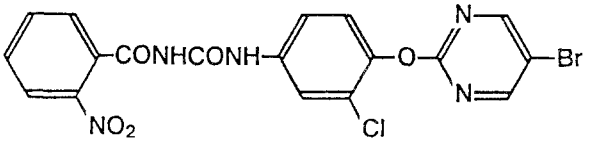

Fig. 1. Chemical structure of HO-221

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Table 1. Activity of HO-221 against L1210 leukemia

Tumor	Treatment schedule[b]	Dose (mg kg^{-1} day^{-1})	ILS[c] (%)
L1210 (i.p.)[a]	Days 1–9	1.6	0
		3.13	2
		6.25	81
		12.5	–31
		25	–40
	Day 1	6.25	0
		12.5	28
		25	45
		50	37
		100	17
		200	–13
	Days 1, 5, 9	6.25	17
		12.5	91
		25	63
		50	–7
		100	–4
	Days 1, 8	12.5	71
		25	108
		50	123 (1/6)[d]
		100	123
		200	109
		400	–47
L1210 (s.c.)[a]	Days 1, 8	6.25	27
		12.5	68
		25	81
		50	94
		100	119 (1/6)[d]
		200	80

[a] Route of tumor-cell implantation
[b] Tumor cells were implanted on day 0. HO-221 was given according to the schedules shown
[c] The mean survival of control animals was 7.8 (L1210, i.p.) and 9.7 days (L1210, s.c.)
[d] 45-day survivors

Table 3. Activity of HO-221 against Lewis lung carcinoma

Treatment schedule[a]	Dose (mg kg^{-1} day^{-1})	T/C (%)
Days 1–9	0.8	90.3
	1.6	39.3
	3.13	36.7
	6.25	53.6
	12.5	Toxic[b]
	25	Toxic[b]
Day 1	6.25	65.3
	12.5	25.2*
	25	5.2**
	50	1.1**
	100	1.5**
	200	Toxic[b]
	400	Toxic[b]
Days 1, 8, 15	12.5	67.1
	25	3.1**
	50	0.9**
	100	0.3**
	200	Toxic[b]

[a] Tumor cells were implanted s.c. on day 0. HO-221 was given according to the schedules shown
[b] The treatment was considered to be toxic if one or more of the animals had died by the final day of testing
* $P<0.05$; ** $P<0.01$

Ltd. (Tokyo); and cisplatin (CDDP) was obtained from Nippon Kayaku Co., Ltd. (Tokyo).

Animals and tumor cells. Male C57BL/6× DBA/2 F1 mice (hereafter termed BDF1; body weight, 19–21 g; age, 5–7 weeks), male ICR mice (19–21 g, 5–7 weeks old), male SD rats (80–100 g, 5–6 weeks old), and female BALB/c-nu/nu mice (4–5 weeks old) were purchased from Charles River Japan (Kanagawa, Japan) and Clea Japan Inc. (Tokyo). Food and drinking water were provided ad libitum. L1210 leukemia (L1210), P388 leukemia (P3888), M5076 reticulum-cell sarcoma

Table 2. Activity of HO-221 against various tumors

Tumors[a]	Treatment schedule[b]	Dose range (mg kg^{-1} day^{-1})	Optimal dose (mg kg^{-1} day^{-1})	ILS[c] (%)
P388 (i.p.)	Days 1–9	1.6 – 25	6.25	35
	Day 1	12.5 –200	50	70 (1/6)[d]
	Days 1, 5, 9	6.25–100	25	88
	Days 1, 8	12.5 –200	50	97 (1/6)[d]
B16 (i.p.)	Days 1–9	0.8 – 25	1.6	15
	Day 1	12.5 –400	50	21
	Days 1, 8, 15	6.25–200	25	48 (1/6)[d]
M5076 (i.p.)	Days 1, 5, 9, 13	6.25–200	50	56
B16 (s.c.)	Days 1–9	0.8 – 25	1.6	36 (1/6)[d]
	Day 1	12.5 –400	50	17
	Days 1, 8, 15	6.25–200	12.5	23
LL (i.v.)	Days 1–9	1.6 – 25	3.13	26
	Days 1, 5, 9	3.13– 50	25	47
	Days 1, 8, 15	6.25–100	50	44 (1/6)[d]
LL (s.c.)	Days 1–9	0.8 – 25	1.6	22
	Day 1	12.5 –400	25	40 (1/6)[d]
	Days 1, 8, 15	6.25–400	100	118 (3/6)[d]

[a] Route of tumor-cell implantation is indicated in parentheses
[b] Tumor cells were implanted on day 0. HO-221 was given according to the schedules shown
[c] ILS(%) at the optimal dose. The mean survival of control animals was 9.4 (P388, i.p.), 21.6 (B16, i.p.), 23 (M5076, i.p.), 29.2 (B16, s.c.), 26 (LL, i.v.) and 27 days (LL, s.c.), respectively
[d] 45- (P388) and 60-day survivors

Table 4. Activity of HO-221 against solid tumors

Tumors	Treatment schedule[a]	Dose range (mg kg^{-1} day^{-1})	Optimal dose (mg kg^{-1} day^{-1})	T/C[b] (%)
C38	Days 2, 9	3.13–100	25	2.8*
M5076	Days 1, 5, 9, 13	3.13–100	50	2.1**
	Days 1, 8, 15	6.25–100	50	1.7**
S180	Days 1–9	3.13–100	25	10.5**
	Days 1, 5, 9	12.5 –400	200	10.5**
	Days 1, 8	50 –400	400	14.1*
W256	Days 1–5	50 –400	200	3.5**
	Days 1, 5, 9	50 –400	400	0**
MX-1	Day 0	50 –400	400	43.1*
	Days 0, 4, 8	12.5 –400	200	28.9*
	Days 0, 7, 14	25 –400	200	20.5*
LX-1	Days 0, 4, 8	12.5 –400	100	6.5**

[a] Tumor cells were implanted s.c. on day 0. HO-221 was given according to the schedules shown. For MX-1 and LX-1 xenografts, HO-221 was given after the tumor volume had increased to about 150–200 mm^3; that time is expressed as day 0

[b] T/C(%) at the optimal dose

* $P<0.05$; ** $P<0.01$

(M5076), B16 melanosarcoma (B16), colon 38 (C38) carcinoma, human mammary xenograft MX-1 (MX-1), and lung xenograft LX-1 (LX-1) were obtained from the Cancer Chemotherapy Center, Cancer Institute, Japanese Foundation for Cancer Research (Tokyo). Human gastrointestinal xenografts Ga-1 (Ga-1) and Co-1 (Co-1) were established by transplanting the tumors from patients into nude mice. Lewis lung carcinoma (LL), sarcoma 180 (S180) and Walker 256 carcinosarcoma (W256) were obtained from the Sasaki Foundation Institute (Tokyo). Sublines of L1210 that were resistant to DM, MMC, CDDP, 5-FU, Ara-C, MTX, CPA, and CQ were established by treatment with a maximally tolerated dose of the antitumor agents. Sublines of P388 that were resistant to ADM and VCR were supplied by the Cancer Chemotherapy Center, Cancer Institute.

Unless noted otherwise, tests were carried out according to the protocols published by the National Cancer Institute (USA) [1]. The parent and drug-resistant sublines of L1210 were implanted i.p. or s.c. at 1×10^5 cells into BDF1 mice, and P388 was implanted i.p. at 1×10^6 cells. B16 was implanted i.p. or s.c. as 0.2 ml of a 10% (w/v) tumor brei into BDF1 mice, and M5076 was implanted i.p. or s.c. at 1×10^6 cells. LL was implanted i.v. (1×10^4 cells) or s.c. (5×10^5 cells) into BDF1 mice, and C38 was implanted s.c. as fragments. S180 was implanted s.c. at 1×10^6 cells into ICR mice and W256 was implanted s.c. at 1×10^6

Table 5. Effect of HO-221 on human xenografts

Drugs[a]	Treatment schedule[b]	Dose[c] (mg/kg)	Ga-1		Co-1		Lx-1	
			T/C (%)	B.w.loss[d] (%)	T/C (%)	B.w.loss[d] (%)	T/C (%)	B.w.loss[d] (%)
HO-221 (p.o.)	Days 0, 4, 8	50	52.6	3.5	53.9	–	22.4*	5.7
		100	57.9	–	46.3	3.9	Toxic[e]	–
ADM (i.v.)	Days 0, 4, 8	2.5	77.4	–	56.2	–	60.8	5
		5	84.4	5.9	51.7	0.9	56.9	10.3
MMC (i.p.)	Days 0, 4, 8	1.5	72.1	–	42.3	1.3	43.9	2.9
		3	45	–	20.3*	–	22.6*	3.8
CDDP (i.p.)	Days 0, 4, 8	3	72.3	4.3	33	0.5	53.8	10.6
		6	42.7	15.2	17.4*	6.3	34.3*	14.6
TGF (p.o.)	Days 0–8	50	99.9	–	72	–		
		100	78.5	–	68	–		
CPA (i.p.)	Days 0, 4, 8	40					54.4	9.2
		80					65.4	7.9
VCR (i.p.)	Days 0, 4, 8	0.7					44	13.2
		1.4					Toxic[e]	–

[a] Route of drug administration is indicated in parentheses

[b] Drug was given after the tumor volume had increased by ca. 150–200 mm^3; that time is expressed as day 0

[c] The dose amounted to 1/3 and 1/6 of the LD_{50} in mice treated on the intermittent schedule (days 0, 4, 8) and was 1/9 and 1/18 of that in animals treated on the consecutive schedule (days 0–8)

[d] Maximal body weight loss

[e] The treatment was considered to be toxic if one or more of the animals had died by the final day of testing

* $P<0.05$

Table 6. Effect of HO-221, TGF, and Ava-C on advanced-stage L1210 or Lewis lung carcinoma in mice

Drugs	Treatment schedule[a]	Dose (mg kg^{-1} day^{-1})	ILS[b] (%)	T/C (%)
L1210:				
HO-221	Days 5, 9	6.25	5	–
		12.5	27	–
		25	67	–
		50	64	–
		100	63	–
TGF	Days 5–9	100	9	–
		200	44	–
		400	46	–
		800	42	–
Ara-C	Days 5–9	25	88	–
		50	98	–
		100	126	–
		200	94	–
Lewis lung carcinoma:				
HO-221	Days 12, 16, 20	12.5	1	38.3
		25	61	39.7
		50	94	33.5
		100	60	17.6*
		200	61	16.6*
TGF	Days 12–16	50	12	66.7
		100	17	49.6
		200	–22	26.2*
		400	–61	Toxic
Ara-C	Days 12–16	25	0	86.4
		50	0	46
		100	–61	Toxic
		200	–60	Toxic

[a] Tumor cells were implanted on day 0. Drugs were given according to the schedules shown

[b] The mean survival of control animals was 8.1 (L1210) and 23 days (LL)

* $P<0.05$

cells into Sprague-Dawley rats. MX-1 and LX-1 xenografts were implanted s.c. as fragments into BALB/c-nu/nu mice.

Antitumor effect of HO-221 in survival experiments. L1210, P388, B16, M5076, and LL were implanted on day 0. Treatment with HO-221 was initiated at 1 day after implantation and was continued either daily for 9 days, every 4th or 7th day, or on day 1 only. Antitumor activity was assessed on the basis of the percentage of increase in life span (ILS) and the incidence of long-term survivors (45 or 60 days). The mean life span was calculated from grouped mean survival data (MS), and the percentage of ILS was calculated as ILS% = (MS of treated animals/MS of control animals) $\times$100–100. The criteria for effective activity in these models were the same as those used in NCI protocols.

Antitumor effect of HO-221 in tumor-growth inhibition experiments. LL, C38, M5076, S180, and W256 were implanted on day 0. Thereafter, HO-221 was given according to the schedules used in the survival experiment. In human tumor xenografts, treatment was begun after tumor volume had increased by about 150–200 mm^3 [5]. Antitumor activity was assessed according to the mean tumor volume derived from caliper measurements [1]. The percentage of mean tumor volume in treated (T) as compared with control (C) animals was calculated as T/C(%) = (mean tumor volume in treated animals/mean tumor volume in control animals) $\times$100. Significance was evaluated using Student's *t*-test.

Comparison of the antitumor effects on human tumor xenografts. LX-1, Ga-1 and Co-1, which were poorly differentiated adenocarcinomas, were implanted. After the tumor volume had increased by about 150–200 mm^3, HO-221 and the antitumor agents were given three times on every 4th day or daily for 9 days. The total dose of the individual agents was divided into 3 or 9 doses at levels that were lethal to 50% of the mouse population (LD_{50}).

Effect on advanced-stage tumors. TGF and Ara-C as reference drugs and HO-221 were given to animals with advanced-stage tumors. L1210 was implanted i.p. and LL was implanted s.c. into mice on day 0. Thereafter, treatment with HO-221, TGF (p.o.) and Ara-C (i.p.) was initiated on days 5, 12, and 12, respectively. Antitumor activity was assessed from the percentage of ILS and T/C.

Antitumor effect of HO-221 on drug-resistant tumors. Cells from the parental lines and the resistant sublines of L1210 or P388 were implanted into mice on day 0. HO-221 and the antitumor agents (i.p.) were given according to the treatment schedules shown in Tables 1 and 2.

In vitro assay of growth inhibition in drug-resistant tumors. Cells of the drug-resistant sublines P388/ADM, L1210/MMC, and L1210/CDDP were suspended in culture medium with antitumor agents, seeded at a final cell density of 2×10^4/ml, and incubated in a CO_2 incubator at 37° C for 48 h. The growth-inhibition rate was assessed according to the tetrazolium (MTT) dye-reduction assay of Mosman [2].

Results

Effect of HO-221 on survival models

The antitumor effect of HO-221 was evaluated in a variety of murine tumor models. HO-221 exhibited activity against L1210 in mice as shown in Table 1. The results of other survival experiments are shown in Table 2. The optimal dose of HO-221 resulted in 97% ILS in P388, 56% ILS in M5076, 48% (i.p.) and 36% (s.c.) ILS in B16, and 47% (i.v.) and 118% (s.c.) ILS in LL. Intermittent administration of HO-221 produced good results.

Effect of HO-221 on solid tumors

HO-221 showed significant antitumor activity against LL and other solid tumors as shown in Tables 3 and 4, respectively. The optimal dose of HO-221 resulted in 0.3% T/C in LL, 2.8% T/C in C38, 1.7% T/C in M5076, 10.5% T/C in S180, 20.5% T/C in MX-1, and 6.5% T/C in LX-1. Complete tumor regression was observed in W256 at this dose.

Effect of HO-221 on human tumor xenografts

As shown in Table 5, HO-221 showed significant activity against lung xenograft LX-1. None of the drugs was effective against gastrointestinal tumor Ga-1. On the other hand, HO-221 was more effective than ADM or TGF against Co-1, although its antitumor activity was lower than that of either CDDP or MMC. HO-221 caused less body weight change than did CDDP.

Table 7. Effect of HO-221 on the survival of mice bearing P388 or L1210 leukemia either resistant or sensitive to antitumor agents

Tumors[a]	Drugs	Schedule (days)	Dose range (mg/kg)	Resistant		Sensitive	
				OED[b] (mg/kg)	ILS (%)	OED[b] (mg/kg)	ILS (%)
P388, P388/ADM	ADM	1, 8	1.25 – 40	5	0	5	233 (3/6)[c]
	HO-221	1, 8	25 – 800	400	73	400	161
P388, P388/VCR	VCR	1, 5	0.13 – 4	2	12	2	100
	HO-221	1, 8	25 – 1,600	200	88	200	161
L1210, L1210/DM	DM	1, 8	1.25 – 40	10	25	5	49
	HO-221	1, 8	25 – 800	800	137	800	148
L1210, L1210/MMC	MMC	1, 8	1 – 32	4	2	8	54
	HO-221	1, 8	25 – 1,600	50	222 (6/6)[c]	200	105
L1210, L1210/CDDP	CDDP	1, 8	1.25 – 40	2.5	17	20	118
	HO-221	1, 8	25 – 1,600	25	168 (5/6)[c]	200	105
L1210, L1210/5-FU	5-FU	1 – 4	25 – 800	50	13	200	87
	HO-221	1, 8	25 – 800	800	115	800	148
L1210, L1210/Ara-C	Ara-C	1 – 4	25 – 800	100	0	400	122
	HO-221	1, 8	6.25 – 200	200	190	100	154
L1210, L1210/MTX	MTX	1 – 4	0.63 – 80	40	11	40	52 (1/6)[c]
	HO-221	1, 8	12.5 – 1,600	800	127	800	106 (1/6)[c]
L1210, L1210/CPA	CPA	1	3.13 – 400	200	21	200	64
	HO-221	1, 8	12.5 – 1,600	200	94 (1/6)[c]	400	110
L1210, L1210/CQ	CQ	1	0.13 – 4	2	17	2	59
	HO-221	1, 8	12.5 – 1,600	400	91	400	117 (2/6)[c]

[a] Tumor cells were implanted i. p. on day 0
[b] Optimal effective dose
[c] Survivors on day 60

Effect of HO-221 on advanced-stage tumors in mice

As shown in Table 6, all drugs were effective in increasing the survival of L1210-bearing animals, although the ILS values obtained were lower than those resulting from earlier-stage treatment. In LL, HO-221 also inhibited the growth of advanced-stage tumors. On the other hand, TGF and Ara-C were ineffective in increasing the life span of mice, although they inhibited the growth of advanced tumors.

Effect of HO-221 on drug-resistant tumor cells

The response of L1210 and its eight resistant sublines to three alkylating agents, three antimetabolites, and two antibiotics and the response of P388 and its two resistant sublines to one antibiotic and an alkaloid are shown in Table 7. The sensitivity of ADM- or VCR-resistant sublines to HO-221 was slightly decreased. However, the individual sensitivities of DM-, 5-FU-, Ara-C-, MTX-, CPA-, or CQ-resistant sublines to HO-221 were about the same as those of the parent lines. Furthermore, the response of MMC- or CDDP-resistant sublines to the compound was greatly increased as compared with that of the parent lines; that is, the collateral sensitivities to HO-221 were shown in vivo. Numerous 60-day survivors were also observed among mice bearing the MMC-, CDDP-, and CPA-resistant sublines as compared with those bearing the parent lines following treatment with HO-221. The ADM-, MMC-, and CDDP-resistant sublines, which showed multidrug resistance and collateral sensitivities in vivo, were used for in vitro study. As shown in Table 8, no cross-resistance was observed between HO-221 and the ADM-, MMC-, and CDDP-resistant sublines; furthermore, the collateral sensitivities observed in vivo did not occur in vitro.

Discussion

Among many benzoylphenylurea compounds, HO-221 was chosen for testing as an agent with promising antitumor activity. In this study, HO-221 showed significant activity against various experimental tumor models, including eight survival models (Tables 1, 2) and seven solid tumor models (Tables 3, 4) in mice and rats. The effect was especially marked against solid tumors. In these tumor models, the optimal HO-221 dose that produced the best results varied from 25 to 200 mg/kg daily given intermittently. The wide range of values obtained is considered to be attributable to the varying susceptibility to HO-221 (maximally tolerated dose) of the animal species tested, including Sprague-Dawley rats and BDF1, ICR, and BALB/c-nu/nu mice. The effect of HO-221 was compared with that of other known antitumor agents in three human tumor xenografts. HO-221 showed higher activity against LX-1 than did ADM, CDDP, or CPA. In a gastrointestinal tumor, the compound was also more effective than either

Table 8. Cytotoxicity of HO-221, ADM, CDDP, and MMC in murine leukemias exhibiting sensitivity or resistance to ADM, CDDP, and MMC

Tumors	IC_{50} (nM)			
	HO-221	ADM	CDDP	MMC
P388	160±36	130±10		
P388/ADM	140±12 (0.9)	8,590±140 (66)		
L1210	57±2.6		1,060±109	
L1210/CDDP	54±5.2 (0.9)		12,560±350 (12)	
L1210	71±0.7			480±16
L1210/MMC	65±0.5 (0.9)			2,490±60 (5)

Values represent the mean ± SD of 3 determinations. Numbers in parentheses represent the degree (n = fold) of resistance as compared with sensitive cells. IC_{50}, Concentration of drug that inhibits the growth of 50% of the cell population

ADM or TGF (Table 5). Furthermore, in L1210 and LL, the compound was effective in increasing the life span of animals and in inhibiting the growth of advanced-stage disease (Table 6).

The effect of HO-221 on the response of drug-resistant sublines to ten antitumor agents proved to be almost the same as that of the parent lines, and collateral sensitivity to HO-221 was observed in the MMC-, CDDP-, and CPA-resistant sublines (Table 7). Thus, no cross-resistance was found between HO-221 and other known antitumor agents. Collateral sensitivity has been reported for a number of resistant tumor cell lines but has been observed only in in vivo experiments [6]. In the present study, these phenomena could not be proven on a cellular basis in vitro using drug-resistant sublines (Table 8) and were diminished in vivo following pretreatment of the mice with CPA at 3 days before tumor-cell implantation (data not shown). Therefore, the collateral sensitivities observed in this study seem to be due to the elevated immunosensitivity of the drug-resistant cells to the tumor-bearing host [4, 8]. In addition, it has been shown that although HO-221 strongly inhibits the activity of DNA polymerase-α, it does not diminish DNA polymerase-β or -γ activity, RNA polymerase activity, or protein synthesis in cell-free systems [3]. As a novel antitumor agent for cancer chemotherapy, HO-221 exhibits significant, reproducible, broad-spectrum antitumor activity against experimental tumor models.

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