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Antitumor activity of MST-16, a novel derivative of bis(2,6-dioxopiperazine), in murine tumor models

Toshiharu Narita[1], Shin-ichi Yaguchi[1], Toshihiko Komatsu[1], Muneaki Takase[1], Akira Hoshino[2], Makoto Inaba[3], and Shigeru Tsukagoshi[3]

[1] Research Laboratory, Zenyaku Kogyo Co., Ltd., 33-7 Ohizumi-machi 2-chome, Nerima-ku, Tokyo 178, Japan
[2] Anjo Kosei Hospital, Anjo-shi, Aichi 446, Japan
[3] Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Kami-Ikebukuro, Toshima-ku, Tokyo 170, Japan

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Summary. We studied the antitumor activity of newly synthesized bis(1-acyloxymethyl) derivatives of 4,4'-(1,2-ethanediyl)bis(2,6-piperazinedione) using i.p.-i.p. models of P388 leukemia and B16 melanoma. As a result, we found 4,4'-(1,2-ethanediyl)bis(1-isobutoxycarbonyloxymethyl-2,6-piperazinedione) (MST-16) to possess considerable therapeutic activity. MST-16 showed not only marked life-prolonging effects in both P388 leukemia- and B16 melanoma-bearing mice but also a greater therapeutic ratio than did its parent compounds, ICRF-154 and ICRF-159. Further studies revealed that MST-16 has considerable therapeutic activity against a number of other tumors such as ascitic forms of L1210 leukemia, colon 26 adenocarcinoma, and MH-134 hepatoma and solid forms of B16 melanoma, Lewis lung carcinoma, colon 38 adenocarcinoma, and M5076 fibrosarcoma. These results suggest that MST-16 is very promising as an antitumor agent.

Introduction

The antitumor activity of bis(2,6-dioxopiperazine) derivatives, such as ICRF-159 and -154, has been studied extensively over the past several years, and the chemical, biochemical, and pharmacological properties of these compounds were recently reviewed [13]. These agents show significant antitumor activity against several kinds of murine tumor model [6, 23], have unique antimetastatic action [10, 12, 18, 24] and protect against anthracycline-induced toxicity [3, 7, 8, 19, 22]. However, their inadequate bioavailability, presumably due to poor solubility in both water and organic solvents, seems to have limited their clinical effectiveness [4, 11].

Offprint requests to: T. Narita

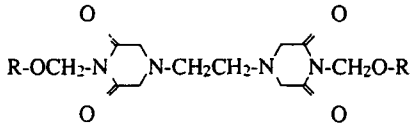
Ren et al. [16, 17] synthesized bimolane (AT-1727), a bis(morpholinomethyl) derivative of ICRF-154, and found that it has antitumor activity against various kinds of murine tumors [15–17]. In addition, the results of clinical investigation in China indicate that bimolane might be useful in the treatment of malignant lymphoma and psoriasis [25]. However, bimolane also showed poor solubility in protic solvents such as water and alcohol. Therefore, to enhance the solubility and therapeutic efficacy of this type of compound, we synthesized several derivatives of ICRF-154 and examined their antitumor activity against two tumor models, P388 leukemia and B16 melanoma. Further additional studies are also described.

Materials and methods

Tumor models. DBA/2, C57BL/6, BALB/c, C_3H/He, ICR, BALB/c × DBA/2 (CDF_1), and C57BL/6 × DBA/2 (BDF_1) mice, 6 weeks of age, were purchased from Charles River Japan Inc. (Kanagawa, Japan). They were maintained under specific pathogen-free conditions at a temperature of 23° ± 2° C and a humidity of 50% ± 10%. P388 leukemia (P388) and L1210 leukemia (L1210) were maintained by i.p. serial passage in DBA/2 mice. B16 melanoma (B16), Lewis lung carcinoma (LL), colon 26 adenocarcinoma (C-26), colon 38 adenocarcinoma (C-38), and M5076 fibrosarcoma (M5076) were serially passaged by s.c. injection in either C57BL/6 (for B16, LL, C-38, and M5076) or BALB/c mice (for C-26). MH-134 hepatoma (MH-134) was maintained by i.p. serial passage in C_3H/He mice and Ehrlich carcinoma, in ICR mice. All tumor lines were supplied by the Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, except for MH-134, which was obtained from Sasaki Institute, Sasaki Foundation (Tokyo, Japan).

Substances. MST-16 and other bis(1-acyloxymethyl) derivatives of 4,4'-(1,2-ethanediyl)bis(2,6-piperazinedione) were prepared as described below. An excess of aqueous formaldehyde was added slowly to 4,4'-(1,2-ethanediyl)bis(2,6-piperazinedione) preheated in a dimethylformamide solution, and the mixture was heated at 130° C for 1.5 h. The 4,4'-(1,2-ethanediyl)bis(1-hydroxymethyl-2,6-piperazinedione) thus produced was precipitated from the reaction mixture solution by cooling. Various bis(1-acyloxymethyl) derivatives of 4,4'-(1,2-ethanediyl)bis(2,6-piper-

Table 1. Bis(1-acyloxymethyl) derivatives of 4,4'-(1,2-ethanediyl)-bis(2,6-piperazinedione) and their antitumor activity against P388 leukemia and B16 melanoma



Compound	R	P388[a]		B16[b]	
		Optimal dose (mg/kg)	Survival (T/C%)	Optimal dose (mg/kg)	Survival (T/C%)
I	$COCH_3$	500	240	60	202
II	$COCH_2$-C$_6$H$_5$	200	303	40	210
III	$COCH_2O$-C$_6$H$_4$-Cl	200	194		ND
IV	$COCH_2CH_2COOH$	400	110		ND
V	$COCH_2NH_3^+CF_3COO^-$	400	103		ND
VI	CO-C$_6$H$_5$	400	244	20	186
VII	CO-C$_4$H$_3$S	300	222	40	215
VIII	COCH=CH-C$_6$H$_5$	200	134		ND
IX	$COOCH_3$	300	211	40	177
X	$COOCH_2CH(CH_3)_2$	250	306	40	216
XI	CONH-C$_6$H$_5$	400	105		ND
ICRF-154 (see Fig. 1)		133	255	30	213
ICRF-159 (see Fig. 1)		200	171	20	125

[a] P388 cells were inoculated i.p. on day 0, and each drug was given i.p. on days 1 and 5
[b] B16 cells were inoculated i.p. on day 0, and each drug was given i.p. daily on days 1–8
ND, not done

azinedione) were obtained by acylation of the hydroxymethylated compound [1, 2].

In vivo treatment. This study was carried out according to the standard protocols of the Drug Research and Development Program, National Cancer Institute (USA) [9]. P388 (1×10^6 cells), L1210 (1×10^5 cells), or C-26 (3×10^5 cells) were implanted i.p. into CDF_1 mice. MH-134 (2×10^6 cells) and Ehrlich (5×10^5 cells) were implanted i.p. into C_3H/He and ICR mice, respectively. LL (5×10^5 cells) was implanted s.c. into BDF_1 mice. B16 (2×10^6 cells) was injected i.p. or s.c. into BDF_1 mice. Nonnecrotic tissue fragments of C-38 and M5076 were implanted s.c. with a trocar into BDF_1 and C57BL/6 mice, respectively. Each test group contained at least seven mice.

Various derivatives including MST-16 were suspended in a 1% hydroxypropylcellulose solution (HPC) and injected i.p. into tumor-bearing mice in a volume of 0.1 ml/10 g body weight by various schedules. Survival effects were evaluated for i.p. implanted tumors, and tumor growth-inhibitory effects were assessed for s.c. implanted tumors. The latter (B16, LL, C-38) were excised from each mouse on day 21, and their weights were measured. In these experiments metastatic colonies in the lung were also counted.

In vitro assay of growth inhibition. P388 (5×10^4 cells) were incubated for various periods with MST-16 in RPMI 1640 culture medium supplemented with 10% fetal calf serum, kanamycin (100 μg/ml) and 5 μM 2-hydroxyethyldisulfide at 37° C in a humidified atmosphere containing 5% CO_2. For the 2.5-h exposure to the drug, cells were harvested by centrifugation after incubation with MST-16 and washed twice with Hanks' solution. They were then resuspended in fresh culture medium and kept under the same conditions for 48 h. B16 (2×10^4) cells in 35-mm tissue-culture clusters were precultured for 24 h in MEM medium containing 10% fetal calf serum, then cultured with graded concentrations of MST-16 for 48 h. The number of either cell type was counted with a Coulter counter (Model ZM; Coulter Electronics, Inc.).

Results

Structure-activity relationship of N-acyloxymethyl derivatives of 4,4'-(1,2-ethanediyl)bis(2,6-piperazinedione)

A total of 11 bis(1-acyloxymethyl) derivatives of 4,4'-(1,2-ethanediyl)bis(2,6-piperazinedione) were examined for their antitumor activity in two murine models, P388 and B16. Both types of tumor cell were inoculated i.p., drugs were injected by the same route, and the life-prolonging effects of the compounds were compared. As a result, we found that the acyl moiety of the derivatives profoundly affected their antitumor activity (Table 1).

In the treatment of P388-bearing mice, the alkyl or aryl carboxylates, i.e., acetate (I), phenylacetate (II), phenoxyacetate (III), benzoate (VI), and heterocyclic carboxylate (VII) showed significant activity against P388 leukemia. Two carbonates, isobutyl carbonate MST-16 (X) and methyl carbonate (IX), also exhibited high activity. Although compounds VIII and XI are also lipophilic, their antitumor activity was limited. In contrast, the compounds with a hydrophilic acyl moiety, such as the carboxyl (IV) or amino (V) group, did not show any appreciable activity. The therapeutic efficacy of compounds I, III, VI, VII, and IX were comparable with those of ICRF-154 and ICRF-159, but compounds II and X (MST-16) seemed to be more active than any of these.

On the other hand, when B16 was used as a target, even the compounds remarkably active against P388 showed only moderate activity. However, the therapeutic efficacy

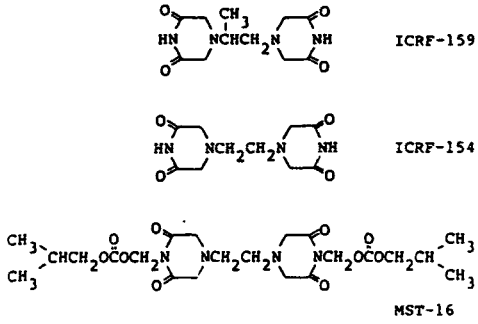


Fig. 1. Chemical structures of ICRF-154, ICRF-159, and MST-16

Table 2. Therapeutic ratios of MST-16, ICRF-154, and ICRF-159 in the P388 leukemia model

	MST-16	ICRF-154	ICRF-159
MTD (mg/kg)	600	500	1,000
ILS_{30} (mg/kg)	53	80	227
ILS_{max} (mg/kg)	400	300	800
MTD/ILS_{30}	11.3	6.3	4.4
ILS_{max}/ILS_{30}	7.5	3.8	3.5

P388 leukemia cells were implanted i.p. on day 0, and each drug was given i.p. once on day 1. MTD, maximum tolerated dose; ILS_{30}, dose producing a 30% increase in life span; ILS_{max}, dose producing the maximal increase in life span

of these compounds against B16 were comparable with that of ICRF-154 but much better than that of ICRF-159. Based on these results, we tentatively chose MST-16 as a promising candidate for becoming a new antitumor agent and further investigated its therapeutic activity against several other tumors. Chemical structures of ICRF-159, ICRF-154, and MST-16 are shown in Fig. 1.

Therapeutic ratio of MST-16

In the P388 leukemia model, a comparison was made between the therapeutic ratios of MST-16 and ICRF-154 or -159. The therapeutic ratio attained by a single i.p. injection was expressed as the ratio of a maximum tolerated dose (MTD) or a dose giving the maximal increase in life span (ILS_{max}) to that giving a 30% increase in life span (ILS_{30}) (Table 2). For either MTD/ILS_{30} or ILS_{max}/ILS_{30}, MST-16 registered a therapeutic ratio almost 2-fold that of both parent compounds.

Schedule dependency of MST-16 in the P388 model

Next, we examined the extent to which the therapeutic effect of MST-16 depends on the treatment schedule in the i.p.-i.p. P388 leukemia model. MST-16 was given on the following three schedules: once on day 1, once on day 1 and once on day 5, and daily for 9 consecutive days (Fig. 2). On these schedules, the optimal doses were 400, 200 and 40 mg/kg, respectively, and their maximal effects were almost equal (ILS, 220%–240%). These results indicate that optimal doses on the three schedules are identical in terms of cumulative total dose. Although the chemotherapeutic ratio of the single administration appeared overall to be somewhat higher than that of the other schedules, no evident schedule dependency was observed in this model.

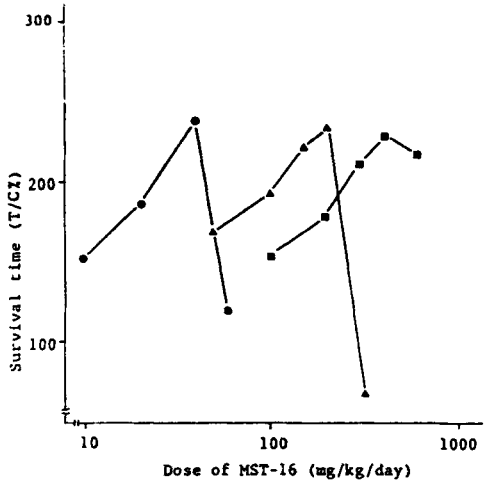


Fig. 2. Antitumor effects of MST-16 given on different treatment schedules on P388 leukemia. Mice that had been inoculated i.p. with P388 leukemia cells on day 0 were given i.p. injections of MST-16 on the following schedules: (■), once on day 1; (▲), once on day 1 and once on day 5; (●), daily on days 1–9.

Table 3. Life-prolonging activity of MST-16 injected i.p. in mice bearing i.p. implanted murine ascites tumors

Tumor	Schedule	Dose (mg/kg)	Survival[a] (T/C%)	Survivors on day 30
B16	Days 1–8	60	113	
		50	182	
		40	186	
		20	156	
L1210	Days 1 and 5	300	339	2/7
		250	>357	4/7
		200	273	1/7
		100	226	0/7
		50	190	0/7
		25	176	0/7
		12.5	131	0/7
C-26	Days 1 and 5	250	236	
		125	190	
MH-134	Days 1–9	50	120	
		25	218	
Ehrlich carcinoma	Days 1–9	60	70	
		30	105	

[a] Survival of untreated mice bearing B16, L1210, C-26, MH-134, and Ehrlich carcinoma amounted to 23.3, 9.6, 15.0, 15.0, and 19.0 days, respectively

Therapeutic effects on i.p. implanted tumors

The therapeutic efficacy of MST-16 on i.p.-i.p. models of L1210, B16, C-26, MH-134, and Ehrlich carcinoma was investigated next (Table 3). MST-16 was the most active

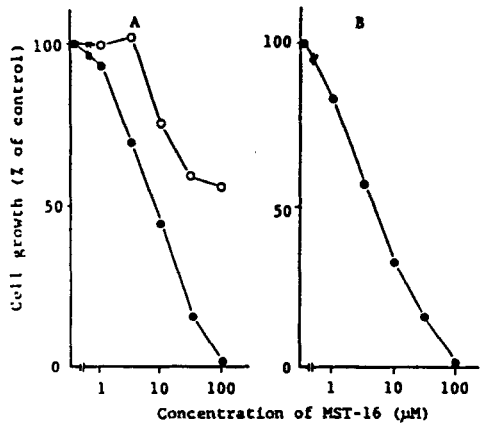


Fig. 3. In vitro growth-inhibitory effects of MST-16 on P388 leukemia and B16 melanoma. P388 (*A*) or B16 (*B*) cells were cultured in vitro in the presence of various concentrations of MST-16 for 2.5 (○) or 48 h (●) and counted by a Coulter counter after 48 h

against L1210, demonstrating even a curing effect on some leukemic mice at its optimal doses. Although the treatment schedules were not uniform, MST-16 exhibited significant life-prolonging effects on B16, C-26, and MH-134. In the case of B16, remarkable effects were observed over a relatively wide dose range (20–50 mg/kg). However, the drug was quite ineffective against Ehrlich carcinoma.

Therapeutic effects on s.c. implanted tumors

MST-16 was injected i.p., and its effects on the growth of solid tumors were examined (Table 4). In some tumors, antimetastatic effects were also studied. MST-16 showed significant growth-inhibitory effects on LL and B16, with respective maximal inhibitions of 94% and 92%, at the daily dose of 60–80 mg/kg given for 8 consecutive days; lung metastases were also effectively suppressed over a relatively wide dose range. MST-16 was moderately effective against M-5076 and C-38, maximally giving 71% and 51% growth inhibition, respectively.

In vitro growth-inhibitory activity

When either P388 or B16 cells were cultured with MST-16 for 48 h, their growth was suppressed in a manner dependent on the drug concentration (Fig. 3). For P388 and B16 cells the IC_{90} was 47 and 56 μM, respectively. In contrast, when P388 cells were exposed to MST-16 for only 2.5 h and cultured for 48 h, not even 50% growth inhibition was attained, even at the maximal concentration of 100 μM.

Discussion

The poor bioavailability of both ICRF-159 and -154 limits the clinical therapeutic efficacy of these drugs in spite of their considerable activity against several experimental tumors [22]. Therefore, in the present study, we synthesized several derivatives of ICRF-154 by introducing acyloxymethyl groups into its 2,6-dioxopiperazine ring and examined their antitumor activity in comparison with that of ICRF-154 and ICRF-159 (Table 1). As a result, 7 of the 11 derivatives studied exhibited activity comparable with or superior to that of ICRF-154 against P388 leukemia. Since two derivatives with a hydrophilic acyl moiety in which alkyl or aryl groups including carboxylate or amine (IV, V) were ineffective, it seemed valuable to give a lipophilic property to the mother structure. All seven compounds found to be active against P388 also showed remarkable therapeutic effects on B16. Compounds II and X (MST-16) exhibited the highest therapeutic activity against P388 and B16, with results comparable with those obtained using ICRF-154. Since MST-16 showed an improved therapeutic ratio as compared with ICRF-159 and -154 (Table 2), we selected it as a candidate for further testing as an antitumor agent. MST-16 was also found to possess considerable activity against several other ascites and solid tumors following its i.p. administration (Tables 3, 4).

MST-16 was shown in the present study to inhibit metastases of Lewis lung carcinoma and B16 melanoma. These effects were observed even at a relatively low dose, one that exerted only a moderate influence on the growth rate of the s.c. implanted tumor. In their studies on the mechanism of the antimetastatic effect of ICRF-159, Le

Table 4. Growth-inhibitory and antimetastatic activities of MST-16 injected i.p. in mice bearing s.c. implanted murine solid tumors

Tumor	Schedule	Dose (mg/kg)	Inhibition of tumor growth (%)[a]	Metastasis	
				Colonies[b]	Inhibition (%)
B16	Days 1–8	60	91.8	0.3 ± 0.03	>95.0
		40	81.4	0.1 ± 0.1	>95.0
		20	66.0	0.6 ± 0.4	>95.0
		0		14.2 ± 2.1	
LL	Days 1–8	80	93.7	0.3 ± 0.3	>97.0
		60	88.3	0.9 ± 0.7	>97.0
		40	61.2	2.1 ± 1.1	92.3
		20	42.9	3.4 ± 1.2	87.5
		0		27.1 ± 3.4	
C-38	Days 1–8	50	50.0		
M5076	Days 1, 5, 9, and 13	150	71.0		
		100	49.0		
		50	35.0		

[a] Tumor weights of untreated mice bearing B16, LL, C-38, or M5076 tumors on day 21 were 2,857, 4,890, 3,358, and 2,765 mg, respectively

[b] Number of metastatic colonies in the lung on day 21 (mean ± SE)

Serve and Hellman [14] proposed that the compound inhibited metastasis by normalizing tumor blood vessels. The antimetastatic mechanism of MST-16 would be expected to be similar to that of ICRF-159.

Evident schedule dependency of MST-16 was not observed among the three different treatment schedules examined, i.e., single, intermittent, and daily administration (Fig. 2). Optimal MST-16 doses observed using these three schedules were equal in terms of their total doses, suggesting a schedule-independent activity for this drug. On the other hand, our preliminary in vitro study indicated that a long exposure time is required for its efficient cytotoxic action (Fig. 3). This observation is in agreement with the cell cycle phase-specific action of ICRF-159 previously reported on the basis of flow-cytometric study [21]. This difference between the in vivo and in vitro effects of MST-16 seems to result from the routes of administration used in the in vivo system, i.e., the i.p.-i.p. system. When MST-16 is injected as a suspension directly into the intraperitoneal cavity where leukemic cells exist, cell exposure to the drug is expected to last for a relatively long time because of its gradual dissolution in the peritoneal fluid. Therefore, to confirm the presence or absence of schedule dependency in the in vivo administration of MST-16, it may be necessary that different types of therapeutic experiments are carried out, e.g., those using an i.p.-p.o. system.

The in vitro study of MST-16 also revealed that the IC_{90} values obtained for MST-16 against both P388 and B16 cells were as high as approximately 50–60 μM (around 30 μg/ml), even with a 48-h exposure. These remarkable therapeutic effects could be obtained because the maximum tolerated dose of the drug in mice is sufficiently high.

The mechanism of action of MST-16 remains to be clarified. Testing ICRF-159, Sharpe et al. [20] reported that it induces mitotic arrest, for the drug-sensitive phase of the cell cycle of PHA-stimulated lymphocytes is the late G_2 or early prophase. On the other hand, Creighton and Birnie [5] suggested that its mode of action involves bifunctional acylation: an imide ring of the bis(dioxopiperazine) cleaves and reacts with nucleophilic groups such as amines, thiols, and phosphates of cellular components. MST-16 seems to act in a manner similar to that of ICRF-159; a study of the precise mechanism is now under way.

Since MST-16 hardly dissolves in water, oral administration would be expected to be optimal for its application in the clinical stage. Therefore, it is important that therapeutic efficacy be observed following oral administration. Experiments on the oral administration of MST-16 are currently in progress and will be presented in the near future.

References

1. Cai JC, Takase M (1985) Kokai 60-97963. Japanese Patent Office, Tokyo
2. Cai JC, Takase M (1987) Patent 4650799. U. S. Patent Office, Washington D. C.
3. Chlebowski R, Pugh R, McCracken J (1979) A phase I–II trial of combination therapy with ADM and ICRF. Proc Am Assoc Cancer Res 20: 199
4. Creaven PJ, Allen LM, Alfold DA (1975) The bioavailability in man of ICRF-159, a new oral antineoplastic agent. J Pharm Pharmacol 27: 914
5. Creighton AM, Birnie GD (1970) Biochemical studies on growth inhibitory bisdioxopiperazines. Effect on DNA, RNA and protein synthesis in mouse embryo fibroblasts. Int J Cancer 5: 47
6. Creighton AM, Hellmann K, Whitecross S (1969) Antitumor activity in a series of bisdiketopiperazines. Nature 222: 384
7. Fischer VW, Wang GM, Hobart NH (1986) Mitigation of an anthracycline-induced cardiomyopathy by pretreatment with razoxane: a quantitative morphological assessment. Virchows Arch [Cell Pathol] 51: 353
8. Giuliani F, Casazza AM, Marco AD, Savi G (1981) Studies in mice treated with ICRF-159 combined with daunorubicin or doxorubicin. Cancer Treat Rep 65: 265
9. Goldin A, Venditti JM, Macdonald JS, Muggia FM, Henney JE, Devita VT Jr (1981) Current results of the screening program at the Division of Cancer Treatment, National Cancer Institute. Eur J Cancer Clin Oncol 17: 129
10. Hellmann K, Burrage K (1969) Control of malignant metastases by ICRF-159. Nature 224: 273
11. Hellmann K, Newton KA, Whitmory DW, Hanman WF, Bond JV (1969) Preliminary clinical assessment of ICRF-159 in acute leukemia and lymphosarcoma. Br Med J 1: 822
12. Hellmann K, Gilbert J, Evans M, Cassell P, Taylor R (1987) Effect of razoxane on metastases from colorectal cancer. Clin Exp Metastasis 5: 3
13. Herman EH, Witiak DT, Hellmann K, Waravdekar VS (1982) Biological properties of ICRF-159 and related bis(dioxopiperazine) compounds. Adv Pharmacol Chemother 19: 249
14. Le Serve AW, Hellman K (1972) Metastasis and the normalization of tumor blood vessels by ICRF159: a new type of drug action. Br Med J 1: 597
15. Li XT, Hutchinson GE, Pym BA, Finch M, Hellmann K (1983) Effect of AT-1727 on growth and metastasis of murine tumors. Eur J Cancer Clin Oncol 19: 283
16. Ren YF, Shu HL, Zhang TM, Cheng ZY, Lin C (1980) Studies on bimolane, a new anticancer agent. Kuexue Tongbao 25: 189
17. Ren YF, Otter GM, Schmid FA (1985) Further evaluation of bimolane and analogs as potential antitumor agents. Eur J Cancer Clin Oncol 21: 493
18. Salsbury AJ, Burrage K, Hellmann K (1970) Inhibition of metastatic spread by ICRF-159: selective deletion of a malignant characteristic. Br Med J 4: 344
19. Shao TH, Elsbeth B, Gerhard Z (1983) Inhibition of cardotoxic, nephrotoxic and neurotoxic effects of doxorubicin by ICRF-159. Pharmacology 26: 210
20. Sharpe HBA, Field EO, Hellman K (1970) Mode of action of the cytostatic agent "ICRF159". Nature 226: 524
21. Trangonos F, Darzynkiewicz Z, Melamed MR (1981) Effects of the L isomer(+)-1,2-bis(3,5-dioxopiperazine-1-yl) propane on cell survival and cell cycle progression of cultured mammalian cells. Cancer Res 41: 4566
22. Wang G, Finch MD, Trevan D, Hellmann K (1981) Reduction of daunomycin toxicity by razoxane. Br J Cancer 43: 871
23. Witiak DT, Lee HJ, Hart RW, Gibson RE (1977) Study of *trans*-cyclopropyl-bis(diketopiperazine) and chelating agents related to ICRF-159. Cytotoxicity, mutagenicity and effects on scheduled and unscheduled DNA synthesis. J Med Chem 20: 630
24. Witiak DT, Lee HJ, Goldman HD, Zwilling BS (1978) Stereoselective effects of *cis*- and *trans*-cyclopropyl-bis(dioxopiperazine)related to ICRF-159 on metastases of a hamster lung adenocarcinoma. J Med Chem 21: 1194
25. Zhang ZY, Liu TF, Zhang XF (1983) Phase II evaluation of AT-1727 in advanced human cancer. Chin Med J [Engl] 96: 601