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Therapeutic activity and tissue distribution of ME2303, a new anthracycline containing fluorine, and its metabolites in mice bearing hepatic metastases of Lewis lung carcinoma

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Doxorubicin (DXR) and ME2303, a new fluorine-containing (*C'*-2) anthracycline derivative, were studied for their tissue distributions—particularly in the plasma, liver and bone marrow—following administration at the maximum tolerated doses to normal mice and mice bearing hepatic metastases of Lewis lung carcinoma. ME2303 was rapidly metabolized and disappeared rapidly from the plasma, liver and bone marrow. Its metabolites—the product of esterolysis (M1) and its reduced derivative at the *C*-14 position (M2)—remained for a long period except in bone marrow. On the other hand DXR remained in the analyzed tissues for a long period; an especially large amount of DXR was found in the bone marrow even at 24 h after administration of the drug while, in the case of ME2303, by this time even its metabolites had disappeared. The concentrations of M1 and DXR in the liver at 2 h were about 50- and 300-fold higher than their plasma concentrations. The tissue distributions in the normal mice and hepatic-metastases-bearing mice showed no significant differences. Regarding the antitumor effects of ME2303, M1, M2 and DXR in the hepatic-metastases-bearing mice, ME2303 was the most effective compound, and M1 was also active; DXR showed only a marginal effect, and M2 showed no effect.

Key words: Anthracycline derivative, hepatic metastases, tissue distribution.

Introduction

Doxorubicin (DXR) is widely used as a chemotherapeutic agent in the treatment of various human neoplasms, and it shows impressive antitumor activities. However, its use is restricted due to its cardiotoxicity. In order to improve the pharmacodynamic properties of clinically-useful antitumor anthracyclines, new doxorubicin analogs that have lower cardiotoxicity but higher antitumor activity have been synthesized or isolated.[1–6]

Recently, Tsuchiya *et al.*[7,8] reported (8S,10S)-8-(6-carboxyhexanoyloxyacetyl)-10-[(2, 6-dideoxy-2-fluoro-α-L-talopyranosyl)oxy]-7,8,9,10-tetra-hydro-6, 8, 11-trihydroxy-1-methoxy-5, 12-naphthacenedione (ME2303, Figure 1), a 2-fluoroglycoside of DXR, which is more resistant to hydrolysis to aglycones and shows marked antitumor effects compared to DXR.

Moreover, ME2303 showed a marked antitumor effect in mice bearing 'artificial' liver metastases of Lewis lung carcinoma and colon carcinoma 26.[9] It is reported that ME2303 is metabolized to the product of esterolysis, (8S,10S)-8-hydroxyacetyl-10-[(2, 6-dideoxy-2-fluoro-α-L-talopyranosyl)oxy]-7, 8, 9, 10-tetrahydro-6, 8, 11-trihydroxy-1-methoxy-5,12-naphthacenedione (M1) and its reduced derivative at the *C*-14 position (M2) due to the action of esterase or to spontaneous hydrolysis.[10] For elucidation of the mechanism of the marked antitumor effect of ME2303, we studied the *in vivo* fate of ME2303 in the plasma, liver and bone marrow in healthy mice and hepatic-metastases-bearing mice. Furthermore, we investigated these drugs and metabolites for antitumor effects in mice bearing established liver metastases of Lewis lung carcinoma.

Materials and methods

Chemicals

DXR was purchased from Kyowa Hakko Kogyo Co. Ltd, Tokyo, Japan. ME2303 and its metabolites

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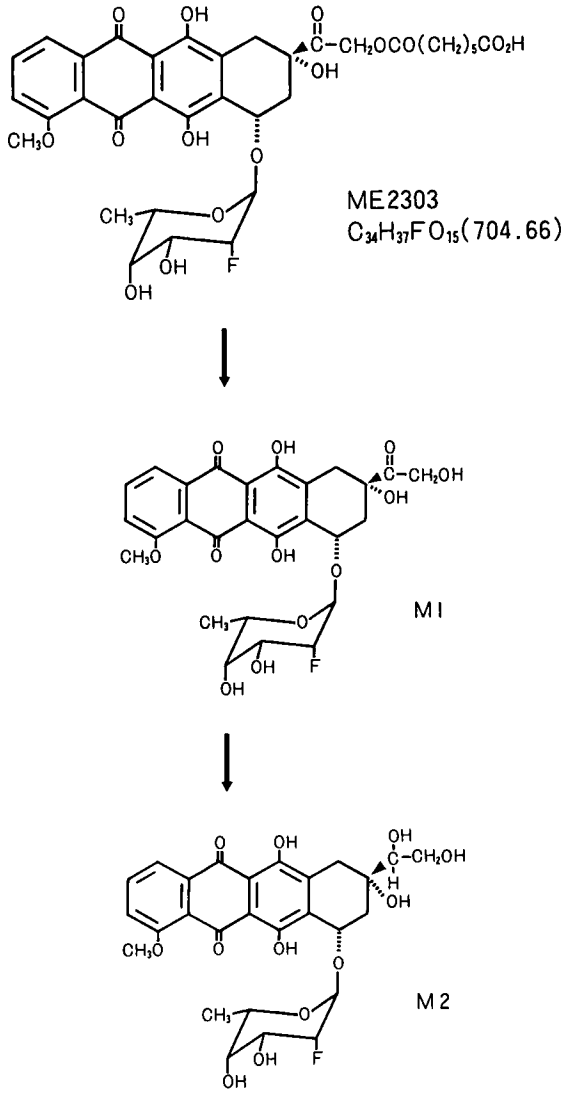

Figure 1. Chemical structures of ME2303 and its metabolites.

(M1 and M2) were provided by Meiji Seika Kaisha Ltd, Tokyo, Japan.

Animals

Groups of six or more specific-pathogen-free male BDF_1 mice weighing 22–24 g each (Japan SLC, Hamamatsu, Japan) were housed in plastic cages with woodchip bedding and were provided CA-1 pellet diets (CLEA Japan, Inc., Tokyo, Japan) and water *ad libitum*. All experiments were performed in an animal laboratory at a controlled temperature (25°C).

Determination of drug concentration by HPLC

Groups of three normal or 10-day-old hepatic-metastases-bearing mice were administered DXR at 12.5 mg/kg or ME2303 at 100 mg/kg in 0.1 ml of physiological saline by i.v. injection. Blood samples (1.0 ml) were collected in heparinized tubes under ether anesthesia at 15 min, 2, 6 and 24 h following treatment, and centrifuged immediately after collection. The plasma supernatants were harvested and frozen at −80°C. The liver was rapidly removed and placed in a flask that was frozen with dry ice–acetone and stored until determination. Bone marrow was collected from the right and left femurs (5 mm × 2) by flushing with 1 ml of saline. The pH of these samples was adjusted to less than 2 with 0.1 N HCl (0.5 ml) and methanol (0.1 ml). Fluorescein (0.01 μg/ml) was added to the sample. The drugs were extracted by shaking with 5 ml of ethyl acetate. The ethyl acetate layer was separated (3500 r.p.m × 10 min, 4°C) and dried in an evaporator. The residue was then dissolved in 0.5 ml of a mixture of Britton–Robinson buffer (pH 3.0) and methyl alcohol (1:2, 200 μl). The samples were injected into a column of Inertsil ODS (4.6 × 250 mm, Gasukuro Kogyo Co., Tokyo, Japan) and then eluted with a mixture of 0.05 M sodium acetate buffer (pH 5.6) and acetonitrile (65:35) at a flow rate of 1.0 ml/min. The detector used was a Spectrofluorometer 820FP, FP-210 (Jasco, Tokyo, Japan). Absorbance was monitored at an excitation wavelength of 472 nm and an emission wavelength of 560 nm. The limit of quantitation of the assay was 5 ng/ml for ME2303 and its metabolites, and 2 ng/ml for DXR.

Experimental procedure for inducing liver metastases

Lewis lung carcinoma was maintained in male C57BL/6 mice. Cell suspensions of Lewis lung carcinoma in saline were prepared from surgically-removed tumors by disaggregating tumor pieces by gentle homogenization in a loosely fitting glass homogenizer, and the cell suspension was passed through a 120-mesh sieve. 'Artificial' multiple hepatic metastases were produced according to the method of Kopper *et al.*[11] Mice were anesthetized with ether, a left subcostal incision (~10 mm) was made, and the spleen was externalized. A 27-gauge needle (Terumo Japan, Tokyo, Japan) was used to puncture the splenic capsule, and

5×10^4 viable tumor cells in 0.1 ml of saline were injected directly into the upper pole of the spleen. Gentle pressure was applied for a period of 10 s to prevent hemorrhage and tumor cell extravasation. The splenic pedicle was then clamped with a medium hemoclip (Edward Weck & Co., Inc., NC, USA), and the spleen was then removed. The abdomen was stitched with surgical sutures, and the skin was closed with disposable skin clip applicators (Avlox 12, Medi Plast, Sweden). The mice were allowed to recover and were then randomized before being distributed to groups.

Drug treatment

Drugs were dissolved in 0.9% saline or Intralipos 10% (soybean oil–egg yolk lecithin–glycerin, Green Cross, Osaka, Japan). The M1 and M2 metabolites of ME2303 are not readily dissolved in water; hence, they were administered as a solution in Intralipos containing 5% DMSO. ME2303 and DXR were dissolved in Intralipos and saline. Drugs were injected i.v. on days 10 and 17. On day 10, liver metastases appeared as distinct, whitish colonies, ~1 mm long. The injection volume was 0.01 ml/g of body weight. Observation was terminated on day 80. All mice surviving at that time were recorded as 'cured'. The antitumor effect was determined by comparing the mean survival time of each treated group with that of the control group and expressed as the increase in life-span (ILS). At death, an autopsy was performed and all mice featured many liver metastases. Cured mice were excluded from the mean survival time calculation.

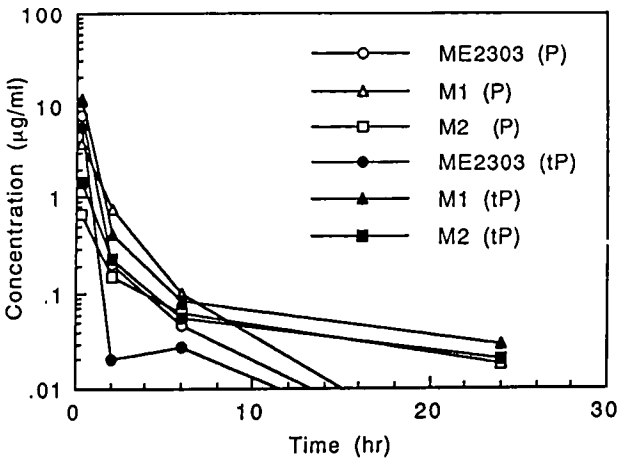

Figure 2. Plasma concentrations of ME2303 and its metabolites in normal and hepatic metastases bearing BDF_1 mice following i.v. administration of ME2303 at 100 mg/kg. P, plasma in normal mice; tP, plasma in hepatic metastases bearing mice.

Results

Plasma concentrations of ME2303 and its metabolites in mice bearing established liver metastases of Lewis lung carcinoma

When the maximum tolerated dose of ME2303 (100 mg/kg) was injected i.v. to hepatic-metastases-bearing BDF_1 mice, the maximum plasma concentrations of ME2303, M1 and M2 were observed at 15 min after the injection (Figure 2). The concentrations were 5.75 ± 1.39 μg/ml (mean ± SE) for ME2303; 11.72 ± 1.83 μg/ml for M1; and 1.51 ± 0.20 μg/ml for M2. Although the level of ME2303 decreased rapidly during the first 6 h and was not detected after 24 h of the administration, M1 and M2 were present in plasma and their concentrations were about 0.03 μg/ml at 24 h. The plasma concentrations of ME2303, M1 and M2 in normal mice were not significantly different from hepatic-metastases-bearing mice. On the other hand, the maximum plasma DXR concentrations were 0.40 ± 0.01 and 0.93 ± 0.32 μg/ml (at 15 min) in the normal and hepatic-metastases-bearing mice, respectively. DXR decreased gradually and could still be found at considerable levels in the plasma at 24 h post-treatment (0.03–0.05 μg/ml) (Figure 3).

Liver concentrations of ME2303 and its metabolites in mice bearing advanced hepatic metastases of Lewis lung carcinoma

The maximum ME2303 concentrations in the liver were observed at 15 min after the drug administration as 13.44 ± 1.62 μg/g for the normal mice and 4.86 ± 0.21 μg/g for the hepatic-metastases-bearing mice. In both groups, < 0.01 μg/g of the drug remained at 24 h after the treatment (Figure 4). The maximum concentrations of M1 and M2 were also observed at 15 min after the treatment; their concentrations were 157.1 ± 14.1 and 19.69 ± 2.39 μg/g, respectively, in the hepatic-metastases-bearing mice. However, these concentrations at 24 h after the treatment were 0.84 ± 0.40 μg/g for M1 and 0.17 ± 0.06 μg/g for M2. On the other hand, DXR showed the maximum concentration of 40.13 ± 3.10 μg/g at 2 h after the drug administration in the hepatic-metastases-bearing mice and remained at the

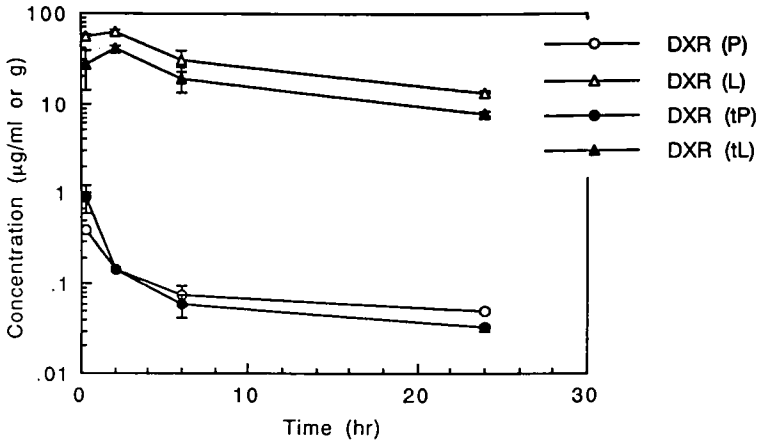

Figure 3. Plasma and liver concentrations of DXR in normal and hepatic metastases bearing BDF_1 mice following i.v. administration of DXR at 12.5 mg/kg. P, plasma in normal mice; L, liver in normal mice; tP, plasma in hepatic metastases bearing mice; tL, liver in hepatic metastases bearing mice.

concentration of 7.90 ± 0.36 μg/g even after 24 h from administration. The normal mice showed a similar tendency (Figure 3).

Bone marrow concentrations of ME2303 and its metabolites in mice bearing hepatic metastases of Lewis lung carcinoma

The maximum ME2303 concentrations in the bone marrow were 0.24 ± 0.04 and 0.37 ± 0.05 μg per femur (at 15 min) in the normal and hepatic-metastases-bearing mice, respectively. Less than 0.01 μg was found at 6 h post-treatment (Figure 5A). The maximum concentrations of M1 were 0.32 μg (at 6 h) and 0.49 μg (at 2 h) in the normal and hepatic-metastases-bearing mice, respectively. Thus, M1 was present at a high level during the first 6 h but decreased rapidly and was found at < 0.01 μg at 24 h post-treatment. On the other hand, DXR remained at a high level in the bone marrow for 24 h (0.15–0.33 μg) (Figure 5B).

Antitumor effect of ME2303 and its metabolites in mice bearing advanced hepatic metastases of Lewis lung carcinoma

The results of the study of the antitumor effects of ME2303 and its metabolites administered on days 10 and 17 are shown in Table 1. Two of the six mice were cured by ME2303 at 100 mg/kg, whereas no mice were cured by DXR at 12.5 mg/kg, which was the maximum tolerated dose under this system. The antitumor effects of ME2303 and DXR were not affected by Intralipos delivery. M1 at 50 mg/kg showed a similar antitumor effect to ME2303 at the same dose, but M2 showed no effect. These observations clearly demonstrate the superiority of ME2303 and M1 over DXR in therapeutic efficacy against hepatic metastases of Lewis lung carcinoma.

Discussion

The tissue distributions of ME2303, a fluorine-containing anthracycline glycoside, and its metabolites, M1 and M2, were investigated in normal and hepatic-metastases-bearing mice. There were no significant differences in the distributions of ME2303 and its metabolites in the two groups of mice—the rate of drug elimination was similar for both the normal and metastasized livers and was not influenced by micrometastases. ME2303 was rapidly metabolized to M1 and M2 in both types of liver. The maximum concentrations of the metabolites in the plasma and liver occurred within 15 min after administration in both groups. The metabolites remained at a high level for 24 h, although ME2303 itself rapidly disappeared. DXR was also concentrated dramatically in the liver. The metabolites, M1 and M2, and DXR in the liver were

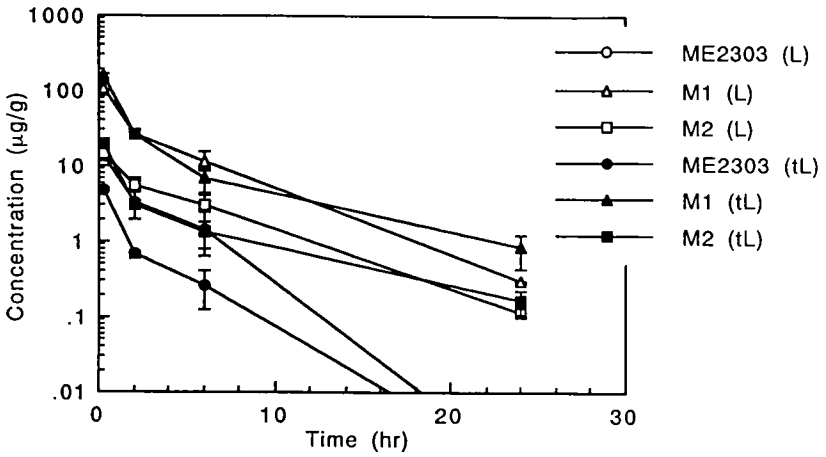

Figure 4. Liver concentrations of ME2303 and its metabolites in normal and hepatic metastases bearing BDF_1 mice following i.v. administration of ME2303 at 100 mg/kg. L, liver in normal mice; tL, liver in hepatic metastases bearing mice.

about 50- and 300-fold higher than their levels in the plasma, respectively. The liver is the main organ metabolizing and accumulating drugs. This function probably means that liver tumors are subject to greater exposure to drugs. Therefore, it should be ascertained whether or not anthracyclines have marked antitumor activity against tumors implanted into the liver, such as hepatic metastases of Lewis lung carcinoma. The maximum tolerated dose of ME2303 is higher than that of DXR. ME2303 at 100 mg/kg resulted in some cured mice. The maximum doses of M1 and M2 were about 50 mg/kg, and the activity of M1 was similar to that of ME2303 at the same dose. But M2 was inactive in this tumor system. In the bone marrow, ME2303 also rapidly disappeared, and M1 was found at a high level during the first 6 h but had disappeared completely by 24 h. The DXR level in the bone marrow was cleared more slowly and was high even at 24 h post-treatment. These results may explain why ME2303 has lower toxicity than DXR. Thus, a larger amount of ME2303 can be administered due to its lower toxicity. Finally, ME2303 showed a marked antitumor effect in the hepatic-metastases-bearing mice, being superior to DXR.

Conclusion

The tissue distributions of ME2303, a newly-synthesized fluorine-containing anthracycline glycoside, and its metabolites M1 and M2 showed no

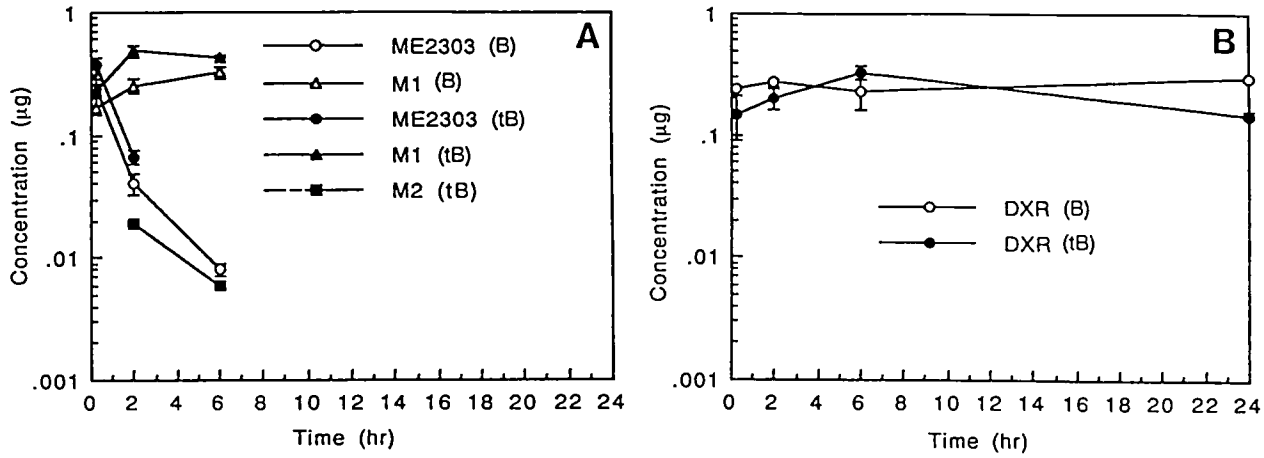

Figure 5. Bone marrow concentrations of ME2303 and its metabolites (A) and DXR (B) in normal and hepatic metastases bearing BDF_1 mice following i.v. administration of ME2303 (100 mg/kg) and DXR (12.5 mg/kg). B, bone marrow in normal mice; tB, bone marrow in hepatic metastases bearing mice.

Table 1. Effect of ME2303 and its metabolites on advanced liver metastases of Lewis lung carcinoma bearing mice

Drugs	Dose (mg/kg/day)	Vehicle	MST (days)[a]	ILS (%)	No. of cured mice[b]
Control		Saline	18.6 ± 0.6[c]		0/9
ME2303	100	Saline	39.0 ± 0.7	110	2/6
	100	Intralipos	40.5 ± 3.6	118	0/6
	50	Saline	29.0 ± 3.2	56	0/6
	50	Intralipos	31.5 ± 2.9	69	0/6
M1	50	Intralipos	32.8 ± 2.3	76	0/6
M2	50	Intralipos	21.5 ± 2.0	16	0/6
DXR	12.5	Saline	24.8 ± 2.2	33	0/6
	12.5	Intralipos	24.2 ± 3.5	30	0/6

Lewis lung carcinoma cells were inoculated into the liver by intrasplenic inoculation, and drugs were administered i.v. on days 10 and 17.
[a] MST, mean survival time of deceased mice.
[b] Number of cured mice per treated mice on day 80.
[c] Mean ± SE.

significant differences between normal and hepatic-metastases-bearing mice. In the plasma and liver, the active metabolite of ME2303, M1, was maintained for a long period, similar to DXR. On the other hand, ME2303 and M1 rapidly disappeared from the bone marrow, but a high level of DXR was found even at 24 h. The result may mean low toxicity of ME2303, enabling administration of a higher dose of ME2303 compared to DXR. The high dose of ME2303 showed marked antitumor activity in the hepatic-metastases-bearing mice, but DXR showed only a marginal effect. M1 also produced a marked antitumor effect.

References

1. Oki T, Matsuzawa Y, Yoshimoto A, *et al.* New antitumor antibiotics, aclacinomycins A and B. *J Antibiotics* 1975; **28**: 830–834.
2. Umezawa H, Takahashi Y, Kinoshita M, *et al.* Tetrahydropyranyl derivatives of daunomycin and adriamycin. *J Antibiotics* 1979; **32**: 1082–1084.
3. Cassinelli G, Configliacchi E, Penco S, *et al.* Separation, characterization, and analysis of epirubicin (4'-epidoxorubicin) and its metabolites from human urine. *Drug Metab Disp* 1984; **12**: 4–20.
4. Weiss RB, Sarosy G, Clagett-Carr K, Russo M, Leyland-Jones B. Anthracycline analogs: the past, present, and future. *Cancer Chemother Pharmacol* 1986; **18**: 185–197.
5. Arcamone F. Clinically useful doxorubicin analogues. *Cancer Treat Rev* 1987; **14**: 159–161.
6. Israel M, Seshadri R, Kaseki Y, Sweatman TW, Idriss JM. Amelioration of adriamycin toxicity through modification of drug-DNA binding properties. *Cancer Treat Rev* 1987; **14**: 163–167.
7. Tsuchiya T, Takagi Y, Ok K, *et al.* Syntheses and antitumor activities of 7-*O*-(2,6-dideoxy-2-fluoro-α-L-talopyranosyl)-daunomycinone and -adriamycinone. *J Antibiotics* 1986; **39**: 731–733.
8. Tsuchiya T, Takagi Y, Umezawa S, *et al.* Synthesis and antitumor activities of 14-*O*-acyl derivatives of 7-*O*-(2,6-dideoxy-2-fluoro-α-L-talopyranosyl) adriamycinone. *J Antibiotics* 1988; **41**: 988–991.
9. Iigo M, Nishikata K, Nakajima Y, Hoshi A. Antitumor effect of anthracycline derivatives in the treatment of established murine hepatic metastases. *Proc Jpn Cancer Assoc* 1990; **49**: 376.
10. Kunimoto S, Komuro K, Nosaka C *et al.* Biological activities of new anthracyclines containing fluorine, FAD104 and its metabolites. *J Antibiotics* 1990; **43**: 556–565.
11. Kopper L, Hanh TV, Lapis K. Experimental model for liver metastasis formation using Lewis lung tumor. *J Cancer Res Clin Oncol* 1982; **103**: 31–38.

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