Incorporation of the Anticancer Agent KRN5500 into Polymeric Micelles Diminishes the Pulmonary Toxicity

Yasuo Mizumura,1,6 Yasuhiro Matsumura,3,7 Masayuki Yokoyama,4 Teruo Okano,4 Takanori Kawaguchi,5 Fuminori Moriyasu6 and Tadao Kakizoe2

1Department of Medicine, 2President, National Cancer Center, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, 3Investigative Treatment Division, National Cancer Center Research Institute East, 6-5-1 Kashiwanoha, Kashiwa, Chiba 277-8577, 4Institute of Advanced Biomedical Engineering and Science, Tokyo Women’s Medical University, 8-1 Kawada-cho, Shinjuku-ku, Tokyo 162-8666, 52nd Department of Pathology, Fukushima Medical University School of Medicine, 1 Hikarigaoka, Fukushima-shi, Fukushima 960-1247 and 64th Department of Medicine, Tokyo Medical University, 6-7-1 Nishi-Shinjuku, Shinjuku-ku, Tokyo 160-0023

KRN5500 is a highly active new semi-synthetic water-insoluble anticancer agent. The only mechanism of anticancer activity of KRN5500 described so far is an inhibitory effect on protein synthesis. At the time of writing, a phase I clinical trial is under way at the National Cancer Center Hospital, Tokyo, and at the National Cancer Institute in the USA. Although preclinical data did not indicate lung toxicity, some cases of severe pulmonary disorder were reported in the phase I clinical trials. This study has been conducted to examine whether incorporation of KRN5500 into polymeric micelles (KRN/m) could reduce the toxic effects caused by the current formulation of KRN5500.

The in vitro and in vivo antitumor activities of KRN5500 and KRN/m were compared. Pulmonary toxicity of KRN5500 and KRN/m was studied using a bleomycin (BLM)-induced lung injury rat model. In BLM-rats, extensive pulmonary hemorrhage with diapedesis was observed with KRN5500 i.v. bolus injection at the dose of 3 mg/kg, which is equivalent to 21.0 mg/m² (level 5) of the Japanese phase I trial. However, toxicity was not observed when rats were administered KRN/m at the equivalent dose to KRN5500 in potency. Electron microscopy of the lung treated with KRN5500 showed disruption of the alveolar type II membrane with release of lamellar debris. Furthermore, in vivo, KRN/m showed similar antitumor activity to KRN5500. These results indicate that KRN/m may be useful for reducing the pulmonary toxicity associated with the current formulation of KRN5500, while fully maintaining its antitumor activity.

Key words: Drug delivery system — Polymer micelles — KRN5500 — Pulmonary toxicity — Tumor targeting

KRN5500, 6-[4-deoxy-4(2E,4E)-tetradeциденоилглцерил]амино-L-глцеро-β-L-манно-гептопираносиламино-9H-пурин, is a spicamycin analogue derived from Streptomyces alanosinusus. KRN5500 was demonstrated to be highly active in a number of experimental solid tumors, including stomach, esophageal and colon cancer.1) KRN5500 itself has, however, only minor effects on protein synthesis in reticulocyte lysates. A metabolite, 4-N-glycylspicamycin aminonucleoside (SAN-Gly), which has no fatty acid chain and is thought to be generated through metabolism of KRN5500 by a cytosomal enzyme, exhibited a marked inhibitory effect on protein synthesis in a cell-free system. On the other hand, SAN-Gly showed 1000-fold weaker cytotoxicity than KRN5500 in vitro because it does not cross cellular membranes as easily as KRN5500. These findings indicate that the intracellular conversion of KRN5500 to SAN-Gly may be responsible for the potent antitumor activity.2,3) Because of the fatty acid chain, KRN5500 is water-insoluble and must be dissolved in organic solvents and chemicals for intravenous injection.

Currently, phase I clinical trials of KRN5500 are ongoing at the National Cancer Center Hospital in Tokyo and the National Cancer Institute in the USA. Their protocols define that the drug must be injected into a central vein in order to minimize vasculitis, probably caused by the organic solvents and chemicals essential for its dissolution. The toxicity profile of the drug has been partially disclosed at scientific meetings.4,5) In addition to nausea, vomiting, diarrhea, fatigue, transaminitis, hyperbilirubinemia and prolongation of coagulation time, grade 4 pulmonary disorder and interstitial pneumonitis, including one death, were reported.4,5) Meanwhile, we have successfully developed KRN5500-containing polymeric micelles (KRN/m).6) Although KRN/m possesses similar antitumor activity to KRN5500 in vitro, as well as in vivo, the vascular damage with fibrin clot observed after KRN5500 i.v. injection was not seen when KRN/m was administered i.v.7) Therefore, we expect that KRN/m would be superior.

7To whom correspondence should be addressed.
E-mail: yhmatsum@east.ncc.go.jp
to KRN5500 for clinical use in the future. In the present study, we examined the antitumor activity and the toxic effects of KRN/m in vitro and in vivo in a rat model in comparison with KRN5500. We paid particular attention to the pulmonary toxicity of KRN/m compared with that of KRN5500 in both bleomycin (BLM)-rat models.

MATERIALS AND METHODS

Chemicals KRN5500 (KRN5500; its chemical structure is shown in Fig. 1A) was kindly supplied by Kirin Brewery Co., Ltd. Also, several organic solutions and chemicals used to dissolve KRN5500 were a gift from the company. Organic solvents used for dissolving KRN5500 consisted of 3.3% N,N-dimethylacetamide, 3% Polysorbate 80, and 0.45% ethanolamine in 0.9% saline for in vivo tests. Bleomycin (BLM) hydrochloride was purchased from Nihon Kayaku Co., Ltd. Other chemicals were of reagent grade and were used as purchased.

Incorporation of KRN5500 into micelles As shown in Fig. 1B, KRN5500 was incorporated into polymeric micelles formed from poly(ethylene glycol)-poly(β-benzyl L-aspartate-co-β-centyl L-aspartate) block copolymer (PEG-P(BLA, C16)) by physical entrapment utilizing hydrophobic interactions between the drug and the poly(amino acid) chain of the block copolymers, as reported previously by Yokoyama et al.6) (Fig. 1B).

In vitro cytotoxicity Colonic cancer cell lines (Colon 26, Widr), gastric cancer cell lines (MKN-45, MKN-74), breast cancer cell line (MCF-7), bladder cancer cell line (RT-112) and lung cancer cell lines (A549, EBC-1, PC-14, SBC-3) were used in this study. All the cell lines were maintained in monolayer cultures in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum in a humidified atmosphere containing 5% CO2 at 37°C. For cytotoxicity analysis, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used. Ten thousand cells of each cell line in 198 µl of culture medium were plated in 96-well plates 24 h prior to drug treatment. Then 2 µl of various doses of free KRN5500 or KRN/m was added. Cells were exposed to the indicated drug concentration in triplicate for 24 h, 48 h or 72 h. Three measurements were taken at every point, and the mean of these was taken to be the value for that measurement time.

Mouse experiments

In vivo antitumor activity: Antitumor activity was evaluated against the human gastric cancer cell line MKN-45 grown in nude mice. BALB/c nu/nu female mice (6 weeks old) were inoculated subcutaneously (s.c.) on the abdominal skin with a half million MKN-45 tumor cells. Four days later, when the tumor length reached approximately 3 mm, the tumor-bearing mice were allocated randomly to drug treatment groups of 5 animals each. A.

Fig. 1. Chemical structure of spicamycin analogue KRN5500, 6-[4-deoxy-4(2E,4E)-tetradecadienoyl]glycyl]amino-L-glycero-β-D-mannoheptopyranosyl]amino-9H-purine, and the method of incorporating KRN5500 into polymeric micelles are shown. A. The fatty acid chain of KRN5500 is pivotal for drug internalization into cancer cells. 4-N-Glycylspicamycin (SAN-Gly), which has no fatty acid and is obtained after metabolism of KRN5500 by a cytosomal enzyme, exhibited a marked inhibitory effect on protein synthesis in the cell-free system. Nevertheless, SAN-Gly showed 1000-fold weaker cytotoxicity than KRN5500 in vitro because of poor intracellular incorporation of SAN-Gly. Since KRN5500 is highly water-insoluble, a mixture of organic solvents and chemicals must be used to dissolve the drug for injection. B. An AB-block copolymer, PEG-P (BLA, C-16), was dissolved in dimethyl sulfoxide (DMSO) and mixed with KRN5500 in DMSO. The mixture was stirred at room temperature for 10 min, and then dialyzed against distilled water for at least 5 h using a cellulose membrane. Sonication was then carried out to obtain uniformly sized micelle particles (approximate size, 70 nm). • drug, ○ hydrophilic segment, ○ hydrophobic segment.
Treatment groups were as follows: free KRN5500 at a dosage level of 5 mg/kg; KRN/m at an equivalent dose of KRN5500; saline as a control. Drugs in a volume of 0.1 ml were injected as a single dose into a tail vein on day 6 after tumor inoculation. The antitumor effect was evaluated by measuring 2 orthogonal diameters of the tumor \((a \times b; a, \text{long diameter}; b, \text{short diameter})\) at various times. Relative tumor growth rates were compared across these 3 treatments, including the control and across time, as represented by these 6 measurements (days 2, 6, 9, 13, 16, 20), using repeated measure two-way ANOVA.

**Evaluation of systemic side effects by measurement of body weight changes in mice:** Adverse effects were also determined by measurement of body weight changes in nude mice after drug treatment. Animals and methods are described above. Body weight was measured when tumor size was measured.

**Rat experiment: Lung toxicity in rats with BLM-induced lung injury** We designed a model for pulmonary fibrosis in rats by intratracheal instillation of BLM. The model has been shown to be similar histopathologically to the pulmonary fibrotic changes in humans. We used Male Sprague-Dawley rats (8 weeks old, 250 g) were used in this study. Animals were intratracheally instilled with 1 mg of BLM hydrochloride diluted in a volume of 0.1 ml of sterile saline under ether anesthesia. On day 7 after BLM instillation, rats were allocated randomly to drug injection groups of 3 animals each, and rats were given each drug as a single dose into a tail vein by bolus injection. Injection groups were as follows: KRN5500 3 mg/kg (0.1 mg/ml at a volume of 7.5 ml); KRN5500 1 mg/kg (0.1 mg/ml at a volume of 2.5 ml); KRN/m 3 mg/kg (at equivalent dose of KRN5500); KRN/m 1 mg/kg (the equivalent dose to that of KRN5500); saline as a control. The 3 mg/kg injection of KRN5500 was compatible with that of 21.0 mg/m² (level 5) of the Japanese clinical trials.

Two toxic deaths out of 3 animals occurred immediately after the drug injection in the KRN5500 treatment group at 3 mg/kg. The surviving rats received the designated dose daily for another 2 days.

One day after the last i.v. injection of each drug, the surviving rats were sacrificed under ether anesthesia and the lungs were collected. Resected lungs were immersed in 10% formalin solution. Paraaffin-embedded sections were stained with hematoxylin and eosin and examined by microscopy. The morphologic changes after KRN5500 treatment were examined by electron microscopy.

**Statistical methods** In vivo data from the mouse experiment were compared across groups by using ANOVA. \(P\) values of 0.05 or less were considered statistically significant.

**RESULTS**

**In vitro cytotoxicity** IC\(_{50}\) values for KRN5500 and KRN/m against various cancer cell lines exposed at the indicated drug concentrations in triplicate for 24 h, 48 h, or 72 h. Each cell line was treated in triplicate for 24 h, 48 h, and 72 h. MTT assay was used for obtaining IC\(_{50}\) value.

| Table I. IC\(_{50}\) Value (µM) of KRN5500 and KRN/m in Various Cell Lines |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|                            | Exposure time               |                            |                            |                            |                            |
|                            | 24 h                        | 48 h                        | 72 h                        | 24 h                        | 48 h                        | 72 h                        |
|                            | KRN5500                     | KRN/m                       | KRN5500                     | KRN/m                       | KRN5500                     | KRN/m                       |
| Lung cancer                | A549                        | >3.0                        | >3.0                        | >3.0                        | >3.0                        | 0.061                       | 0.084                       |
|                            | EBC-1                       | >3.0                        | >3.0                        | >3.0                        | >3.0                        | 0.082                       | 0.11                        |
|                            | PC-14                       | >3.0                        | >3.0                        | >3.0                        | >3.0                        | 0.23                        | 0.367                       |
|                            | SBC-3                       | >3.0                        | >3.0                        | >3.0                        | >3.0                        | 0.079                       | 0.087                       |
| Gastric cancer             | MKN-45                      | >3.0                        | >3.0                        | 0.037                       | 0.042                       | 0.019                       | 0.018                       |
|                            | MKN-74                      | >3.0                        | >3.0                        | >3.0                        | >3.0                        | 1.67                        | 1.95                        |
| Colonic cancer             | Colon 26                    | >3.0                        | >3.0                        | >3.0                        | >3.0                        | 0.47                        | 0.60                        |
|                            | Widr                        | >3.0                        | >3.0                        | >3.0                        | >3.0                        | 0.11                        | 0.13                        |
| Breast cancer              | MCF-7                       | >3.0                        | >3.0                        | >3.0                        | >3.0                        | 1.67                        | 1.07                        |
| Bladder cancer             | RT-112                      | 4.4                         | 1.8                         | 0.38                        | 0.44                        | 0.047                       | 0.064                       |

KRN5500-containing Polymeric Micelles
h are shown in Table I. There was no remarkable difference between the IC₅₀ values of KRN5500 and KRN/m at any exposure time.

**Mouse experiments**

*In vivo antitumor activity*: The relative tumor growth rate in each treatment group after injection of each drug is shown in Fig. 2A. Both KRN5500 and KRN/m exerted significant anticancer activity *in vivo* \( (F=6.4, P=0.013) \). Comparison of relative tumor growth rates after KRN5500 and KRN/m revealed no significant difference \( (F=0.12, P=0.74) \). Comparison of relative tumor growth rates after KRN/m injection and in the control revealed potent inhibition of tumor growth by KRN/m \( (F=16.1, P=0.004) \). Overall, these results show that KRN5500 maintains sufficient antitumor activity after the incorporation of the drug into micelles.

*Adverse effects in terms of body weight changes in mice*: Fig. 2B shows the body weight changes in tumor-bearing mice after i.v. injection of each drug. Mean body weight in the KRN5500 treatment group decreased significantly compared to the KRN/m \( (F=7.0, P=0.03) \) and control groups \( (F=6.4, P=0.03) \). The body weight loss of mice treated with KRN/m was no greater than that of mice treated with saline \( (F=0.01, P=0.92) \). Overall, these results show that KRN/m had far less toxicity than KRN5500.

**Rat experiments: Pulmonary toxicity of each drug in rats with BLM-induced lung injury** KRN5500-injected rats at a dosage level of 3 mg/kg demonstrated extensive pulmonary pathological changes with widespread hemorrhage (Fig. 3A). At a KRN5500 dosage level of 1 mg/kg, focal hemorrhage was observed (Fig. 3B). On the other hand, no such pulmonary pathological changes occurred with KRN/m (Fig. 3C) at either dosage level, and the lungs resembled those in the control group (Fig. 3D). Electron microscopy (EM) showed that alveolar type II cells, which help prevent the development of pulmonary fibrosis, were swollen and contained extensive “lamellar” inclusions within the cytoplasm 7 days following BLM instillation. On the day after KRN5500 injection at the dosage level of 1 mg/kg, EM exhibited more extensive swelling and disruption of the alveolar type II cell membrane with release of lamellar debris (Fig. 3, E and F).

**DISCUSSION**

Following the development of liposomes, it was proposed by Ringsdorf *et al.*\(^{10}\) that AB block copolymer-drug conjugates, composed of hydrophilic and hydrophobic components, might form micelle structures in aqueous media owing to this amphiphilic characteristic. Then the utility of polymeric micelles in cancer chemotherapy was demonstrated in the form of adriamycin (ADR)-incorporated polymeric micelles (ADR/m) by Yokoyama *et al.*\(^{11, 12}\). ADR/m decreased the toxicity of ADR significantly in terms of body weight change and still expressed superior *in vivo* antitumor activity against several solid tumors, due to the EPR effect, in comparison with free ADR. Cisplatin (CDDP)-incorporating polymeric micelles (CDDP/m) have also been developed, in which platinum atoms of CDDP are linked to aspartic acid residues of copolymers by means of a ligand substitution reaction.\(^{13, 14}\) KRN5500, which was examined in this study, possesses a unique chemical structure and cytotoxic mechanism.\(^{15}\) Lee *et al.* reported that CDDP-resistant human lung cancer
cells showed higher sensitivity to KRN5500 as compared with sensitive cells. To explain this, Takara et al. demonstrated that KRN5500 was hardly transported via P-glycoprotein (P-gp). Therefore, KRN5500 may be useful even for the treatment of tumor cells exhibiting P-gp-associated MDR.

**Fig. 3.** On day 7 after BLM instillation, rats were allocated randomly to drug injection groups of 3 animals each, and rats were given each drug daily 3 times by bolus injection into a tail vein. The histological appearance of lungs in rats was evaluated by hematoxylin and eosin (HE) staining. The histological appearance was as follows: (A) KRN5500 at 3 mg/kg (×100), extensive and widespread intra-alveolar hemorrhage was found. (B) KRN5500 1 mg/kg (×100), focal hemorrhage was observed. (C) KRN/m 3 mg/kg (×100), little hemorrhage was seen. (D) Saline as a control (×100). Electron microscopy showed morphologic alveolar type II cell changes after i.v. administration of KRN5500 at 3 mg/kg or saline as a control. (E) Control, alveolar type II cells contained more lamellar structure (thick arrow). (F) KRN5500 at 3 mg/kg, alveolar type II cells showed much vacuolar degeneration (thin arrow) and several disruptions of the alveolar type II cell membrane with release of lamellar debris (thick arrow).
KRN5500 was injected 7 days after BLM instillation. No KRN5500, but not KRN/m. This occurred when 3 mg/kg rats died immediately after 3 mg/kg i.v. injection in the clinical trials of KRN5500. The previous findings indicated that the organic solvents and chemicals used to solubilize KRN5500 were implicated in these adverse effects, although the precise mechanism of the vascular damage is not clear at the moment.

The present comparative study of pulmonary toxicity of KRN5500 and KRN/m using the BLM-rat model revealed that KRN5500 induced pulmonary hemorrhage, while KRN/m did not. The KRN5500 dose used in this experiment was comparable with the maximum level of the Japanese phase I trial. The pulmonary fibrotic damage in humans with chronic lung disease seems to be pathologically very similar to BLM-induced lung injury in rats. Therefore, we would suggest that the lethal lung toxicity that occurred in patients with insufficient lung function could have been caused by KRN5500 and its organic solvents or chemicals, and that extreme caution must be taken in the administration of KRN5500 in patients with abnormal lung function in the clinical trials of KRN5500.

In the BLM-induced lung injury rat model, two of three rats died immediately after 3 mg/kg i.v. injection of KRN5500, but not KRN/m. This occurred when 3 mg/kg KRN5500 was injected 7 days after BLM instillation. No rat died when 3 mg/kg KRN5500 was administered i.v. one day after BLM instillation (data not shown). Namely death had occurred at the time when fibrosis was established in the rat lung, suggesting that acute bleeding in the rat lung was a cause. It is not clear whether KRN5500 itself, or one of the organic solvents and chemicals used to dissolve the drug, was responsible. Further studies are in progress.

Analysis of relative body weight change in the mice treated with KRN5500 or KRN/m indicated that the toxic effect of KRN5500 may be overcome by incorporating KRN5500 into polymeric micelles.

In conclusion, the present study demonstrates that KRN/m is superior to KRN5500 because the toxicity was reduced and the potent antitumor activity of KRN5500 was retained after the incorporation of KRN5500 into micelles. We think that these results justify a clinical phase I trial of KRN/m.

REFERENCES

1) Kamishouhara, M., Kawai, H., Odagawa, A., Isoe, T., Mochizuki, J., Uchida, T., Hayakawa, Y., Seto, H., Tsuruo, T. and Otake, N. Antitumor activity of SPM VIII, derivative of the nucleoside antibiotic spicamycin, against human tumor xenografts. J. Antibiob. (Tokyo), 47, 1305–1311 (1994).  
2) Kamishouhara, M., Kawai, H., Sakai, T., Isoe, H., Hasagawa, K., Mochizuki, J., Uchida, T., Kataoka, S., Yamaki, H., Tsuruo, T. and Otake, N. Antitumor activity of a spicamycin derivative, KRN5500, and its activity metabolite in tumor cells. Oncol. Res., 6, 383–390 (1994).  
3) Sakai, T., Kawai, H., Kamishouhara, M., Odagawa, A., Suzuki, A., Uchida, T., Kawasaki, T., Tsuruo, T. and Otake, N. Structure-antitumor activity relationship of semi-synthetic spicamycin derivatives. J. Antibiob. (Tokyo), 48, 1467–1480 (1995).  
4) Matsumura, Y., Kamiya, Y., Yamamoto, N., Ono, H., Shirao, K., Kondo, H., Tamura, T. and Shimada, Y. A phase I study of KRN5500 in patients with refractory solid tumors (stomach, colon, lung). Proc. Am. Soc. Clin. Oncol., 18, 219 (abstr. 841) (1999).  
5) Eder, J. P., Supko, J. G., Ryan, K., Roper, N., Kinchla, D. P. and Kufe, D. W. A. Phase I trial of the spicamycin analogue KRN5500. Proc. Am. Assoc. Cancer Res., 40, 91 (abstr. 604) (1999).  
6) Yokoyama, M., Satoh, A., Sakurai, Y., Okano, T., Matsumura, Y., Kakizoe, T. and Kataoka, K. Incorporation of water-insoluble anticancer drug into polymeric micelles and control of their particle size. J. Controlled Release, 55, 219–229 (1998).  
7) Matsumura, Y., Yokoyama, M., Kataoka, K., Okano, T., Sakurai, Y., Kawaguchi, T. and Kakizoe, T. Reduction of the side effects of an antitumor agent, KRN5500, by incorporation of the drug into polymeric micelles. Jpn. J. Cancer Res., 90, 122–128 (1999).  
8) Thrall, R. S., McCormick, J. R., Jack, R. M., McReynolds, R. A. and Ward, P. A. Bleomycin-induced pulmonary fibrosis in the rat: induction by indomethacin. Am. J. Pathol., 95, 117–130 (1979).  
9) Adamsom, I. Y. R. and Bowden, D. H. The pathogenesis of bleomycin-inducing pulmonary fibrosis in mice. Am. J. Pathol., 77, 185–198 (1974).  
10) Bader, H., Ringsdorf, H. and Schmidt, B. Water-soluble polymers in medicine. Angew. Makromol. Chem., 123/124, 457–485 (1984).  
11) Yokoyama, M., Miyauchi, M., Yamada, N., Okano, T.,
Sakurai, Y., Kataoka, K. and Inoue, S. Characterization and anticancer activity of micelle-forming polymeric antitumor drug adriamycin-conjugated poly(ethylene glycol)-poly(aspartic acid) block copolymer. Cancer Res., 50, 1693–1700 (1990).
12) Yokoyama, M., Okano, T., Sakurai, Y., Ekimoto, H., Shibazaki, C. and Kataoka, K. Toxicity and antitumor activity against solid tumors of micelle-forming polymeric antitumor drug and its extremely long circulation in blood. Cancer Res., 51, 3229–3236 (1991).
13) Yokoyama, M., Okano, T., Sakurai, Y., Suwa, S. and Kataoka, K. Introduction of cisplatin into micelle. J. Controlled Release, 39, 351–356 (1996).
14) Nishiyama, N., Yokoyama, M., Aoyagi, T., Okano, T., Sakurai, Y. and Kataoka, K. Preparation and characterization of self-assembled polymer-metal complex micelle from cis-dichlorodiammineplatinum (II) and poly(ethylene glycol)-poly(ω,β-aspartic acid) block copolymer in an aqueous medium. Langmuir, 15, 377–383 (1999).
15) Burger, A. M., Kaur, G., Hollingshead, M., Fischer, R. T., Nagashima, K., Malspeis, L., Duncan, K. L. K. and Sausville, E. A. Antiproliferative activity in vitro and in vivo of the spicamycin analogue KRN5500 with altered glycoprotein expression in vitro. Clin. Cancer Res., 3, 455–463 (1997).
16) Lee, Y. S., Nishio, K., Ogasawana, H., Funayama, Y., Ohira, T. and Saijo, N. In vitro cytotoxicity of a novel antitumor antibiotic, spicamycin derivative, in human lung cancer cell lines. Cancer Res., 55, 1075–1079 (1995).
17) Takara, K., Tanigawara, Y., Komada, F., Nishiguchi, K., Sakaeda, T. and Okamura, K. The novel anticancer drug KRN5500 interacts with, but is hardly transported by, human P-glycoprotein. Jpn. J. Cancer Res., 91, 248–254 (2000).