FR901228, A NOVEL ANTITUMOR BICYCLIC DEPSIPEPTIDE PRODUCED BY *Chromobacterium violaceum* No. 968

I. TAXONOMY, FERMENTATION, ISOLATION, PHYSICO-CHEMICAL AND BIOLOGICAL PROPERTIES, AND ANTITUMOR ACTIVITY

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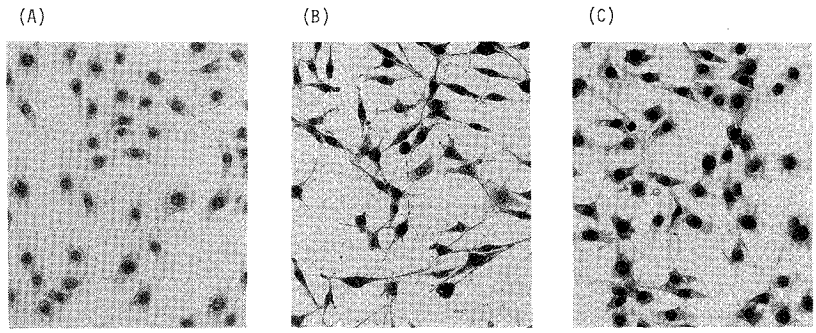
A novel antitumor bicyclic depsipeptide, FR901228, was isolated from a broth culture of *Chromobacterium violaceum* No. 968 as colorless prisms and the molecular formula was determined as $C_{24}H_{36}N_4O_6S_2$. This antibiotic reverted the transformed morphology of a Ha-*ras* transformant to normal, and exhibited prominent antitumor activities against murine and human tumor cell lines both *in vitro* and *in vivo*.

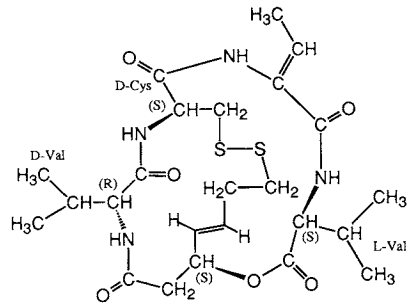
Recent studies showed that a family of *ras* oncogenes was frequently found to harbor mutations in human tumors[1]. The presence of these genes in human tumors play an important role in the development of tumors. A relationship between the levels of expression of the Ha-*ras* oncogene and tumorigenic potential was clearly demonstrated[2,3]. In addition, it was shown that morphological reversion of the *ras* transformed phenotype was achieved through micro-injection of antibodies specific for the Ha-*ras* gene product[4]. Consequently, activated *ras* expression is thought to be critical in maintaining the transformed phenotype. These data show that if an agent selectively reversed the phenotype of a *ras* transformant, it would be a new class of anticancer drug targeting the components of *ras*-mediated signaling transduction pathways. For this reason, we have searched for novel products from fermentation broths which would reverse a *ras*-transformed phenotype to normal, and which could also show inhibitory effects on the growth of tumor cells.

Fig. 1. Morphology of NIH3T3 cells and Ha-*ras* transformant.
NIH3T3 cells (A), Ha-*ras* transformant, Ras-1 cells without (B), or with FR901228 (C).



During the course of our research program, we found a novel antitumor antibiotic, FR901228 from the fermentation broth of a strain of *Chromobacterium violaceum* No. 968. This compound reversed the transformed morphology of Ha-*ras* transformant to normal (Fig. 1). The chemical structure of this compound was determined on the basis of physico-chemical and spectroscopic data as shown in Fig. 2.

Fig. 2. Structure of FR901228.



In this paper, we describe the taxonomy of the producing strain, the fermentation, isolation procedure and physico-chemical properties and some biological activities as well as antitumor activities of FR901228. The structural elucidation of this compound[5], the actions of FR901228 on Ha-*ras* transformed cells[6] and precise antitumor activity of this compound[7] will be reported in the accompanying papers.

Materials and Methods

Taxonomic Studies

The taxonomic studies were based on the methods described in BERGEY's Manual of Systematic Bacteriology 9th Edition (Vol. 1.)[8] and Manual for Identification of Medical Bacteria[9]. Morphological observation of strain No. 968 was carried out using a light microscope and a scanning electron microscope with the cells grown on Nutrient agar for 20 hours at 30°C. DNA was isolated by the phenol method of SAITO and MIURA[10], with some modifications. DNA base and quinone compositions were determined by HPLC[11~13]. Cellular fatty acid composition was prepared and analyzed according to the method of SUZUKI and KOMAGATA[14].

Fermentation

A culture medium (160 ml) consisting of 1% glucose and 1% Nutrient broth (Kyokuto Seiyaku Kogyo Co., Ltd., Tokyo, Japan) was sterilized in a 500-ml Erlenmeyer flasks at 120°C for 30 minutes. A loopful from a slant culture of strain No. 968 was inoculated into each of the flasks and cultured at 30°C for 24 hours on a rotary shaker (250 rpm, 5.1 cm-throw). The resultant seed culture was inoculated into a 30-liter stainless steel jar-fermentor containing 20 liters of a sterile production medium consisting of 1% glucose, 1% Nutrient broth and 0.05% Adekanol LG109 (antifoaming agent, Asahi Denka Kogyo Co., Ltd., Tokyo, Japan). The fermentation was carried out at 30°C under aeration of 20 liters/minute, inner pressure of 1.0 kg/cm^2 and agitation of 200 rpm for 48 hours.

Assay for FR901228 Production

The amount of FR901228 in the fermentation broth was determined by HPLC using a Hibar LiChrosorb RP-18 Column (5 μm, 4 mm i.d. $\times$ 200 mm length, E. Merck, Darmstadt, Germany) with 60% aqueous CH_3OH and detected by UV absorption at 210 nm. The sample for the HPLC assay was prepared as follows: 2 ml of acetone was added to 1 ml of the fermentation broth and the mixture was stirred vigorously for 1 minute, then centrifuged at 3,000 rpm for 5 minutes. Five μl of the supernatant was injected in to a Hitachi Model L-6000 HPLC.

Antimicrobial Activity

Antimicrobial activity was determined by a serial broth dilution method in Nutrient broth for Gram-positive and Gram-negative bacteria and in Sabouraud broth (Difco Laboratories, Detroit, MI, U.S.A.) for fungi and yeast. The inoculum was adjusted to 5×10^5 cfu/ml for bacteria and 1×10^6 cfu/ml for fungi and yeast. Minimum inhibitory concentration (MIC) is expressed in terms of μg/ml after 18 hours

at 37°C for bacteria and 48~72 hours incubation at 28°C for fungi and yeast.

Ha-*ras* Transformed Cells

Human bladder carcinoma EJ cells were kindly provided by Dr. M. TACHIBANA, Keio University. Mouse established normal fibroblast NIH3T3 cells were a kind gift from professor M. SAITO, Jichi Medical School. The Ha-*ras* transformed NIH3T3 cell line was obtained from transfection of DNA from EJ cells to NIH3T3 cells according to the method described by PARADA *et al.*[15]. Ras-1 cells, a clonal cell line used in these studies, were obtained from the parental transformant by the limiting-dilution method and had more malignant properties. Ras-1 cells expressed high levels of the Ha-*ras* product, exhibited the transformed phenotype, and was tumorigenic in nude mice. To determine the effect of FR901228 on the morphology of Ras-1 cells, Ras-1 cells (1×10^6) in 10 ml of complete MEM were cultured in a 25-cm^2 flask with various concentrations of FR901228 for 24 hours, fixed by ethanol, and stained by Wright-Giemsa. Complete MEM comprised EAGLE's minimum essential medium (MEM, Flow Laboratories, Rockville, MD, U.S.A.) supplemented with 10% fetal calf serum (Gibco, Gland Island, NY, U.S.A.) and kanamycin (60 μg/ml).

In Vitro Cytotoxic Activity of FR901228

The cells used for the experiments were as follows (the media used were shown in parenthesis); human lung adenocarcinoma A549 (complete DMEM) and PC-9 (complete RPMI), human lung squamous cell carcinoma PC-1 and PC-10 (complete RPMI), human small cell lung carcinoma ADH (complete RPMI) and LX-1 (complete DMEM supplemented with 5×10^{-5} M 2-mercaptoethanol), human stomach adenocarcinoma MKN28 and MKN74 (complete RPMI), human mammary adenocarcinoma MCF-7 and ZR-75-1 (complete DMEM supplemented with 4 μg/ml insulin and 10^{-8} M 17β-estradiol), human colon adenocarcinoma Colo201 (complete RPMI) and SW480 (complete DMEM), human normal fibroblast HNF cells (complete DMEM), human normal endothelium HE-9 cells (complete MCDB), mouse normal fibroblast cells (complete DMEM), and mouse established normal fibroblast Balb/c3T3 and NIH3T3 cells (complete DMEM). Unless otherwise indicated, complete DMEM comprised DULBECCO's modified EAGLE's medium (Flow Laboratories) supplemented with 10% heat-inactivated fetal bovine serum (Gibco), 50 U/ml benzylpenicillin and 50 μg/ml streptomycin, complete RPMI comprised RPMI-1640 medium (Flow Laboratories) supplemented with 10% heat-inactivated fetal bovine serum, 50 U/ml benzylpenicillin and 50 μg/ml streptomycin, and complete MCDB comprised MCDB-151 medium (Gibco) supplemented with 15% heat-inactivated fetal bovine serum, 100 μg/ml endothelial cell growth supplement (Collaborative Research Inc., Bedford, MA, U.S.A.), 10 μg/ml heparin, 60 μg/ml kanamycin. All cell lines were cultured at 37°C in a humidified atmosphere of 5% CO_2-95% air.

In vitro cytotoxic activity was tested in 96-well microtiter plate, with each well containing 1×10^3 Colo201 cells, 2×10^3 ZR-75-1 or HE-9 cells, 4×10^3 other cells in 100 μl medium. For HE-9 cells, a 96-well microtiter plate was precoated with 125 μg/ml human plasma fibronectin (Green Cross Co., Osaka, Japan). The cells were incubated at 37°C for 4 days and growth was measured by the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay described by MOSMANN[16]. Briefly, MTT was dissolved in DULBECCO's phosphate buffered saline (pH 7.4) at 5 mg/ml and filtered to sterilize and remove a small amount of insoluble residue. On the fourth day of the culture, the MTT solution (10 μl per 100 μl medium) was added to all wells, and the plates were further incubated at 37°C for 4 hours. After removal of the medium from all wells, 2-propanol was added and mixed thoroughly to dissolve the dark blue crystals. After all crystals were dissolved, the plates were measured on a two-wavelength microplate photometer (Model MTP-100; Corona Electric Co., Ltd., Ibaraki, Japan) at 550 nm with a reference wavelength at 660 nm.

In Vivo Antitumor Activity of FR901228

A two-week Subrenal Capsule (SRC) assay using the immunosuppressive agent FK-506 was performed according to the method described by NISHIMURA *et al.*[17,18]. In brief, a 1-mm^3 tumor fragment of A549 or MCF-7 maintained in nude mice was implanted under the kidney capsule of BDF_1 mice. For the mice implanted with MCF-7, 0.1 ml of E.P. Hormone Depo. (hydroxyprogesterone caproate 50 mg/ml and estradiol dipropionate 1 mg/ml, Teikoku Zoki Co., Ltd., Japan) was injected intramuscularly at day-0.

FR901228 suspended in 0.5% methyl cellulose was administered intraperitoneally at day-1, -5 and -9. FK-506 (32 mg/kg) was suspended in saline and given subcutaneously at day-1, -2, -5, -7, -9 and -12 which completely inhibited the host immune response for rejecting the xenograft[19]. Eight mice were used in each group. Fourteen days after tumor implantation, the length and width of each tumor was measured using a slide caliper and the tumor weight was calculated by the following formula:

$$\text{Tumor weight (mg)} = 1/2 \times a \times b^2$$

where a represents the length and b represents the width (mm). The efficacy of the drug was assessed by the calculating percent of growth inhibition using the following formula:

$$\text{Growth inhibition (\%)} = (1 - T/C) \times 100$$

where T represents mean tumor weight of the treated group and C represents that of the control group.

Animals

Specific pathogen free (SPF) BDF_1 mice (C57BL/6 × DBA/2) (female, 8 weeks old) were purchased from Charles River Japan Inc. (Kanagawa, Japan).

Results

Taxonomic Studies on the Strain No. 968

Strain No. 968 was isolated from a soil sample obtained from Yamagata-prefecture, Japan. The morphological characteristics of strain No. 968 are summarized in Table 1. Strain No. 968 was a Gram-negative, motile bacterium with a single polar flagellum. Colonies of No. 968 on Nutrient agar were smooth, grayish-orange in color, circular and entire-edged. Spores were not formed. The cells had a rod shape with a size of 0.5~0.6 × 1.2~1.8 μm (Fig. 3).

The physiological characteristics of strain No. 968 are summarized in Table 2. The growth temperature range was from 15°C to 40°C. Strain No. 968 gave positive results in tests for catalase, oxidase and casein hydrolysis. β-Galactosidase, indole, Voges-Proskauer test and esculin hydrolysis were negative. Strain No. 968 did not produce violet pigment on Nutrient agar and was resistant to vibriostatic agent 0/129 (2,4-diamino-6,7-diisopropylpteridine, 150 μg/disc). Acid formation was observed from D-glucose, D-fructose and D-trehalose. The guanine-plus-cytosine (G+C) content of the DNA was 62.7 mol%. The predominant isoprenoid quinone was the ubiquinone Q-8. The major component of the cellular fatty acid was $C_{16:1}$ acid.

According to BERGEY's Manual of Systematic Bacteriology 9th Edition (Vol. 1.)[8], the strain No. 968 was considered to belong to the genus *Chromobacterium* based on the observed characteristics. Thus, strain

Table 1. Morphological characteristics of strain No. 968.

Gram stain	Negative
Color of colony	Grayish orange
Cell shape	Rod
Cell size	0.5~0.6 × 1.2~1.8 μm
Spore formation	Negative
Motility	Positive
Flagella	Single polar flagellum

Fig. 3. Electron micrograph of strain No. 968.

Scale: 1 μm.

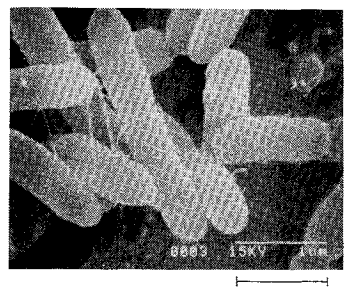


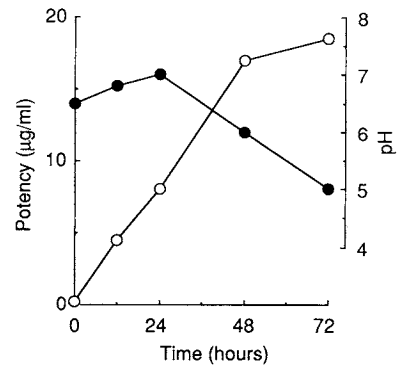
Table 2. Physiological characteristics of strain No. 968.

Growth temperature	15~40°C	Gelatin liquefaction	Positive
Growth in air	Positive	Casein hydrolysis	Positive
Growth in peptone water without NaCl	Positive	Esculin hydrolysis	Negative
Growth in 6% NaCl	Negative	Lysine decarboxylase	Negative
Growth in KCN broth	Positive	Ornithine decarboxylase	Negative
Violet pigment	Negative	Arginine dihydrolase	Positive
Catalase	Positive	G+C content of DNA	62.7 mol%
Oxidase	Positive	Major cellular fatty acid	$C_{16:1}$
O-F test	Fermentative	Quinone type	Q-8
Gas from glucose	Negative	Acid formation from	
0/129 sensitivity (150 μg)	Negative	D-Glucose	Positive
Nitrate reduction	Positive	L-Arabinose	Negative
Tween 80	Positive	D-Mannitol	Negative
Production of H_2S (TSI)	Negative	D-Fructose	Positive
Indole	Negative	D-Galactose	Negative
MR test	Negative	D-Sorbitol	Negative
VP test	Negative	D-Trehalose	Positive
Simmons citrate	Positive	Sucrose	Negative
ONPG test	Negative	Lactose	Negative
Urease	Positive	Salicin	Negative
DNase	Positive	Maltose	Negative
Starch hydrolysis	Negative	Cellobiose	Negative

No. 968 was compared with *Chromobacterium* species described in literature. As a result, strain No. 968 was found to be almost identical to *Chromobacterium violaceum* JCM-1249 except for the difference in pigmentation. It was considered that this difference was too small to regard strain No. 968 as a different species. Therefore, we identified strain No. 968 as *Chromobacterium violaceum* and designated it *Chromobacterium violaceum* No. 968. A culture of *Chromobacterium violaceum* No. 968 has been deposited with National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Japan, under the accession number of FERM BP-1968.

Fig. 4. Time course of FR901228 production.

○ Potency, ● pH.



Fermentation

Fig. 4 presents the data from a typical time course of a 30-liter stainless steel jar-fermentation. The organism reached the stationary phase of growth at 72 hours after inoculation. FR901228 production started at 12 hours and the maximum accumulation was observed at 72 hours. A maximal yield of 19 μg/ml was observed after 72 hours of cultivation. The pH of the medium rose gradually from 6.5 to 7.0 for 24 hours and then fell to 5.0 at 72 hours.

Isolation and Purification Procedure

After the fermentation was terminated, each jar-fermentor was sterilized at 120°C for 30 minutes. The cultured broth thus obtained was filtered with the aid of diatomaceous earth (10 kg). The filtrate (150 liters) was extracted twice with EtOAc (150 liters). The extract was evaporated under reduced pressure to give an

oily residue. The oily residue was mixed with 500 g of silica gel (Kieselgel 60, 70~230 mesh, E. Merck), and this mixture was slurried in methanol. After evaporating the solvent, the resultant dry powder was poured on top of the column of the same silica gel (500 g) which was prepacked with *n*-hexane. The column was washed with *n*-hexane (2 liters), a mixture of *n*-hexane - EtOAc (3 : 1, 3 liters), *n*-hexane - EtOAc (1 : 1, 3 liters), *n*-hexane - EtOAc (1 : 2, 3 liters). FR901228 was eluted with EtOAc (5 liters). Fractions containing FR901228 were collected and concentrated under reduced pressure to give a slightly yellowish powder. This powder was dissolved in a mixture of dichloromethane - methanol - acetonitrile (10 : 1 : 20, 30 ml). This solution was kept at room temperature to get colorless prisms of FR901228 (920 mg).

Physico-chemical Properties of FR901228

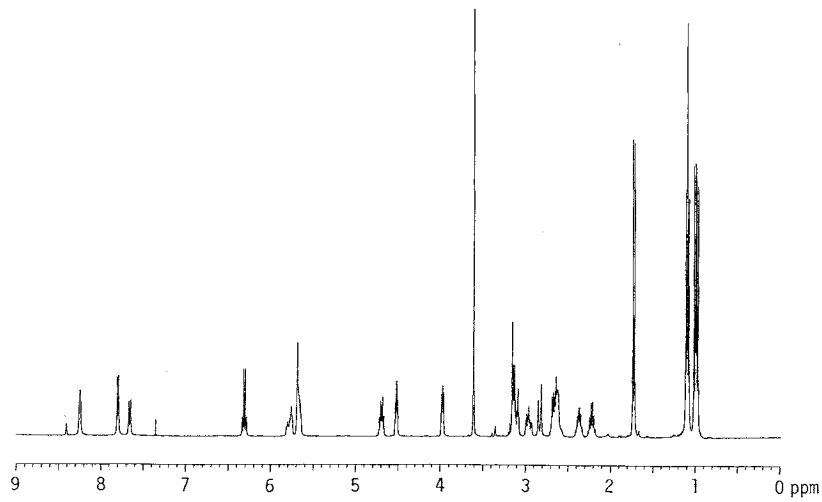
The physico-chemical properties of FR901228 are summarized in Table 3. FR901228 was soluble in chloroform and EtOAc. It was sparingly soluble in methanol and ethanol and was insoluble in water and *n*-hexane. Color reactions are as follows: Positive for ceric sulfate, sulfuric acid and iodine vapor reactions, and negative for Ninhydrin, ferric chloride, Ehrlich and Molisch reactions. It's Rf value on TLC of silica gel plate (Kieselgel 60 F_{254}, E. Merck) developed with a mixture of dichloromethane - methanol (10 : 1) was 0.65. The result of the amino acid analysis for FR901228 revealed the presence of valine and ammonia (the molar ratio of valine and ammonia is 2 : 1). The ^{1}H and ^{13}C NMR spectra of FR901228 are shown in Figs. 5 and 6, respectively. From the data of the ^{13}C NMR spectrum and

Table 3. Physico-chemical properties of FR901228.

Appearance	Colorless prisms
MP	235~245°C (dec)
$[\alpha]_D^{23}$	+39° (*c* 1.0, $CHCl_3$)
FAB-MS *m/z*:	541 $(M+H)^+$
Molecular formula	$C_{24}H_{36}N_4O_6S_2$
Elemental analysis (%)	
Calcd for:	$C_{24}H_{36}N_4O_6S_2 \cdot CH_3CN$
	C 53.68, H 6.76, N 12.04, S 11.02
Found:	C 53.68, H 6.71, N 11.80, S 11.09
UV λ_{max}^{MeOH} nm (ε)	End absorption
IR ν_{max}^{KBr} cm^{-1}	3360, 3320, 2950, 2910, 1740, 1690, 1660, 1650, 1630, 1520, 1460, 1440, 1400, 1390, 1370, 1350, 1300, 1250, 1220, 1170, 1100, 1040, 1020, 1000, 980, 910
TLC Rf value[a] (I)	0.65
(II)	0.45

[a] Plate; Silica gel 60 F_{254} (E. Merck, Art. 5715), solvent system; (I) CH_2Cl_2 - MeOH (10 : 1). (II) EtOAc - acetone (5 : 1).

Fig. 5. 400 MHz ^{1}H NMR spectrum of FR901228 in $CDCl_3$ - CD_3OD (10 : 1).



Signals at 8.41, 8.25, 7.80 and 7.66 ppm are due to slowly exchanging amide protons.

Fig. 6. 100 MHz ^{13}C NMR spectrum of FR901228 in $CDCl_3$-CD_3OD (10:1).

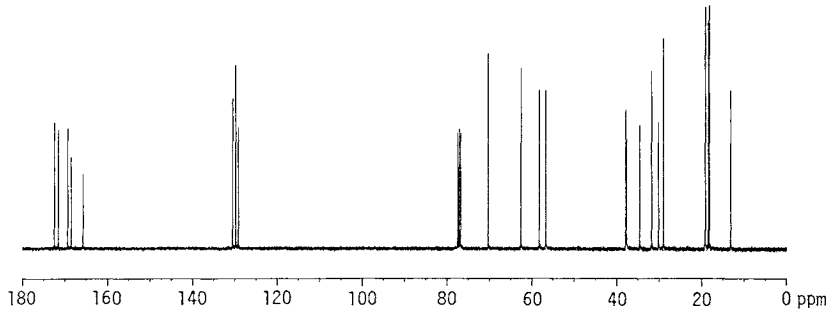


Table 4. Antimicrobial activity of FR901228.

Organisms	MIC (μg/ml)
Shizosaccharomyces pombe	10
Aureobasidium pullulans IFO 4466	10
Aspergillus niger	100
Candida albicans	>100
Staphylococcus aureus	>100
Bacillus subtilis	>100
Pseudomonas aeruginosa	>100
Escherichia coli	>100

Table 5. Antitumor activity of FR901228 against human tumor cell lines (*in vitro*).

Cell line		IC_{50} (ng/ml)	
		Exp. 1	Exp. 2
Lung adenocarcinoma	A549	0.7	0.8
	PC-9	0.3	0.5
Lung squamous cell carcinoma	PC-1	1.2	0.9
	PC-10	2.4	1.9
Small cell lung carcinoma	ADH	1.0	0.6
	LX-1	1.2	1.3
Stomach adenocarcinoma	MKN28	0.9	3.2
	MKN74	0.7	2.3
Mammary adenocarcinoma	MCF-7	0.6	0.8
	ZR-75-1	0.7	1.0
Colon adenocarcinoma	Colo201	0.3	0.3
	SW480	1.0	1.1

Table 6. Cytotoxic activity of FR901228 against normal cell lines (*in vitro*).

Cell line		IC_{50} (ng/ml)	
		Exp. 1	Exp. 2
Human normal fibroblast	HNF	>1000	>1000
Human normal endothelium	HE-9	7.0	6.0
Mouse normal fibroblast	Stromal	>500	>500
Mouse established normal fibroblast	Balb/c3T3	9.2	7.9
	NIH3T3	3.2	2.7

FAB-MS, together with the elementary analysis, the molecular formula of FR901228 was determined to be $C_{24}H_{36}N_4O_6S_2$. On the basis of these physicochemical properties and spectroscopic data, the structure of FR901228 was elucidated as (*E*)-(1*S*,4*S*,10*S*,21*R*)-7-[(*Z*)-ethylidene]-4,21-diisopropyl-2-oxa-12,13-dithia-5,8,20,23-tetraazabicyclo[8,7,6]-tricos-16-ene-3,6,9,19,22-pentanone as shown in Fig. 2. The full account of structural elucidation of FR901228 will be published in the succeeding paper[5].

Biological Properties of FR901228

Antimicrobial Activities

Antimicrobial activities of FR901228 are shown in Table 4. FR901228 exhibited weak antimicrobial activities against *Shizosaccharomyces pombe*, *Aureobasidium pullulans* IFO 4466 and *Aspergillus niger*, but were not effective on *Candida albicans*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli* at 100 μg/ml.

Antitumor Activities (*In Vitro*)

The cytotoxic activities of FR901228 substances determined by the MTT assay are shown in Table 5 and 6. FR901228 showed potent cytotoxic activities against human lung adenocarcinoma A549 and PC-9, human lung squamous cell carcinoma PC-1 and PC-10, human small cell lung carcinoma ADH and

Table 7. Antitumor activity of FR901228 against human adenocarcinomas (SRC assay).

Dose (mg/kg)	MCF-7			A549		
	D/T	MTW±SE (mg)	Inhibition (%)	D/T	MTW±SE (mg)	Inhibition (%)
Vehicle	0/8	44.3±7.2	0.0	0/8	27.2±4.7	0.0
0.32	0/8	30.5±5.7	32.3	0/7	24.3±3.1	10.6
0.56	0/8	21.4±3.2*	51.7	0/8	22.5±2.8	17.3
1.0	0/6	17.1±2.6**	61.4	0/8	14.5±2.7*	46.7
1.8	0/8	12.5±2.7**	71.8	0/7	9.4±1.3**	65.3
3.2	6/8	6.3±0.8*	85.8	2/8	3.9±0.5**	85.6

MCF-7: Mammary adenocarcinoma, A549: lung adenocarcinoma, D/T: dead animal number/treated animal number, MTW: mean tumor weight.
* $P<0.05$, ** $P<0.01$.

LX-1, human stomach adenocarcinoma MKN28 and MKN74, human mammary adenocarcinoma MCF-7 and ZR-75-1, and human colon adenocarcinoma Colo201 and SW480. FR901228 had weak cytotoxic activities against the human normal endothelial cell HE-9, mouse fibroblasts Balb/c3T3 and NIH3T3, and showed almost no cytotoxic activities against human and mouse normal fibroblast cells.

Antitumor Activities (*In Vivo*)

The antitumor activity of FR901228 was examined against two kinds of human tumors, A549 and MCF-7, implanted under the kidney capsule of immunosuppressed BDF_1 mice (SRC assay). Tumor weight was measured on day 14. As shown in Table 7, FR901228 significantly inhibited the growth of human tumors A549 and MCF-7 in a dose dependent manner at doses from 0.56 to 3.2 mg/kg and from 1.0 to 3.2 mg/kg, respectively.

Acute Toxicity of FR901228

Acute toxicities of FR901228 were examined in BDF_1 mice (female, 5-week-old). The LD_{50} values of FR901228 when given intraperitoneally and intravenously to mice were 6.4 and 10.0 mg/kg, respectively.

Discussion

We have searched for novel products of microbial origin which can reverse the *ras*-transformed phenotype to normal. As a result of screening, we isolated a novel antitumor bicyclic depsipeptide, FR901228, from the culture broth of *Chromobacterium violaceum* No. 968. It was a colorless prism which was found to have a novel structure as shown in Fig. 2. Details on the structural elucidation will be published in another paper[5].

In vitro, FR901228 showed potent cytotoxic activities against human lung adenocarcinoma A549 and PC-9, human lung squamous cell carcinoma PC-1 and PC-10, human small cell lung carcinoma ADH and LX-1, human stomach adenocarcinoma MKN28 and MKN74, human mammary adenocarcinoma MCF-7 and ZR-75-1, and human colon adenocarcinoma Colo201 and SW480. However, FR901228 had weak cytotoxic activities against human normal endothelial cell HE-9, mouse fibroblasts Balb/c3T3 and NIH3T3, and the activity was completely absent against human and mouse normal fibroblast cells. Known antitumor drugs such as doxorubicin or mitomycin C did not show this type of spectrum, but had almost the same cytotoxic activities against the cells used above (data not shown). Based on these results, FR901228 seemed a new type of antitumor drug having a novel mode of action. The actions of FR901228 on Ha-*ras* transformed cells will be reported in other papers[6].

Several compounds which reversed the transformed phenotype of oncogene transformants have previously been isolated as pharmacological agents of microbial origin[20~25], and some of them have also

showed antitumor activity *in vivo*, such as azatyrosin which is a chemopreventive agent for carcinogenesis[26]. To evaluate antitumor activity of this compound, the chemical carcinogenesis using transgenic mice was necessary. Oxanosine in combination with 5-fluorouracil decreased the progression of K-*ras* NIH3T3 solid tumors[27], but the efficacy of these compounds was not as significant as known antitumor drugs. As shown in Table 7, FR901228 had potent antitumor activity against mammary adenocarcinoma MCF-7 and lung adenocarcinoma A549 in the SRC assay system. These antitumor activities were comparable with those of known antitumor drugs[18]. Precise antitumor activity of this novel compound will be reported in a subsequent paper[7].

Acknowledgments

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