VOL. 57 NO. 11, NOV. 2004 THE JOURNAL OF ANTIBIOTICS pp. 748 — 754

Directed Biosynthesis of Fluorinated Pseurotin A, Synerazol and Gliotoxin

Yasuhiro Igarashi*, Yukihiro Yabuta, Akira Sekine, Kiyonaga Fujii[a], Ken-Ichi Harada[a], Tsutomu Oikawa[b,††], Mayumi Sato[b], Tamotsu Furumai and Toshikazu Oki[†]

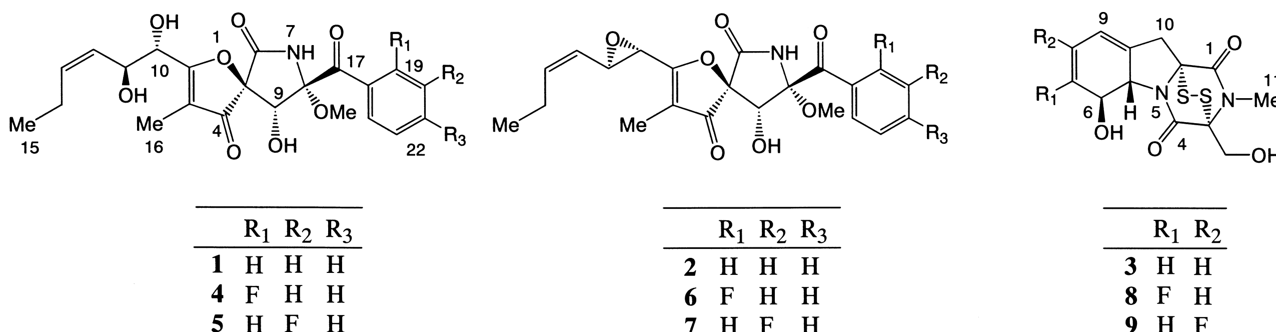
Biotechnology Research Center, Toyama Prefectural University,
5180 Kurokawa, Kosugi, Toyama 939-0398, Japan
[a] Faculty of Pharmacy, Meijo University,
150 Yagotoyama, Tempaku-ku, Nagoya, Aichi 468-8503, Japan
[b] Biomedical Research and Development Center, The Tokyo Metropolitan Institute of
Medical Science, Tokyo Metropolitan Organization for Medical Research,
3-18-22 Honkomagome, Bunkyo-ku, Tokyo 113-8613, Japan

(Received for publication April 8, 2004)

Aspergillus fumigatus TP-F0196 produces pseurotin A, synerazol and gliotoxin. Phenylalanine is a common biosynthetic precursor of these antibiotics. Feeding fluorophenylalanine to the culture induced the production of novel fluorinated analogs. These fluorinated antibiotics were obtained from the culture broth by solvent extraction and purified by chromatographies, and their antimicrobial and antitumor activities were investigated. Among the novel fluorinated analogs, 19- and 20-fluorosynerazols exhibited potent anti-angiogenic activity in the chorioallantoic membrane assay. In addition, 19-fluorosynerazol showed more potent cytocidal activity against several cancer cell lines than synerazol.

Aspergillus fumigatus TP-F0196, isolated from a seawater sample by our group, produces pseurotin A (**1**), synerazol (**2**) and gliotoxin (**3**) (Fig. 1). Pseurotin A was first isolated as a metabolite of *Pseudeurotium ovalis*[1)] and was later rediscovered as a chitin synthase inhibitor[2)] or a neuritogenic substance[3)]. Synerazol is an antifungal antibiotic from *A. fumigatus* and has an epoxy moiety instead of the diol group in pseurotin A[4)]. Gliotoxin was originally isolated from a fungus *Trichoderma viride*, and various biological activities including antiviral and antifungal activities and inhibition of farnesyltransferase have been reported[5)]. Biosynthetic studies using stable isotopes revealed that pseurotin A is biosynthesized from one methylmalonate, four malonates, one phenylalanine

Fig. 1. Structures of pseurotin A (**1**), synerazol (**2**), gliotoxin (**3**) and their fluorinated analogs.



	R_1	R_2	R_3
1	H	H	H
4	F	H	H
5	H	F	H

	R_1	R_2	R_3
2	H	H	H
6	F	H	H
7	H	F	H

	R_1	R_2
3	H	H
8	F	H
9	H	F

* Corresponding author: yas@pu-toyama.ac.jp
† Present address: Tamagawa University Research Institute, 6-1-1 Tamagawa-Gakuen, Machida, Tokyo 194-8610, Japan.
†† Present address: Kanagawa University of Human Services, 1-10-1 Heisei-cho, Yokosuka, Kanagawa 238-8522, Japan.

and two methionines[6]. The carbon skeleton of synerazol is identical to pseurotin A and hence, although there is no experimental confirmation, synerazol is presumably biosynthesized from the same precursors. Gliotoxin is a derivative of diketopiperazine biosynthesized from one molecule of phenylalanine and serine[7].

Replacement of hydrogen atoms by fluorine atoms in appropriate compounds can lead to drastic changes in the pharmacological and biological properties while the structural dimensions of these compounds remain largely unchanged. It also causes an increase in hydrophobicity owing to the low polarizability of the fluorine atoms. In the biosynthesis of secondary metabolites fluorinated precursor analogs are often recognized as the original substrate. Numerous publications on the preparation of fluorinated metabolites by feeding the microorganisms with fluorinated precursors have been reported[8~14]. Introduction of fluorine atom can improve the pharmacokinetics, stability or affinity to the target protein by altering the physico-chemical properties[9,14]. Additionally, fluorine-containing compounds include pharmaceutically important agents such as 5-fluorouracil, fluconazole, synthetic HMG-CoA reductase inhibitors and steroid analogs. In this paper, we report the directed biosynthesis and biological properties of fluorinated analogs of pseurotin A, synerazol and gliotoxin.

Results and Discussion

Production of Fluoroantibiotics by Directed Biosynthesis

Production of fluoroantibiotics was carried out in an A-3M medium. Racemic fluoro-substituted phenylalanine was added at day 2 of the fermentation to yield a final concentration of 0.5 mg/ml, and the production was monitored at days 4, 5, 6, 7 and 8 by HPLC. When the fed precursor was incorporated into the antibiotics, new metabolites showing the UV spectra similar to pseurotin A, synerazol or gliotoxin were detected with retention times different from the parent compounds. In most cases, the production reached a maximum at day 5 or 6. Even at higher concentrations of the fluoroprecursor, the production of fluoroantibiotics did not increase. When the production was low or the retention time of the new metabolite was close to that of the parent compound, the crude extract was roughly separated into fractions by silica gel column chromatography and the fractions were analyzed by HPLC. The new metabolites were purified by silica gel column chromatography and preparative HPLC and their structures were analyzed using NMR and MS techniques.

The production of fluoroanalogs is summarized in Table 1. 2-Fluorophenylalanine was incorporated into pseurotin A and synerazol with isolation yields comparable to that of the control. 3-Fluorophenylalanine was incorporated into all three antibiotics. In contrast, 4-fluorophenylalanine was incorporated into gliotoxin, but not into pseurotin A and synerazol. In the biosynthesis of gliotoxin, epoxidation of the benzene ring is supposed to take place prior to the bond formation between 5-nitrogen and 5a-carbon. It is noteworthy that the fluoro-substituent did not affect the reactivity of a monoxygenase to the aromatic ring. The stereochemistry of the fluorinated analogs, following feeding of racemic substrates is assumed to be that of the natural parent. This assumption is supported by detection of only one diastereomer from each addition and by a comparison of the optical rotations of the analogs with that of the appropriate parent.

Biological Properties

Antimicrobial Activity of Fluorinated Analogs

Antimicrobial activity of fluorinated analogs was investigated in comparison with the non-fluorinated

Table 1. Production of fluorinated pseurotin A, synerazol and gliotoxin.

	Isolation yield (mg/L)										
	R_1	R_2	R_3	Pseurotin A		Synerazol		R_1	R_2	Gliotoxin	
Control (no addition)	H	H	H	**1**	9.0	**2**	23.0			**3**	9.4
2-Fluorophenylalanine	F	H	H	**4**	10.1 (9.8)	**6**	29.0 (18.2)				0 (4.6)
3-Fluorophenylalanine	H	F	H	**5**	8.3 (7.5)	**7**	8.2 (6.5)	H	F	**9**	4.5 (4.2)
4-Fluorophenylalanine	H	H	F		0 (3.8)		0 (5.1)	F	H	**8**	4.5 (6.3)

Table 2. Antimicrobial activity of fluorinated gliotoxins.

Compound	MIC (μg/ml)					
	Bacillus subtilis M45 (rec-)	*Micrococcus luteus* ATCC9341	*Staphylococcus aureus* FDA209P	*Escherichia coli* NIHJ	*Candida albicans* 3147	*Saccharomyces cerevisiae* ATCC9763
Gliotoxin (**3**)	6.25	0.20	3.13	12.5	3.13	0.78
8	12.5	0.39	12.5	50	100	6.25
9	6.25	0.39	12.5	25	12.5	1.56

Table 3. Antimicrobial activity of fluorinated analogs of pseurotin A and synerazol.

Compound	MIC (μg/ml)	
	Candida albicans 3147	*Saccharomyces cerevisiae* ATCC9763
Pseurotin A (**1**)	>100	50
4	>100	50
5	>100	50
Synerazol (**2**)	25	12.5
6	12.5	12.5
7	100	25

Table 4. Cytocidal activity of synerazol and its fluoroderivatives (**6** and **7**).

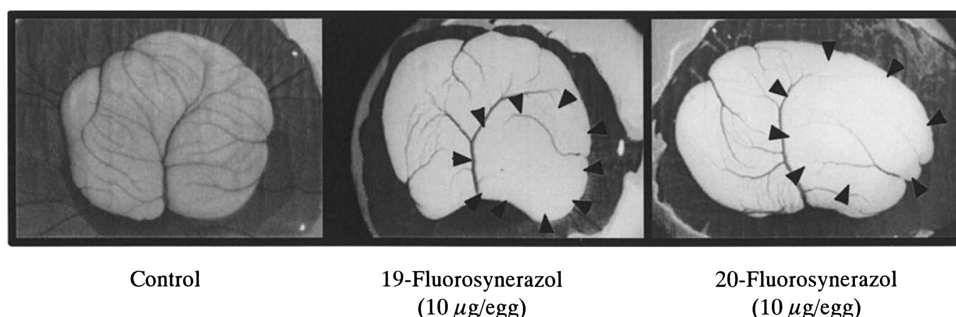
Cancer cell lines	IC_{50} (μM)		
	Synerazol	**6**	**7**
HBC-4	15	1.8	60
HBC-5	2.6	0.20	32
SNB-75	4.2	0.25	30
HCT-15	6.5	1.3	55
NCI-H226	0.44	0.22	2.1
NCI-H460	0.91	2.9	38
OVCAR-3	0.68	1.6	15
SKOV-3	31	28	86
PC-3	16	0.45	17

compounds (Tables 2 and 3). Antibacterial activity of 7- and 8-fluorogliotoxins (**8**, **9**) was equivalent to or less potent than gliotoxin (**3**). Especially the antifungal activity of **8** was lessened. Fluorinated pseurotin A (**4**, **5**) and synerazol (**6**, **7**) showed no activity against Gram-positive and -negative bacteria as is also the case with the nonfluorinated parent compounds. Fluoro-substitution had little effect on the antifungal activity of pseurotin A. The antifungal potency of synerazol varied depending on the substitution positions of fluorine atom, but no obvious structure-activity relationship was observed.

Cytocidal Activity of Fluorinated Analogs

The cytocidal activity of fluorinated antibiotics was first assessed against human myeloid leukemia U937 cells. Gliotoxin and its fluoroderivatives (**8**, **9**) showed potent activity with the IC_{50} ranging from 0.01 to 0.02 μg/ml, suggesting that the fluoro-substitution does not affect the activity. On the other hand, fluoro-substituion in the phenyl group of synerazol induced the drastic change of cytotoxicity. The IC_{50} of 19-fluorosynerazol (**6**) and 20-fluorosynerazol (**7**) was 0.37 and 1.25 μg/ml, respectively, whereas that of synerazol (**1**) was 0.09 μg/ml. Furthermore, the cytocidal activity of **6** and **7** was investigated against 39 human tumor cell lines. The IC_{50} of **1** ranged from 0.44 to 31 μM, that of **6** from 0.2 to 28 μM, and that of **7** from 2.1 to 86 μM (Selected data are shown in Table 4). Notably, the introduction of a fluorine atom at the *ortho*-position of the phenyl group enhanced the cytocidal activity against several cancer cell lines, while the fluoro-substitution at the *meta*-position lessened the activity. This result suggests that the precursor-directed biosynthesis can provide more potent cytocidal analogs of synerazol that are difficult to prepare

Fig. 2. Effect of 19- and 20-fluorosynerazols on angiogenesis in CAMs.



Control | 19-Fluorosynerazol (10 μg/egg) | 20-Fluorosynerazol (10 μg/egg)

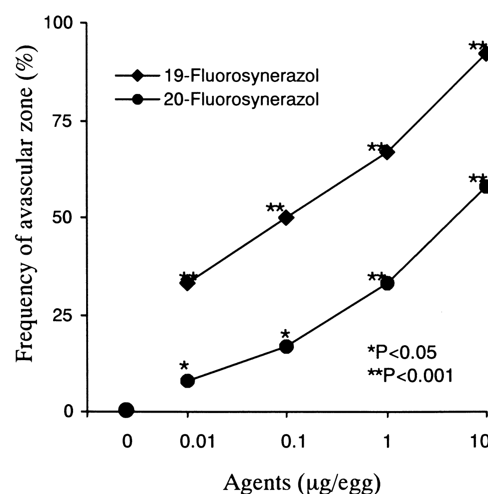
An appropriate volume of a fat emulsion was injected into the chorioallantois to show the vascular network clear. Fluorosynerazol-containing EV pellets led to the formation of a significant avascular zone (indicated by arrowheads). Control EV pellets did not show such an effect.

syntheticly.

Anti-angiogenic Activity of Fluorosynerazols

Synerazol shows antifungal and cytocidal activities but its mode of action is unknown. We tested the antitumor activity of fluorinated synerazols in several target-based assays including the inhibition of DNA topoisomerase, protein kinases and tublin function, and found that they have anti-angiogenic activity. In the preliminary experiment, fluorosynerazols exhibited the significant inhibition of the tube formation of bovine capillary endothelial cells induced by basic fibroblast growth factor[15] (data not shown). 19-Fluorosynerazol (**6**) suppressed 60% of the tube growth at 0.1 μM. Then, we investigated the anti-angiogenic activity in a chorioallantoic membrane (CAM) assay system[16,17]. The CAMs of growing chick embryos were treated for 2 days with EV pellets containing 19- or 20-fluorosynerazol (**6**, **7**), ranging from 0.01 to 10 μg/egg. Both compounds showed significant inhibitory effect on neovascularization in a dose-dependent manner with the ID_{50} values of 0.23 and 13 nmol/egg for **6** and **7**, respectively (Figs. 2 and 3). The proteasome is related to angiogenesis and the neovascularization in the CAM assay is inhibited by the proteasome inhibitors such as eponemycin (ID_{50} 0.25 pmol/egg) and lactacystin (ID_{50} 9.6 nmol/egg)[18]. The inhibitory effect of eponemycin and lactacystin is caused by their covalent binding to the proteasome through its epoxide[19] and the ester bond[20], respectively. It is likely that fluorosynerazol exhibits the anti-angiogenic activity in a similar fashion because its epoxide is highly reactive to nucleophiles[21]. Recently,

Fig. 3. Inhibitory effect of 19- and 20-fluorosynerazols on embryonic angiogenesis.



Osada *et al.* reported the anti-angiogenic activity of azaspirene that possesses the carbon skeleton identical to pseurotin A and synerazol[22]. Azaspirene shows the inhibitory effect on VEGF-induced cell migration of human umbilical vein endotherial cells. This effect is presumably induced by the inhibition of VEGF-induced signal transduction.

Neovascularization is an attractive target for pharmaceutical development since it plays an important role in a variety of human pathologies. In addition to tumor growth and metastatic control, diabetic retinopathy and rheumatoid arthritis are also examples of diseases currently

being targeted using an anti-angiogenic pharmacological strategy. Synerazol derivatives comprise a new group of anti-angiogenic compounds and structure-activity relationship will provide new insight into the design of angiogenic inhibitors.

Experimental

Fermentation

The producing strain *Aspergillus fumigatus* TP-F0196 was isolated from a seawater sample collected 2,600 meters off the shore and 321 meters in depth at Namerikawa, Toyama, Japan by a membrane filter method. A loopful of a mature slant culture of strain TP-F0196 was inoculated into a 500-ml K-1 flask containing 100 ml of A-3M medium composed of glucose 0.5%, glycerol 2%, soluble starch 2%, Pharmamedia (Traders Protein) 1.5%, yeast extract 0.3% and Diaion HP-20 1%. The pH of the medium was adjusted to 7.0 before sterilization. The seed culture was incubated for 2 days at 30°C on a rotary shaker at 200 rpm. One-ml aliquot of the seed culture was inoculated into 500-ml K-1 flasks containing 100 ml of A-3M medium. After 24 hours of incubation at 30°C, a sterilized solution of fluorinated phenylalanine was added to each flask (0.05% w/v, 50 mg/flask). The fermentation was carried out for 3 days at 30°C on a rotary shaker at 200 rpm.

HPLC Analysis

A small portion of the fermentation broth was extracted with ethyl acetate and the organic layer was evaporated. The extract was dissolved in DMSO and analyzed on an Agilent HP1100 HPLC system. The column was Cosmosil 5C18-AR-II (250×4.6 mm, i.d., Nacalai Tesque), the eluent acetonitrile - 0.15% KH_2PO_4 buffer (pH 3.5) (60 : 40), flow rate 0.7 ml/minute, detection at 254 nm and temperature 30°C.

Isolation

A typical isolation procedure is described for 19-fluorosynerazol (**10**). The fermentation broth (5 liters) was extracted with ethyl acetate (2.5 liters) twice. The organic layers were concentrated *in vacuo* to give a crude extract (3.8 g). It was then chromatographed on a silica gel column (60×5 cm, i.d.) with *n*-hexane - ethyl acetate (10 : 1~1 : 1). Fractions containing **10** were combined and evaporated to give crude material (0.8 g). This was further purified by preparative HPLC (Cosmosil 5C18-AR-II, 250×20 mm, i.d., Nacalai Tesque) on a Shimadzu LC-10A with 50% aqueous methanol. The collected fraction was evaporated and the remaining aqueous solution was extracted with ethyl acetate. The organic layer was evaporated to give **10** (119.8 mg) as a colorless powder.

Chemicals

2-, 3-, and 4-DL-Fluorophenylalanines were purchased from Aldrich Chemical Company.

Biological Assay

The minimum inhibitory concentrations against bacteria and yeasts were determined by the 2-fold serial dilution method against laboratory strains. Cytocidal activity against human myeloid leukemia U937 cells and 39 kinds of human cancer cell lines was determined by MTT method or sulforhodamine B after incubation for 2 days. Anti-angiogenic activity was determined by the chick embryo chorioallantoic membrane assay[16,17]. The chorioallantoic membranes of 5-day-old chick embryos were treated with ethylene-vinyl acetate (EV) copolymer 40 pellets containing, or not containing, various doses of 19- or 20-fluorosynerazol at 37°C for 2 days in a humidified egg incubator. Then, an appropriate volume of a 20% fat emulsion was injected into the chorioallantois to show the vascular network better. When the diameter of the avascular zone was 3 mm or more, the anti-angiogenic response was taken as effective.

Instrumental Analysis

NMR experiments were performed on a JEOL JNM-LA400 NMR spectrometer in $CDCl_3$ (fluoropseurotins and fluorosynerazols) or DMSO-d_6 (fluorogliotoxins) with TMS as an internal standard. $CFBr_3$ (7.4 ppm) was used as a reference for ^{19}F chemical shifts. The chemical shifts are given in the order of the position numbering. The position numbering is fully indicated in parenthesis for 19-fluoropseurotin A (**4**), 19-fluorosynerazol (**6**) and 7-fluorogliotoxin (**8**). Interchangeable assignments are marked with asterisk (*). MS spectra were measured on a JEOL JMS-HX110A spectrometer. Optical rotations were measured on a Horiba SEPA-300 polarimeter. All of the fluorinated analogs were obtained as a colorless powder. Their UV and IR spectra were almost identical to that of the parent compounds.

19-Fluoropseurotin A (4)

$[\alpha]_D^{25}$ +48 (*c* 2, $CHCl_3$); HRFAB-MS *m/z* 450.1568 $[M+H]^+$ (calcd *m/z* 450.1564 for $C_{22}H_{25}NO_8F$); ^{13}C NMR δ 185.9 (C-2), 113.5 (C-3), 196.3 (C-4), 92.4 (C-5), 166.8 (C-6), 90.0 (C-8), 73.3 (C-9), 70.5 (C-10), 70.7 (C-11), 126.5 (C-12), 137.0 (C-13), 21.4 (C-14), 14.1 (C-15), 6.0

(C-16), 194.0 (d, 2.4 Hz, C-17), 122.6 (d, 9.9 Hz, C-18), 161.3 (d, 259.2 Hz, C-19), 116.9 (d, 21.4 Hz, C-20), 135.2 (d, 9.8 Hz, C-21), 124.1 (d, 4.1 Hz, C-22), 131.3 (C-23), 51.7 (8-OMe); ^{1}H NMR δ 4.74 (s, H-9), 4.59 (d, 5.1 Hz, H-10), 4.76 (dd, 5.1 and 9.3 Hz, H-11), 5.31 (ddt, 9.3, 11.0 and 1.7 Hz, H-12), 5.61 (dt, 11.0 and 7.1 Hz, H-13), 2.09 (dddq, 1.7, 7.1, 15.2 and 7.6 Hz, H-14), 2.17 (dddq, 1.7, 7.1, 15.2 and 7.6 Hz, H-14), 0.99 (t, 7.6 Hz, H-15), 1.67 (s, H-16), 7.17 (ddd, 1.0, 7.6 and 11.0 Hz, H-20), 7.56 (ddt, 1.7, 5.1 and 7.6 Hz, H-21), 7.23 (dt, 1.0 and 7.6 Hz, H-22), 8.16 (ddd, 1.7, 7.6 and 7.6 Hz, H-23), 8.18 (s, 7-NH), 3.47 (s, 8-OMe), 4.10 (s, 9-OH); ^{19}F NMR δ −111.8 (C-19).

20-Fluoropseurotin A (**5**)

$[\alpha]_D^{25}$ +73 (*c* 2, $CHCl_3$); HRFAB-MS *m/z* 450.1554 $[M+H]^+$ (calcd *m/z* 450.1564 for $C_{22}H_{25}NO_8F$); ^{13}C NMR δ 186.0, 113.2, 196.3, 92.5, 166.7, 90.5, 72.9, 70.6, 70.8, 126.4, 136.7, 21.3, 14.1, 6.0, 194.4 (d, 1.7 Hz), 134.1 (d, 6.5 Hz), 117.4 (d, 21.4 Hz), 162.5 (d, 246.9 Hz, C-20), 121.8 (d, 23.8 Hz), 130.3 (d, 7.5 Hz), 126.6 (d, 2.5 Hz), 51.7; ^{1}H NMR δ 4.69 (s), 4.59 (d, 4.4 Hz), 4.75 (dd, 4.4 and 9.0 Hz), 5.25 (ddt, 9.0, 10.8 and 1.7 Hz), 5.57 (dt, 10.8 and 7.3 Hz), 2.08 (dddq, 1.7, 7.3, 15.6 and 7.6 Hz), 2.16 (dddq, 1.7, 7.3, 15.6 and 7.6 Hz), 0.98 (t, 7.6 Hz), 1.66 (s), 8.02 (ddd, 1.5, 2.4 and 9.6 Hz, H-19), 7.34 (dddd, 1.0, 2.4, 8.0 and 8.3 Hz, H-21), 7.47 (dt, 5.6 and 8.0 Hz, H-22), 8.14 (ddd, 1.0, 1.5 and 8.0 Hz, H-23), 8.44 (s), 3.43 (s), 4.31 (s); ^{19}F NMR δ −111.0 (C-20).

19-Fluorosynerazol (**6**)

$[\alpha]_D^{25}$ −7 (*c* 10, $CHCl_3$); HRFAB-MS *m/z* 432.1476 $[M+H]^+$ (calcd *m/z* 432.1459 for $C_{22}H_{23}NO_7F$); ^{13}C NMR δ 182.0 (C-2), 114.4 (C-3), 196.4 (C-4), 91.6 (C-5), 165.2 (C-6), 89.1 (C-8), 73.9 (C-9), 52.7 (C-10), 55.1 (C-11), 123.5 (C-12), 141.6 (C-13), 21.3 (C-14), 14.1 (C-15), 5.2 (C-16), 193.1 (C-17), 123.5 (C-18), 161.1 (d, 258.3 Hz, C-19), 116.9 (d, 21.0 Hz, C-20), 135.1 (d, 9.1 Hz, C-21), 124.1 (d, 2.3 Hz, C-22), 130.9 (C-23), 51.8 (8-OMe); ^{1}H NMR δ 4.69 (d, 12.0 Hz, H-9), 3.77 (d, 2.0 Hz, H-10), 4.12 (dd, 2.0 and 8.8 Hz, H-11), 5.08 (ddt, 8.8, 11.0 and 1.7 Hz, H-12), 5.87 (dt, 11.0 and 7.3 Hz, H-13), 2.25 (dddq, 1.7, 7.3, 14.9 and 7.6 Hz), 2.31 (dddq, 1.7, 7.3, 14.9 and 7.6 Hz, H-14), 1.05 (t, 7.6 Hz, H-15), 1.81 (s, H-16), 7.18 (ddd, 1.0, 8.0 and 11.2 Hz, H-20), 7.57 (dddd, 1.4, 5.1, 8.0 and 8.4 Hz, H-21), 7.24 (ddd, 1.0, 7.6 and 8.0 Hz, H-22), 8.07 (ddd, 1.4, 7.6 and 7.6 Hz, H-23), 7.29 (s, 7-NH), 3.47 (s, 8-OMe), 3.84 (d, 12.0 Hz, 9-OH); ^{19}F NMR δ −112.0 (C-19).

20-Fluorosynerazol (**7**)

$[\alpha]_D^{24}$ −11 (*c* 1, $CHCl_3$); HRFAB-MS *m/z* 432.1454 $[M+H]^+$ (calcd *m/z* 432.1459 for $C_{22}H_{23}NO_7F$); ^{13}C NMR δ 182.2, 114.3, 196.7, 91.6, 165.2, 89.6, 73.8, 52.6, 55.1, 123.5, 141.5, 21.3, 14.0, 5.1, 193.4 (d, 2.5 Hz), 134.1 (d, 7.4 Hz), 117.3 (d, 23.9 Hz), 162.6 (d, 273.1 Hz, C-20), 121.7 (d, 21.4 Hz), 130.4 (d, 7.4 Hz), 126.3 (d, 3.3 Hz), 51.7; ^{1}H NMR δ 4.63 (d, 12.4 Hz), 3.77 (d, 2.0 Hz), 4.10 (dd, 2.0 and 8.8 Hz), 5.07 (ddt, 8.8, 11.0 and 1.7 Hz), 5.86 (dt, 11.0 and 7.3 Hz), 2.23 (dddq, 1.7, 7.3, 14.6 and 7.6 Hz), 2.30 (dddq, 1.7, 7.3, 14.6 and 7.6 Hz), 1.04 (t, 7.6 Hz), 1.83 (s), 8.00 (ddd, 1.5, 2.7 and 9.8 Hz, H-19), 7.35 (dddd, 1.1, 2.7, 8.0 and 8.3 Hz, H-21), 7.48 (ddd, 6.6, 8.0 and 8.0 Hz, H-22), 8.10 (ddd, 1.1, 1.5 and 8.0 Hz, H-23), 7.39 (s, 7-NH), 3.38 (s, 8-OMe), 4.06 (d, 12.4 Hz, 9-OH); ^{19}F NMR δ −110.9 (C-20).

7-Fluorogliotoxin (**8**)

$[\alpha]_D^{25}$ −116 (*c* 1, $CHCl_3$); HRFAB-MS *m/z* 345.0412 $[M+H]^+$ (calcd *m/z* 345.0379 for $C_{13}H_{14}N_2O_4FS_2$); ^{13}C NMR (DMSO-d_6) δ 165.4 (C-1), 78.2 (C-3), 163.7 (C-4), 70.3 (C-5a), 70.2 (d, 15.6 Hz, C-6), 158.9 (d, 273.9 Hz, C-7), 102.1 (d, 18.9 Hz, C-8), 116.9 (d, 8.2 Hz, C-9), 128.6 (d, 4.1 Hz, C-9a), 35.4 (C-10), 76.1 (C-10a), 27.6 (C-11), 58.7 (C-12); ^{1}H NMR δ 5.09 (d, 12.4 Hz, H-5a), 4.81 (dm, 12.4 Hz, H-6), 5.69 (ddd, 1.5, 5.8 and 12.1 Hz, H-8), 5.94 (m, H-9), 3.07 (d, 17.6 Hz, H-10), 3.59 (dm, 17.6 Hz, H-10), 3.09 (s, H-11), 4.22 (dd, 5.6 and 12.7 Hz, H-12), 4.32 (dd, 5.6 and 12.7 Hz, H-12), 5.83 (d, 1.5 Hz, 6-OH), 5.97 (t, 5.6 Hz, 12-OH); ^{19}F NMR δ −116.0 (C-7).

8-Fluorogliotoxin (**9**)

$[\alpha]_D^{25}$ −232 (*c* 0.5, $CHCl_3$); HRFAB-MS *m/z* 345.0433 $[M+H]^+$ (calcd *m/z* 345.0379 for $C_{13}H_{14}N_2O_4FS_2$); ^{13}C NMR (DMSO-d_6) δ 165.7, 72.7, 165.0, 69.9, 72.5 (d, 9.0 Hz), 104.2 (d, 17.2 Hz, C-7), 155.5 (d, 253.4 Hz, C-8), 117.2 (d, 38.3 Hz), 135.3 (d, 9.1 Hz), 33.2, 75.6 , 27.6, 60.3; ^{1}H NMR δ 4.87 (d, 12.7 Hz), 4.98 (dd, 4.9 and 12.7 Hz, H-6), 5.25 (d, 11.7 Hz, H-7), 5.97 (d, 1.7 Hz, H-9), 2.98 (d, 18.0 Hz), 3.78 (d, 18.0 Hz), 3.21 (s), 4.23 (dd, 9.5 and 13.0 Hz), 4.43 (dd, 5.9 and 13.0 Hz), 5.85 (m, 6-OH), 4.00 (dd, 5.9 and 9.5 Hz, 12-OH); ^{19}F NMR δ −115.8 (C-8).

Acknowledgements

We thank to Screening Committee of New Anticancer Agents supported by Grant-in-Aid for Scientific Research on Priority Area "Cancer" from The Ministry of Education, Culture, Sports, Science and Technology, Japan for the evaluation of antitumor activity of fluorinated synerazols. We also thank to Dr. Y. SATO at Institute of Development, Aging and Cancer, Tohoku University, Japan for evaluating the anti-angiogenic activity of fluorosynerazols.

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