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New Triene-ansamycins, Thiazinotrienomycins F and G and a Diene-ansamycin, Benzoxazomycin

NOBUO HOSOKAWA, HIROSHI NAGANAWA, MASA HAMADA, HIRONOBU IINUMA and TOMIO TAKEUCHI

Institute of Microbial Chemistry,
3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141-0021, Japan

KAYOKO S. TSUCHIYA and MAKOTO HORI

Showa College of Pharmaceutical Sciences,
Machida City, Tokyo 194-8543, Japan

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New triene-ansamycins designated thiazinotrienomycins F (TT-F) and G (TT-G) and a new diene-ansamycin, benzoxazomycin, were isolated from a culture broth of *Streptomyces* sp. MJ672-m3 and their structures were elucidated by spectroscopic analyses. The Mean Graphs of TT-G suggests that the tumor growth inhibitory activities are almost as strong as TT-B, in respect of GI_{50} and TGI against several human cancer cell lines.

In the course of screening for new microbial secondary metabolites with antitumor activities, thiazinotrienomycins, new members of the ansamycin family of antibiotics produced by a *Streptomyces* sp. MJ672-m3, were isolated. They were identified by their cell-growth inhibitory activities *in vitro*, and showed some cell line specificities among the human cancer cell lines tested[1]. We recently isolated three new ansamycin antibiotics from a fermentation broth of the same strain. We report here production, isolation, physico-chemical properties, structures and some biological properties of these new antibiotics.

Results and Discussion

Isolation of the Antibiotics

Fermentation was carried out using the same method as reported in the previous paper[1]. A 10-liter culture broth was filtered to separate the mycelial cake. The mycelial cake was stirred successively with 3 liters of MeOH and 3 liters of 66% aqueous acetone for one hour each, and filtered. The filtrates were combined and concentrated under reduced pressure to remove the organic solvents resulting in an aqueous solution. The condensed solution was mixed with the broth filtrate and antibiotics were extracted with an equal volume of EtOAc (10 liters). The EtOAc extract was concentrated under reduced pressure to give an oily residue (6.0 g), which was purified using chromatography on a silica gel column (150 g, 5×20 cm) eluted by a linear gradient from $CHCl_3$ to $CHCl_3$-MeOH (50 : 1, v/v, 1 liter). Active fractions were combined and purified using a Sephadex LH-20 column (1.6×100 cm) developed with MeOH. Active fractions from the Sephadex column were collected and the solvent was evaporated under reduced pressure to give a sticky oil (wet weight 120 mg) which contained thiazinotrienomycin B (TT-B) as a major component (35 mg). We isolated three new active substances from the same sticky oil by preparative HPLC using a Capcell Pak (C_{18} column, 5 μm-100 Å, 19×150 mm, Shiseido Co. Ltd.) developed with 75% MeOH at a flow rate of 9.9 ml/minute at a room temperature, by monitoring the absorption at 250 nm. The three substances were named thiazinotrienomycin F (**1**) and G (**2**) and a diene-ansamycin, benzoxazomycin (**3**). The antibiotics **1**, **2** and **3** were obtained at amounts of 2 mg, 5 mg and 3 mg, respectively. Purity was confirmed by HPLC and TLC.

Table 1. Physico-chemical properties of **1**, **2** and **3**.

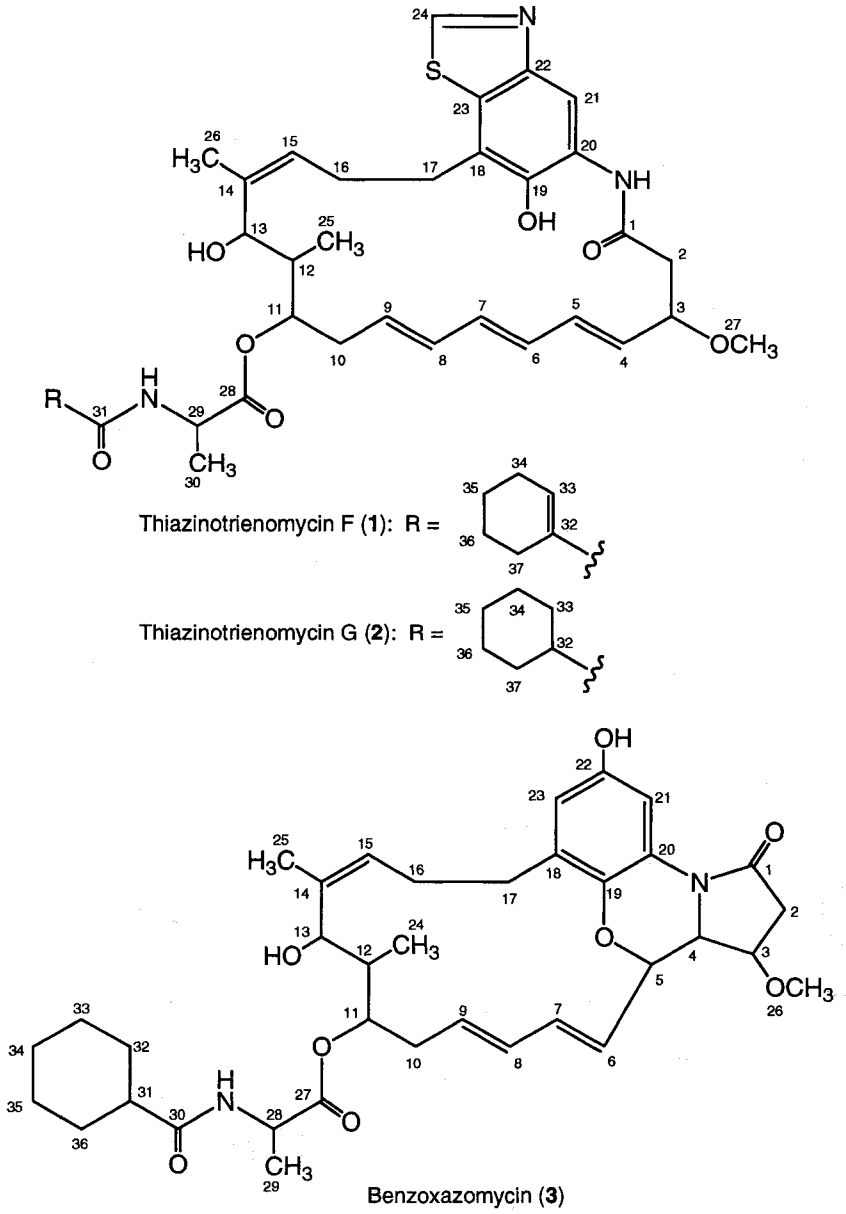
	1	**2**	**3**
Appearance	White powder	White powder	White powder
Molecular formula	$C_{37}H_{47}N_3O_7S$	$C_{37}H_{49}N_3O_7S$	$C_{36}H_{48}N_2O_8$
FAB-MS (*m/z*, Pos.)	678 $(M+H)^+$	680 $(M+H)^+$	636 (M^+)
FAB-MS (*m/z*, Neg.)	676 $(M-H)^-$	678 $(M-H)^-$	635 $(M-H)^-$
HRFAB-MS (Pos., *m/z*)			
Calcd.		680.3369 (as $C_{37}H_{50}N_3O_7S$)	636.3411 (as $C_{36}H_{48}N_2O_8$)
Found.		680.3387 $(M+H)^+$	636.3429 (M^+)
$[\alpha]_D^{25}$	+179.3° (*c* 0.10, MeOH)	+245.8° (*c* 0.29, MeOH)	-165.7° (*c* 0.31, MeOH)
UV λ_{max}^{MeOH} nm (log ε)	210 (4.09), 248 (4.80), 258 (4.77), 270 (4.77), 282 (4.65), 310 (3.36)	210 (4.31), 249 (4.79), 258 (4.75), 270 (4.75), 282 (4.61), 315 (3.71)	215 (4.23), 240 (sh, 4.15), 308 (3.16)
IR $_{max}\nu$ cm^{-1} (KBr)	3350, 2920, 2850, 1730, 1660, 1550, 1450, 1300, 1205, 1160, 1100, 1000	3400, 2950, 2850, 1740, 1660, 1580, 1540, 1460, 1390, 1210, 1100, 1000	3400, 2940, 2850, 1690, 1480, 1270, 1210, 1100, 1000
Rf value on TLC[a]			
1) $CHCl_3$-MeOH=10:1	0.57	0.59	0.56
2) Toluene-acetone=3:2	0.43	0.45	0.40
HPLC retention time (min)[b]	9.0	10	9.6
Solubility Soluble:	DMSO, $CHCl_3$, MeOH, Me_2CO	DMSO, $CHCl_3$, MeOH, Me_2CO	DMSO, MeOH, $CHCl_3$, Me_2CO
Insoluble:	H_2O, Hexane	H_2O, Hexane	H_2O, Hexane

a) Silica gel Art. 5715 (Merck)
b) Senshu-pak 5 μ C_{18}-100 Å (3.9 mm x 15 cm), mobile phase: 65% MeOH (pH 5.0), flow rate: 2.0 ml/min, detection: 250 nm, temperature: 40°C

Structures of **1** and **2**

The physico-chemical properties of **1** and **2** are shown in Table 1. The structural study was started with **2**, a major congener. The molecular formula of **2** was determined to be $C_{37}H_{49}N_3O_7S$ (MW 679) from the high resolution FAB-MS measurement of the molecular ion peak at *m/z* 680.3387 [calcd. for $C_{37}H_{50}N_3O_7S$, *m/z* 680.3369, $(M+H)^+$]. The UV absorption maxima in MeOH at 258 nm (log ε 4.75), 270 nm (log ε 4.75) and 282 nm (log ε 4.61) indicated the presence of a triene structure. The IR spectrum showed a strong absorption band at 1000 cm^{-1} which was attributed to the triene structure. The UV absorption profile exhibited a bathochromic shift from 315 nm (log ε 3.71) in MeOH to 325 nm (log ε 3.40) in an alkaline MeOH solution suggested the presence of a phenol group. Analysis of ^1H-^1H COSY spectrum of **2** indicated four partial structures that were composed of the carbon numbers from 2 to 13, from 15 to 17, 29, 30 and from 32 to 37. The ^1H-^1H COSY spectrum also showed that 13-OH (δ_H 5.63) was free because of its coupling to 13-H (δ_H 5.23). Connectivities of the four partial structures were revealed in the HMBC spectrum as shown in Fig. 2. The HMBC spectrum showed connectivities between 20-NH (δ_H 11.2) and carbons C-1 (δ_C 170.8), C-19 (δ_C 146.7) and C-21 (δ_C 114.8). The ^1H and ^{13}C NMR spectra of **2** were closely related to those of thiazinotrienomycin E[1] that has a triene structure. The ansa ring moiety from C-1 to C-17 and the *N*-(cyclohexylcarbonyl) alanine moiety from C-28 to C-37 in **2** were the same as those of TT-E. These results suggested that **2** was structurally different from TT-E only in the phenolic chromophore. The phenolic chromophore was deduced to have a composition of C_7H_3NOS by considering the molecular formula. The HMBC spectrum of **2** indicated that 17-H at δ_H 3.22 was coupled to C-19 (δ_C 146.7) and C-23 (δ_C 133.6). An aromatic proton (21-H) at δ_H 8.62 showed coupling to C-19, C-20 and C-23. An aromatic proton (24-H) at δ_H 9.18 was coupled to C-23 through a sulfide bond and to C-22 (δ_C 147.3) *via* a nitrogen atom. Thus, the chromophore was found to be a derivative of benzothiazole. The geometry of the triene was all *E* in view of the coupling constants of $J_{4,5}$, $J_{6,7}$ and $J_{8,9}$, which were

Fig. 1. Structures of thiazinotrienomycins F (**1**), G (**2**) and benzoxazomycin (**3**).



15.3 Hz, 15.0 Hz and 15.0 Hz, respectively. NOE's were observed between methyl group at C-26 (δ_H 1.99) and an olefinic proton 15-H (δ_H 5.33), which indicated 14 *Z*.

The molecular formula of **1** was established as $C_{37}H_{47}N_3O_7S$ (MW 677) by FAB-MS and NMR data. From the analyses of NMR spectra, the structural difference between **1** and **2** was revealed that the cyclohexane moiety in **2** was replaced by a cyclohexene in **1** as shown in Fig. 1. The stereochemistries of **1** and **2** at the positions of C-3, 11, 12, 13 and 29 are not defined. But the ^1H and ^{13}C NMR data of **1** and **2** for coupling constants and chemical shifts of above positions were essentially the same as those of TT-E[1,2] as shown in Table 2. Consequently, the configurations in **1** and **2** are assumed to be the same as those of TT-E. It should also be added that the first total synthesis of (+)-TT-E has been achieved[3,4].

Structure of **3**

The physico-chemical properties of **3** are shown in Table 1. The molecular formula of **3** was determined to be $C_{36}H_{48}N_2O_8$ (MW 636) by FAB-MS and NMR data. High

Table 2. ^{13}C and ^{1}H NMR data of **1**, **2**, **3** and TT-E in pyridine-d_5.

Carbon No.	1		2	
	δ_C ppm (125 MHz)	δ_H ppm (*J* in Hz, 500 MHz)	δ_C ppm (125 Hz)	δ_H ppm (*J* in Hz, 500 MHz)
1	170.8 (s)	—	170.8 (s)	—
2	43.7 (t)	3.04 (1H, dd, 10.7, 12.5) 3.24 (1H, dd, 4.5, 12.5)	43.7 (t)	3.03 (1H, dd, 10.7, 12.5) 3.23 (1H, dd, 4.6, 12.5)
3	80.8 (d)	4.50 (1H, ddd, 4.5, 10.7, 8.5)	80.7 (d)	4.48 (1H, ddd, 4.6, 8.5, 10.7)
4	131.9 (d)	5.81 (1H, dd, 8.5, 15.6)	131.7 (d)	5.80 (1H, dd, 8.5, 15.3)
5	135.3 (d)	6.60 (1H, dd, 9.5, 15.6)	135.5 (d)	6.59 (1H, dd, 10.1, 15.3)
6	130.1 (d)	6.34 (1H, m)	130.1 (d)	6.32 (1H, dd, 10.1, 15.0)
7	134.7 (d)	6.34 (1H, m)	134.7 (d)	6.38 (1H, dd, 10.1, 15.0)
8	133.8 (d)	6.20 (1H, 9.5, 15.5)	133.8 (d)	6.20 (1H, dd, 10.1, 15.0)
9	130.6 (d)	5.87 (1H, ddd, 5.1, 10.1, 15.5)	130.6 (d)	5.94 (1H, ddd, 5.2, 10.1, 15.0)
10	33.4 (t)	2.35 (1H, ddd, 2.5, 10.0, 14.0) 2.83 (1H, ddd, 5.0, 5.0, 14.0)	33.5 (t)	2.37 (1H, ddd, 2.0, 10.1, 14.0) 2.81 (1H, ddd, 5.0, 5.2, 14.0)
11	75.4 (d)	5.32 (1H, m)	75.4 (d)	5.33 (1H, ddd, 2.0, 5.0, 9.5)
12	39.1 (d)	2.25 (1H, q, d, d, 6.7, 3.4, 9.5)	39.2 (d)	2.26 (1H, d, d, q, 3.5, 9.5, 6.7)
13	68.1 (d)	5.17 (1H, br), 5.49 (13-OH, br)	68.2 (d)	5.23 (1H, brs), 5.63 (13-OH, br)
14	141.0 (s)	—	141.0 (s)	—
15	123.8 (d)	5.32 (1H, m)	123.8 (d)	5.33 (1H, m)
16	26.9 (t)	2.75, 2.80 (2H, m)	26.9 (t)	2.75, 2.80 (2H, m)
17	33.1 (t)	2.95, 3.19 (2H, m)	33.1 (t)	2.90, 3.22 (2H, m)
18	133.6 (s)	—	133.6 (s)	—
19	146.6 (s)	— 10.3 (19-OH, br)	146.7 (s)	— 10.2 (19-OH, br)
20	128.5 (s)	— 11.08 (20-NH, s)	128.5 (s)	— 11.2 (20-NH, s)
21	114.8 (s)	8.66 (1H, s)	114.8 (s)	8.62 (1H, s)
22	147.3 (s)	—	147.3 (s)	—
23	133.5 (s)	—	133.6 (s)	—
24	152.6 (d)	9.18 (1H, s)	152.6 (d)	9.18 (1H, s)
25	10.2 (q)	0.92 (3H, d, 6.7)	10.2 (q)	0.95 (3H, d, 6.7)
26	21.3 (q)	1.98 (3H, brs)	21.2 (q)	1.99 (3H, brs)
27	56.3 (q)	3.30 (3H, s)	56.2 (q)	3.29 (3H, s)
28	173.0 (s)	—	173.2 (s)	—
29	49.9 (d)	4.75 (1H, d, q, 7.0, 7.0) 8.92 (29-NH, d, 7.0)	49.6 (d)	4.77 (1H, d, q, 6.4, 7.3) 9.02 (29-NH, d, 6.4)
30	17.2 (q)	1.60 (3H, d, 7.0)	17.3 (q)	1.54 (3H, d, 7.3)
31	169.8 (s)	—	176.9 (s)	—
32	131.5 (s)	—	44.9 (d)	2.43 (1H, dddd, 3.5, 3.5, 11.0, 12.0)
33	134.0 (d)	6.86 (1H, m)	30.1 (t)	1.72, 2.03 (2H, m)
34	25.6 (t)	2.02 (2H, m)	26.0 (t)	1.72, 1.16 (2H, m)
35	22.0 (t)	1.48 (2H, m)	26.2 (t)	1.72 (2H, m)
36	22.5 (t)	1.55 (2H, m)	26.0 (t)	1.72, 1.16 (2H, m)
37	24.7 (t)	2.43 (2H, m)	30.0 (t)	1.72, 1.96 (2H, m)

resolution measurement of the molecular ion peak in FAB-MS gave *m/z* 636.3429 [calcd. for $C_{36}H_{48}N_2O_8$, *m/z* 636.3411]. The UV absorption maximum at 240 nm (log ε 4.15) in MeOH indicated the presence of a diene structure in the molecule. A bathochromic shift from 308 nm (log ε 3.16) to 320 nm (log ε 3.15) in an alkaline MeOH solution suggested the presence of a phenol group.

Analysis of ^{1}H-^{1}H COSY spectrum indicated four partial structures that were composed of the carbon number from 2 to 13, from 15 to 17, 28, 29 and from 31 to 36. A doublet signal of a hydroxy group (13-OH, δ_H 5.77) was coupled to 13-H (δ_H 4.76). Connectivities of the four partial structures were determined by the HMBC spectrum as shown in Fig. 2. The HMBC spectrum of **3** indicated that the 17-H at δ_H 3.05 showed coupling to C-19 (δ_C 138.1) and a signal of 21-H (δ_H 8.49) was coupled to C-19, C-20 (δ_C 126.9) and C-22 (δ_C 152.3). A hydroxy group attached to C-22 of the phenolic chromophore was observed at δ_H 11.2 in the ^{1}H NMR spectrum. The ^{1}H and ^{13}C NMR data of spin systems from C-1 to C-17 and from C-28 to C-36 were very similar to those of **2**. But comparison of NMR data for **3** with **2** showed that **3** was structurally different from **2** in the phenolic chromophore moiety and the triene part. The phenolic chromophore of **3** was deduced to be $C_6H_3O_2$ by

Table 2. (Continued)

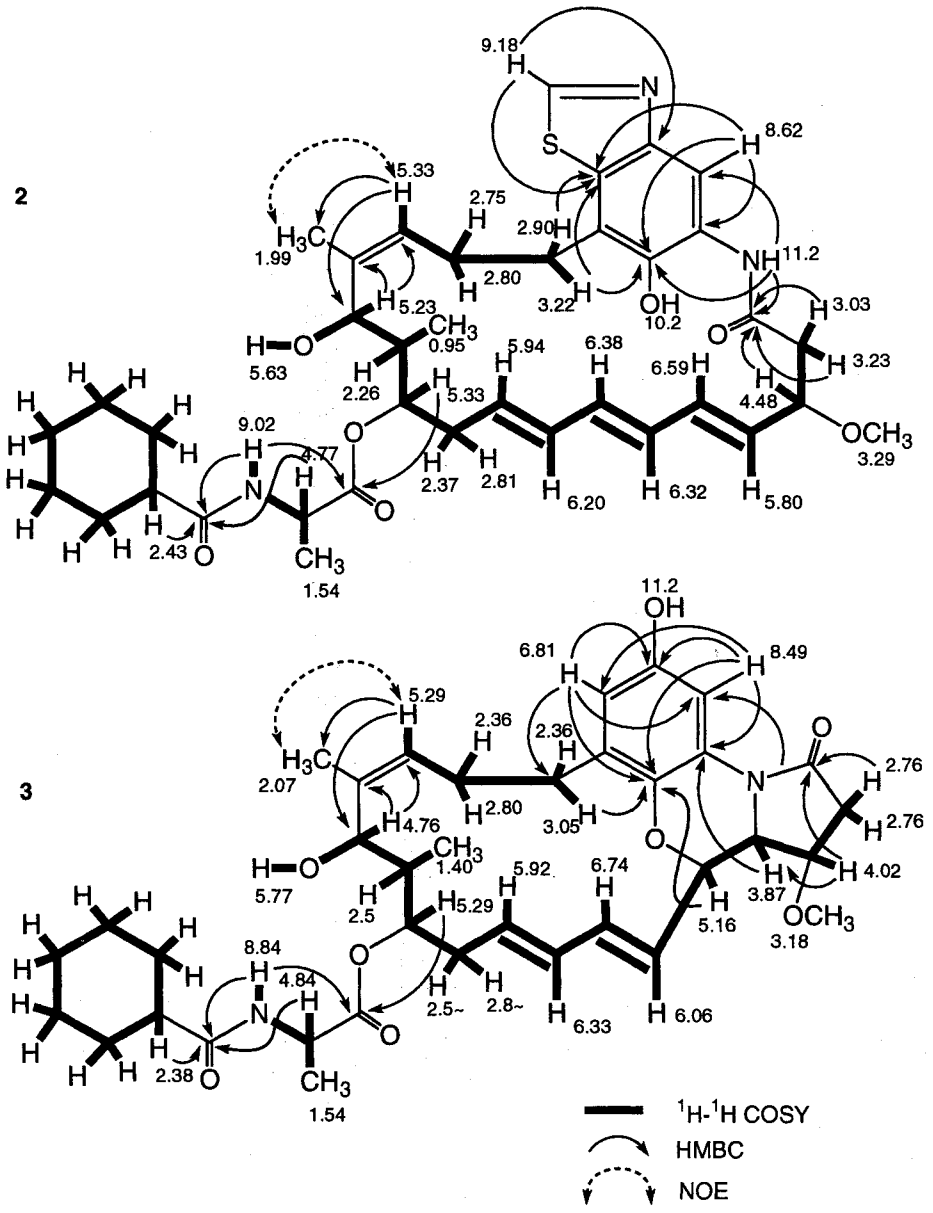
Carbon No.	3		TT-E	
	δ_C ppm (125 MHz)	δ_H ppm (*J* in Hz, 500 MHz)	δ_C ppm (125 Hz)	δ_H ppm (*J* in Hz, 500 MHz)
1	171.1 (s)	—	170.5 (s)	—
2	38.2 (t)	2.76 (2H, d, 3.3)	43.0 (t)	2.95 (1H, dd, 10.7, 12.2) 3.16 (1H, dd, 4.3, 12.2)
3	74.3 (d)	4.02 (1H, d, t, 5.0, 3.3)	80.8 (d)	4.45 (1H, ddd, 4.3, 9.0, 10.9)
4	62.5 (d)	3.87 (1H, dd, 5.0, 8.2)	131.7 (d)	5.75 (1H, dd, 8.9, 15.6)
5	74.0 (d)	5.16 (1H, dd, 6.0, 8.2)	135.5 (d)	6.56 (1H, dd, 10.1, 15.6)
6	126.9 (d)	6.06 (1H, d, d, 6.0, 15.4)	129.9 (d)	6.32 (1H, dd, 10.0, 15.0)
7	138.1 (d)	6.74 (1H, dd, 10.4, 15.4)	134.8 (d)	6.37 (1H, dd, 10.0, 15.0)
8	132.1 (d)	6.33 (1H, dd, 10.4, 15.4)	133.7 (d)	6.20 (1H, dd, 10.1, 15.3)
9	132.9 (d)	5.92 (1H, t, d, 7.6, 15.4)	130.8 (d)	5.95 (1H, ddd, 5.0, 10.5, 15.2)
10	33.8 (t)	2.50 (1H, m), 2.80 (1H, m)	33.5 (t)	2.36 (1H, ddd, 2.0, 10.5, 14.0) 2.84 (1H, ddd, 4.6, 5.4, 14.0)
11	75.2 (d)	5.29 (1H, m)	75.4 (d)	5.34 (1H, ddd, 2.0, 5.4, 9.5)
12	41.5 (d)	2.50 (1H, m)	38.9 (d)	2.27 (1H, ddd, 3.4, 6.7, 9.5)
13	69.3 (d)	4.76 (1H, brd, 9.5), 5.77 (13-OH, br)	68.3 (d)	5.23 (1H, brs), 5.56 (13-OH, br, 4.6)
14	138.2 (s)	—	140.6 (s)	—
15	129.5 (d)	5.29 (1H, m)	124.0 (d)	5.41 (1H, m)
16	28.1 (t)	2.36 (1H, m) 2.80 (1H, m)	27.2 (t)	2.64, 2.75 (2H, m)
17	33.2 (t)	2.36 (1H, m) 3.05 (1H, m)	29.8 (t)	3.06, 3.17 (2H, m)
18	131.4 (s)	—	129.6 (s)	—
19	138.1 (s)	—	144.0 (s)	— 9.74 (19-OH, br)
20	126.9 (s)	—	126.8 (s)	— 10.83 (20-NH, s)
21	105.4 (d)	8.49 (1H, d, 2.5)	109.5 (d)	7.63 (1H, s)
22	152.3 (s)	— 11.2 (22-OH, br)	131.5 (s)	— 11.80 (22-NH, s)
23	114.0 (d)	6.81 (1H, d, 2.5)	117.9 (s)	—
24	11.5 (q)	1.40 (3H, d, 6.7)	30.7 (t)	3.54 (1H, d, 14.6) 3.60 (1H, d, 14.6)
25	19.8 (q)	2.07 (3H, brs)	165.9 (s)	—
26	56.7 (q)	3.18 (3H, s)	10.2 (q)	0.94 (3H, d, 6.7)
27	173.1 (s)	—	21.4 (q)	2.03 (3H, s)
28	49.2 (d)	4.84 (1H, q, d, 7.0, 7.0), 8.84 (28-NH, d, 7.0)	56.2 (q)	3.27 (3H, s)
29	17.5 (q)	1.54 (3H, d, 7.0)	173.2 (s)	—
30	176.5 (s)	—	49.6 (d)	4.78 (1H, d, q, 6.0, 7.3) 9.09 (30-NH, d, 6.1)
31	45.0 (d)	2.38 (1H, m)	17.3 (q)	1.58 (3H, d, 7.3)
32	29.9 (t)	1.28, 2.00 (2H, m)	177.0 (s)	—
33	26.1 (t)	1.09, 1.50 (2H, m)	44.9 (d)	2.44 (1H, m)
34	26.0 (t)	1.65, 1.70 (2H, m)	29.2 (t)	1.95, 1.73 (2H, m)
35	26.0 (t)	1.65, 1.70 (2H, m)	26.1 (t)	1.17 (2H, m)
36	30.1 (t)	1.92, 2.00 (2H, m)	26.2 (t)	1.17, 1.55 (2H, m)
37			26.0 (t)	1.70, 2.05 (2H, m)
38			29.9 (t)	1.17 (2H, m)

considering the molecular formula. The analysis of the HMBC spectrum clearly indicated that C-19 was connected to C-5 (δ_C 74.0) through an ether bond and C-20 linked to C-4 (δ_C 62.5) *via* an amide nitrogen. Therefore, the chromophore moiety was deduced to be a derivative of benzoxazole which was fused by a pyrrolidinone ring as shown in Fig. 2. The geometry of the diene was all *E* in view of the large coupling constants of 15.4 Hz for both $J_{6,7}$ and $J_{8,9}$. NOE's were observed between 15-H (δ_H 5.29) and 25-H3 (δ_H 2.07) indicating 14 *Z*. Other configurations in **3** are not defined.

Biological Activity

The Mean Graph pattern using COMPARE analysis[5] (Fig. 3) suggested that the tumor growth-inhibitory

Fig. 2. Structures of **2** and **3** elucidated by ^{1}H-^{1}H COSY, NOE and HMBC experiments.



activities were almost as active as TT-B[6] against several cell lines, such as NCI-H522 and NCI-H460 (lung), OVCAR-5 (ovarian), St-4 (stomach) and BSY-1 (breast). Compound **2** was as strong as TT-B in respect of GI_{50} and TGI against several cancer cell lines (Table 3). Compound **1** was as active as **2** in the growth inhibitory activity against, for example, KB, HeLa-S3, and SC-6 (data not shown). Compound **3** was some hundred times weaker than **1** and **2** in the growth inhibitory activity against, for example, KB, HeLa-S3 and SC-6. Compound **3** was as active as **1** and **2** only against HL-60 (data not shown).

Various microorganisms were insensitive to all these compounds at concentrations as high as 1,000 μg/ml except that **3** at 1,000 μg/ml showed an inhibitory zone (15 mm in diameter) against *Pyricularia oryzae* on the agar plate.

Discussion

As our original data indicated that TT-B was the strongest congener inhibiting growth *in vitro* of some human cancer cell lines and therefore TT-B was chosen for further studies of the mechanism of action[7~9] and the

Table 3. Cell lines that are more sensitive to TT-G (**2**) than to TT-B.

		TT-G		TT-B	
		Log TGI	Log LC_{50}	Log TGI	Log LC_{50}
Lu (lung)	NCI-H23	-7.13	-5.05	-6.523	-5.141
	NCI-H226	-7.05	> -5.00	-6.345	-5.280
	NCI-H522	-7.22	> -5.00	-6.623	> -4.000
	NCI-H460	-6.82	> -5.00	-5.980	> -4.000
	DMS273	-7.32	-5.25	-6.835	-6.109
Ov (ovary)	OVCAR-4	-6.99	> -5.00	-6.639	-4.433
	OVCAR-5	-7.20	> -5.00	-6.086	> -4.000
St (stomach)	St-4	-7.18	> -5.00	-6.134	-4.778
	MKN-1	-6.87	> -5.00	-6.393	> -4.000
	MKN-7	-6.95	> -5.00	-6.401	-4.490
	MKN-74	-7.16	> -5.00	-6.978	> -4.000
Br (breast)	BSY-1	-7.21	-5.51	-6.699	-5.228
CNS (central nervous system)	SF539	-7.11	> -5.00	-6.607	-5.413

inhibitory effect on human tumor cell xenograft in nude mice[6]. However, the Mean Graph of **2** (Fig. 3) suggests that the newly found congener, compound **2**, was as strong as TT-B[6] in respect of GI_{50} and TGI against BSY-1 (breast), NCI-H522 (lung), OVCAR-5 (ovarian), MKN-1 and -74 (stomach), as summarized in Table 3. Since BSY-1 xenograft was completely suppressed by TT-B at the dosage of 50 mg/kg, better results will be expected with **2**. *In vivo* experiments will be undertaken with **1** and **2**.

Experimental

Human Cancer Cell Lines and Culture Conditions

Measurements of cell growth inhibition against a panel of 39 human cancer cell lines and the data analysis of **2** (Fig. 3) were determined as previously reported[5].

General

UV spectra were recorded on a Hitachi U-320 spectrophotometer and IR spectra on a Hitachi 285-spectrophotometer. NMR spectra were recorded on a JEOL JNM-A500 NMR spectrometer at 500 MHz for ^1H NMR and at 125 MHz for ^{13}C NMR. Optical rotations were measured using a Perkin-Elmer 241 polarimeter. MS spectra were measured on a JEOL JMS-SX102 spectrometer.

Analytical Procedure

An HPLC system using a Senshupak (C_{18} column, 5 μm-100 Å, 3.9 mm×150 mm, SSC Co. Ltd.) was developed with 65% MeOH (pH 5.0) at flow rate of 2.0 ml/minute at 40°C and the absorption was monitored at 250 nm. Silica gel TLC (Kieselgel $60F_{254}$ Art. 5715, Merck) was developed with $CHCl_3$-MeOH (10 : 1), Toluene-acetone (3 : 2) or Toluene-$CHCl_3$-MeOH (3 : 7 : 3) and spots on TLC were detected using phosphomolybdate-H_2SO_4.

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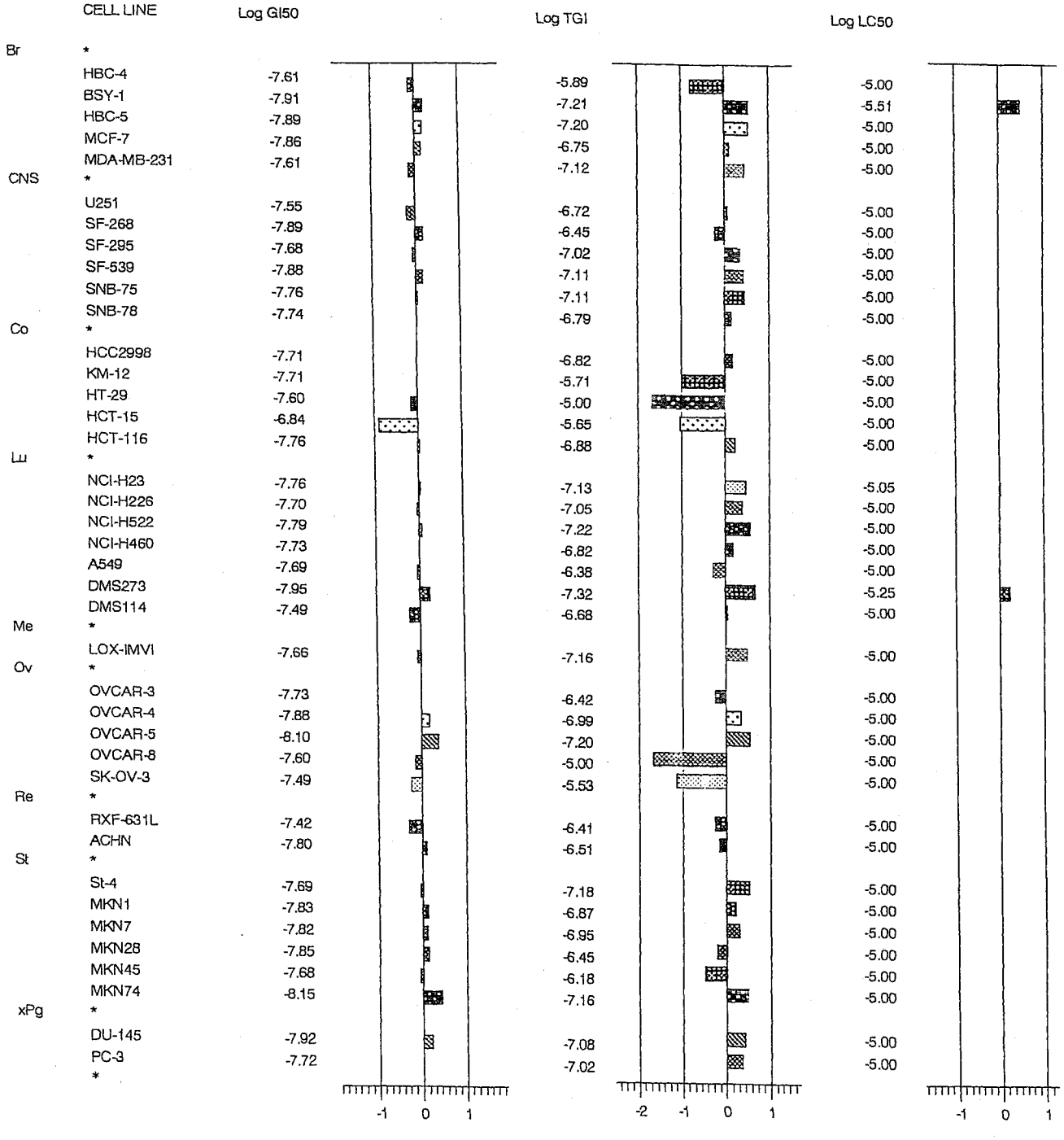
Fig. 3. Growth inhibition by TT-G (**2**) against a panel of 39 human cancer cell lines.



A human cell line panel combined with data base analysis was established by T. YAMORI *et al.*. Original organs of the tumor cell lines are abbreviated as Br (breast), CNS (central nervous system), Co (colon), Lu (lung), Me (melanoma), Ov (ovary), Re (kidney), St (stomach) and xPg (prostate). Growth inhibition (GI_{50}, 50% inhibition; TGI, 100% inhibition; and LC_{50}, 50% killing, *i.e.*, 150% inhibition) was determined and the results were analyzed and graphically displayed as reported[5].

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