New Bisindole Alkaloids Isolated from *Myxomycetes Arcyria cinerea* and *Lycogala epidendrum*

Kazuaki KAMATA,^a Makiko KIYOTA,^a Ayano NAOE,^a Satomi NAKATANI,^a Yukinori YAMAMOTO,^b Masahiko HAYASHI,^c Kanki KOMIYAMA,^c Takao YAMORI,^d and Masami ISHIBASHI*,^a

^a Graduate School of Pharmaceutical Sciences, Chiba University; 1–33 Yayoi-cho, Inage-ku, Chiba 263–8522, Japan:

^b 1010–53 Ohtsu-ko, Kochi 781–5102, Japan: ^c The Kitasato Institute; 5–9–1 Shirokane, Minato-ku, Tokyo 108–8642,

Japan: and ^d Cancer Chemotherapy Center, Japanese Foundation for Cancer Research; 3–10–6 Ariake, Koutou-ku, Tokyo

135–8550, Japan. Received February 10, 2005; accepted February 28, 2005; published online March 15, 2005

Two new bisindole alkaloids, named cinereapyrrole A (**1**) and B (**2**), were isolated from wild fruit bodies of *Arcyria cinerea* and three new bisindole alkaloids (**3**–**5**) were isolated from wild fruit bodies of *Lycogala epidendrum*. Seven known bisindoles (**6**–**12**) were concomitantly obtained from them. The structures of the new compounds were elucidated by spectral data. Among these bisindole alkaloids, compound **12** showed cytotoxicity against cultured tumor cell lines.

Key words myxomycetes; *Arcyria cinerea*; *Lycogala epidendrum*; bisindole; cytotoxicity

The myxomycetes (true slime molds) are an unusual group of primitive organisms that may be assigned to one of the lowest classes of eukaryotes.¹⁾ During our studies on search for bioactive natural products from myxomycetes,^{2–4)} we recently investigated a field-collected sample of fruit bodies of *Arcyria cinerea* and *Lycogala epidendrum* collected at Kochi prefecture. Here we describe isolation and structure elucidation of new bisindole alkaloids (**1**–**5**) along with seven known bisindoles (**6**–**12**), and their cytotoxic activity. Particularly, compound **12** was found to be cytotoxic against a panel of 39 human cancer cell lines.

Results and Discussion

The wild fruit bodies of *Arcyria cinerea*, collected in Kochi Prefecture, Japan, were extracted with 90% MeOH and 90% acetone. The combined extracts were separated by chromatographies on silica gel, ODS, and Sephadex LH-20 to give two new bisindole alkaloids, named cinereapyrrole A (**1**) and B (**2**), together with a known compound, arcyrarubin A (**6**), which was previously isolated from *Arcyria denudata*,⁵⁾ and *Lycogala epedindrum*.⁶⁾

Cinereapyrrole A (**1**) was shown to have the molecular formula C₂₂H₁₇N₃O₄ by the HR-FAB-MS data (*m/z* 387.1208, [M⁺], Δ –1.1 mmu). The UV spectrum of **1** showed absorption maximum at 283 nm, indicating the presence of conjugated system(s), while the IR absorption bands observed at 3396 and 1695 cm^{–1} implied the presence of hydroxy and conjugated carbonyl groups. The ¹H-NMR spectrum of **1** in CD₃OD (Table 1) showed ten signals for nine aromatic protons and one methoxy group. The analysis of the ¹³C-NMR (Table 2) and HMQC spectra of **1** clarified the presence of twenty two carbons including nine *sp*² methines, eleven *sp*² quaternary carbons, one methoxy (δ_C 51.3), and one carboxy or carboxy ester carbon (δ_C 163.6). By analysis of the ¹H–¹H COSY and HMBC spectra of compound **1** suggested the presence of two sets of 5-hydroxyindole moieties by observation of the following cross peaks [¹H–¹H COSY: H-6/H-7 and H-6'/H-7'; HMBC: H-2/C-3, H-2/C-3a, H-2/C-7a, H-4/C-3, H-4/C-5, H-4/C-7a, H-6/C-7a, H-7/C-3a, H-7/C-5, H-2'/C-3', H-2'/C-3a', H-2'/C-7a', H-

4'/C-7a', H-6'/C-7a', H-7'/C-3a', and H-7'/C-5']. The ¹³C-NMR chemical shifts of C-5 (δ_C 151.0) and C-5' (δ_C 151.4) suggested these carbons bore hydroxy groups. These NMR data were reminiscent of those of lycogarubin A (**13**), previously isolated from *Lycogala epidendrum*.⁷⁾ Lycogarubin A (**13**) was an symmetrical compound with one set of NMR signals, while NMR data of **1** implied that compound **1** was an unsymmetrical molecule. Another difference was the observation of an additional *sp*² proton observed at δ_H 7.29 (1H, s) in the ¹H-NMR of **1**, which showed HMBC correlations to C-8, C-8' and C-9'. Thus, this additional hydrogen was placed on the C-9 position, while a methoxycarbonyl group was inferred to be attached on the C-9' position as in case of lycogarubin A (**13**); the methoxy protons at δ_H 3.65 (3H, s) showed an HMBC correlation to the ester carbonyl carbon (δ_C 163.6). An NOE correlation observed between H-9 and H-4 further confirmed the presence of a hydrogen atom at the C-9 position. The structure of cinereapyrrole A was therefore concluded as **1**.

Cinereapyrrole B (**2**) had a molecular formula of C₂₂H₁₇N₃O₃ as shown by the HR-FAB-MS data (*m/z* 371.1278, [M⁺], Δ +0.8 mmu). The UV and IR spectral data

* To whom correspondence should be addressed. e-mail: mish@p.chiba-u.ac.jp

Table 1. ^1H -NMR Spectral Data of Compounds **1**—**5** and **9**

Position	1 (CD_3OD)	2 (CD_3COCD_3)	3 (CD_3COCD_3)	4 (CD_3COCD_3)	5 (CD_3COCD_3)	9 (CD_3COCD_3)
1		9.86 br s	9.99 br s	10 br s	10 br s	10.00 br s
2	7.01 s	6.76 d 2.6	7.03 s	7.11 d 2.4	7.07 d 2.4	7.11 d 2.4
3						
3a						
4	7.01 d 1.9	7.64 d 8.1	7.19 ^{a)} d 7.5	7.16 d 7.7	7.27 d 7.9	7.16 d 7.9
5		6.95 ddd 8.1, 7.0, 1.2	6.79 ^{b)} t 7.5	6.78 t 7.7	6.79 t 7.9	6.81 td 7.9, 0.9
6	6.60 dd 8.4, 1.9	7.04 ddd 8.1, 7.0, 1.2	6.94 ^{c)} t 7.5	6.94 t 7.7	6.95 td 7.9, 1.2	6.97 td 7.9, 1.2
7	7.16 dd 8.4, 0.6	7.31 dt 8.1, 1.2	7.25 dd 7.5, 1.0	7.26 d 7.7	7.27 d 7.9	7.29 dd 7.9, 0.9
7a						
8						
9	7.29 s	7.44 d 3.6				
10		10.9 br s	10.90 br s	10.80 br s	10.8 br s	10.99 br s
1'		9.89 br s	9.99 br s	9.71 br s	9.72 br s	9.70 br s
2'	6.54 s	7.10 d 2.6	7.06 s	6.87 d 2.8	6.91 d 2.4	6.90 d 2.4
3'						
3a'						
4'	6.56 dd 2.6, 0.6	6.62 s	7.21 ^{a)} d 7.3	6.69 d 2.1	6.71 d 2.2	6.71 d 1.9
5'			6.81 ^{b)} t 7.3			
6'	6.61 dd 8.7, 2.6	6.63 dd 8.2, 2.3	6.96 ^{c)} t 7.3	6.59 dd 8.5, 2.1	6.58 dd 8.4, 2.2	6.63 dd 8.3, 1.9
7'	7.08 d 8.7	7.19 d 8.2	7.25 dd 7.3, 1.0	7.08 d 8.5	7.07 d 8.4	7.10 d 8.3
7a'						
8'						
9'						
OMe	3.65	3.57 s	3.62 s	3.63 s	3.62 s	3.63
5'-OH		7.35 br s		7.40 br s	7.40 br s	7.42

a—c) Signals may be reversed.

were parallel to those of **1**, and the ^1H - and ^{13}C -NMR data (Tables 1, 2) were also similar to those of compound **1**. The difference was observed in the ^1H -NMR spectrum of **2**, showing four consecutive aromatic proton signals [δ_{H} 7.64 (H-4), 6.95 (H-5), 7.04 (H-6), and 7.31 (H-7)], which was supported by the ^1H - ^1H COSY spectrum of **2** [cross peaks: H-4/H-5, H-5/H-6, and H-6/H-7]. These aromatic proton signals were assigned to a part of an indole nucleus without any substituents on the benzene ring part, while the ^1H -NMR spectrum of **2** also showed signals assignable to 5-hydroxyindole moiety [δ_{H} 6.62 (H-4'), 6.63 (H-6'), and 7.19 (H-7')], as possessed by compound **1**, which was confirmed by the HMBC spectrum of **2** in acetone- d_6 observed for H-2'/C-3a', H-2'/C-7a', H-4'/C-3', H-4'/C-6', H-6'/C-4', H-6'/C-7a', H-7'/C-5', H-7'/C-3a', OH-5'/C-4', OH-5'/C-5', and OH-5'/C-6'. Thus, compound **2** was suggested to possess one indole and one 5-hydroxyindole moieties. As in case of compound **1**, compound **2** bore a hydrogen atom on C-9 (δ_{H} 7.44, H-9), which was indicated by the HMBC correlations observed for H-9/C-8, H-9/C-9, and H-9/C-9' and the ^1H - ^1H COSY cross peak between H-9 and NH-10 in acetone- d_6 solution. An NOE was observed between H-9 (δ_{H} 7.44) and H-4 (δ_{H} 7.64), thus suggesting that the indole ring without 5-OH group was attached on the adjacent position (C-8) to H-9 and the 5-hydroxyindole ring therefore present at the neighboring position (C-8') to the methoxycarbonyl group. Thus, structure of cinereapyrrole B was revealed as **2**, which corresponded to cinereapyrrole A (**1**) without 5-hydroxy group, and also corresponded to lycogarubin B (**9**)⁷⁾ without 9-methoxycarbonyl group.

The fruit bodies of *Lycogala epidendrum*, collected in Kochi Prefecture, Japan, were also extracted with 90% MeOH and 90% acetone. The combined extracts were partitioned between EtOAc and water, and the EtOAc layer was

Table 2. ^{13}C -NMR Spectral Data of Compounds **1**—**5** and **9**

Position	1 ^{a)}	2 ^{b)}	3 ^{b)}	4 ^{b)}	5 ^{b)}	9 ^{b)}
1						
2	126.5	126.5	125.6	126.0	125.9	126.2
3	110.2	110.8	109.3	109.4	109.4	109.2
3a	129.9	129.9	128.8	129.1	128.9	128.8
4	104.7	120.4	120.6	120.7	120.7	120.5
5	151.0	119.7	119.3	119.3	119.3	119.3
6	112.0	121.8	121.5	121.5	121.5	121.5
7	112.3	111.8	111.8	111.8	111.9	111.8
7a	132.8	137.1	136.8	136.9	136.9	136.9
8	124.8 ^{c)}	123.1 ^{d)}	125.9 ^{e)}	125.8 ^{f)}	126.0 ^{g)}	125.9
9	121.9	121.3	123.3 ^{e)}	123.5 ^{f)}	123.4 ^{g)}	123.6
10						
1'						
2'	124.8	123.5	125.7	126.2	126.3	126.2
3'	110.1	109.9	109.3	108.8	108.8	108.6
3a'	128.7	129.8	129.0	129.9	130.2	129.9
4'	105.5	105.1	120.6	104.8	104.9	104.7
5'	151.4	151.5	119.3	151.5	151.6	151.5
6'	111.9	112.0	121.5	111.8	111.8	111.8
7'	112.5	112.2	111.8	112.1	112.1	112.1
7a'	132.6	132.0	136.8	131.7	131.7	131.6
8'	122.3 ^{c)}	121.6 ^{d)}	126.1 ^{e)}	126.4 ^{f)}	126.2 ^{g)}	125.9
9'	120.7 ^{c)}	121.0 ^{d)}	124.0 ^{e)}	124.1 ^{f)}	124.2 ^{g)}	123.6
COOMe	163.6	162.0	161.9	161.5	161.5	161.5
COOH	—	—	161.5	161.8	161.8	—
OMe	51.3	50.8	51.5	51.5	51.4	51.5

a) In CD_3OD . b) In CD_3COCD_3 . c—g) Signals may be reversed.

then subjected to chromatographies on silica gel, ODS, and Sephadex LH-20 to give three new compounds **3**—**5** as well as six known bisindoles, which were identified as lycogarubin C (**7**),⁷⁾ arcyriaflavin A (**8**),⁶⁾ lycogarubin B (**9**),⁷⁾ stauroporinone (**10**),⁸⁾ lycogarinic acid A (**11**),⁶⁾ and arcyriaflavin B (**12**)⁵⁾ on the basis of comparison of the spectral data.

The molecular formula of compound **3** was revealed as $C_{23}H_{17}N_3O_4$ by the HR-FAB-MS data (m/z 399.1184, $[M]^+$, $\Delta -3.5$ mmu). The 1H -NMR spectral data of **3** were similar to those of lycogarubin C (**7**), but **3** had only one methoxy group, while lycogarubin C (**7**) had two. Lycogarubin C (**7**) was a symmetrical bisindole with two methyl ester groups, whereas the 1H - and ^{13}C -NMR data of **3** (Tables 1, 2) showed that **3** was unsymmetrical. Thus, compound **3** was deduced to have one carboxylic acid and one methyl ester, corresponding to monodemethyl derivative of lycogarubin C (**7**). Compounds **4** and **5** had the same molecular formula of $C_{23}H_{17}N_3O_5$ as revealed by the HR-FAB-MS data (**4**: m/z 415.1179, $[M]^+$, $\Delta +1.1$ mmu; **5**: m/z 415.1179, $[M]^+$, $\Delta +1.1$ mmu). The 1H - and ^{13}C -NMR spectra of **4** and **5** (Tables 1, 2) were similar to each other and both of them also resembled those of lycogarubin B (**9**) (Tables 1, 2),⁷⁾ which was an asymmetric molecule with one indole and one 5-hydroxyindole moieties with two methoxycarbonyl groups at C-9 and C-9' positions. The 1H - and ^{13}C -NMR data showed that both compounds **4** and **5** had only one methoxy groups. This fact as well as interpretation of the 1H - 1H COSY and HMBC spectral data suggested that compounds **4** and **5** were monodemethyl derivatives of lycogarubin B (**9**). The NOE experiment using compound **4** showed an NOE correlation observed between the methoxy protons and H-4', which was on the 5-hydroxyindole group. This result suggested that compound **4** had the 5-hydroxyindole on the adjacent position (C-8') to the methoxycarbonyl group on C-9' while compound **5** was an isomer of **4** possessing the indole group on C-8'.

Compounds **8**, **10**, and **12**, which had a C-C bond connection between C-2 and C-2' positions, showed cytotoxic activity against HeLa cells with IC_{50} values of 47.6, 8.9, and 4.4 $\mu g/ml$, respectively, while compounds **3**, **7**, **9** and **11** without C-2/C-2' bond were almost inactive with IC_{50} values of 93.3, 24.0, >100, >100, respectively. Arcyriaflavin B (**12**) showed cytotoxicity (IC_{50} value, 2.28 $\mu g/ml$) against vincristine (VCR)-resistant KB cells, but didn't show the reversal effect of VCR resistance. Furthermore, arcyriaflavin B (**12**) was evaluated in the Japanese Foundation for Cancer Research 39 cell line panel assay.⁹⁾ Although compound **12** showed low differential cellular sensitivities, NCI-H522 and DMS273 lung cells, BSY-1 breast cancer cells, and SF-539 CNS cancer cells were relatively sensitive to **12** with LC_{50} values of 6.2, 6.7, 6.8, and 6.9 μM , respectively, while SNB-78 CNS cancer cells, HT-29 colon cells, NCI-H226 lung cells, and MKN28 stomach cells were relatively resistant with LC_{50} values of 51, 55, 58, and 55 μM , respectively.

Experimental

General Procedures UV spectra were obtained on a Shimadzu UV mini-1240 spectrometer. IR spectra were measured from samples on a Hitachi 260-10 infrared spectrophotometer. NMR spectra were recorded on JEOL JNM ecp600 spectrometers. HR-FAB-MS were acquired on a JMS HX-110 mass spectrometer.

Organism The fruit bodies of *Arcyria cinerea* were collected in Kochi Prefecture, Japan, in January 2003, and a voucher specimen (#24816 and 24861) is maintained by Y. Y. (Ohtsu-ko, Kochi). The fruit bodies of *Lycogala epidendrum* were also collected in Kochi Prefecture, Japan, in January 2002. A voucher specimen is maintained by Y. Y. (Ohtsu-ko, Kochi).

Extraction and Isolation The air-dried fruit bodies of *Arcyria cinerea* (2.9 g) were extracted with 90% MeOH (150 ml $\times$ 2) and 90% acetone (150 ml $\times$ 1). A portion (191 mg) of the combined extract (200 mg) was subjected to silica gel column chromatography (column A; 17 $\times$ 210 mm) eluted

with 20–100% EtOAc in hexane. A fraction (8.7 mg) of column A eluted with 80% EtOAc in hexane was then separated by ODS column chromatography (column B; 12 $\times$ 190 mm) eluted with 40–100% MeOH in water. A fraction (5.0 mg) of column B eluted with 40% MeOH in water was further purified by sephadex LH-20 column (10 $\times$ 550 mm) eluted with MeOH and HPLC on ODS (Develosil ODS-UG-5, 10 $\times$ 250 mm; eluent, 60% MeOH, flow rate, 1.8 ml/min; detection UV at 254 nm) to give cinereapyrrole A (**1**, 2.0 mg). A fraction (2.7 mg) of column B eluted with 50% MeOH in water was further purified by Sephadex LH-20 column (10 $\times$ 550 mm) eluted with MeOH to give cinereapyrrole B (**2**, 0.8 mg). A fraction (2.8 mg) of column A eluted with 50% EtOAc in hexane was applied on a silica gel column (8 $\times$ 210 mm) eluted with 0–2% MeOH in $CHCl_3$ to give arcyriaflavin A (**6**, 0.6 mg). A fraction (110 mg) of column A eluted with MeOH was subjected to silica gel column (column C; 15 $\times$ 250 mm) eluted with 20–100% acetone in $CHCl_3$. A fraction (5.0 mg) of column C eluted with 80–100% acetone in $CHCl_3$ was further purified by Sephadex LH-20 column (10 $\times$ 550 mm) eluted with MeOH to give **3** (1.6 mg).

The air-dried fruit bodies of *Lycogala epidendrum* (35.3 g) were extracted with 90% MeOH (500 ml $\times$ 2) and 90% acetone (500 ml $\times$ 1). The combined MeOH and acetone extract (6.66 g) was dissolved in 55% EtOAc in water (220 ml), and then partitioned between EtOAc (150 ml $\times$ 3) and distilled water (100 ml). The EtOAc-soluble fraction was evaporated under reduced pressure to give a residue (3.23 g), which was subjected to silica gel column chromatography (column D; 35 $\times$ 300 mm) eluted with 0–100% MeOH in $CHCl_3$. A fraction (1.3 g) of column D eluted with 5–20% MeOH in $CHCl_3$ was further separated on Sephadex LH-20 column (column E; 25 $\times$ 490 mm) eluted with $CHCl_3$ /MeOH (1:1). The fraction (201 mg) of column E was subjected to silica gel column (column F; 15 $\times$ 300 mm) eluted with 5–100% acetone in $CHCl_3$, followed by purification with ODS column chromatography (15 $\times$ 260 mm) eluting with 20–100% acetone in water to give compound **3** (24.2 mg). The fraction (74 mg) of column F eluted with 20% acetone in $CHCl_3$ was also purified by ODS column chromatography (12 $\times$ 310 mm) eluting with 50% acetone in water to give compounds **9** (39.5 mg) and **10** (10.5 mg). The fraction (601 mg) of column D eluted with 5% MeOH in $CHCl_3$ was further separated on Sephadex LH-20 column (25 $\times$ 490 mm) eluted with $CHCl_3$ /MeOH (3:4) to give compounds **7** (52.0 mg) and **8** (1.0 mg). A fraction (574 mg) of column D eluted with 20–50% MeOH in $CHCl_3$ was separated on ODS column (column G; 29 $\times$ 290 mm) eluted with 33–100% MeOH in water to give compound **11** (78.0 mg). A fraction (85 mg) of column G eluted with 50% MeOH in water was separated on Sephadex LH-20 column (12 $\times$ 490 mm) eluted with MeOH, and applied on a silica gel column (10 $\times$ 200 mm, $CHCl_3$ /MeOH/ H_2O , 95:5:0.3–1:1:0.3) to give a 6:4 mixture (8.4 mg) of compounds **4** and **5**. Finally, this mixture was purified by HPLC on ODS (Develosil ODS-UG-5, 10 $\times$ 250 mm; eluent, 30% CH_3CN , flow rate, 1.8 ml/min; detection UV at 254 nm) to give compound **4** (2.7 mg, t_R 19 min) and **5** (2.0 mg, t_R 22 min). A fraction (24 mg) of column G eluted with 70% MeOH in water was further separated on silica gel column (8 $\times$ 140 mm) eluted with 17–100% acetone in $CHCl_3$ to give compound **12** (6.1 mg).

Cinereapyrrole A (1): Dark brown amorphous powder; UV λ_{max} (MeOH) 283 (ϵ 11000); IR (film) ν_{max} 3396, 1695, 1630, 1583 and 1377 cm^{-1} ; 1H - and ^{13}C -NMR (Tables 1, 2); FAB-MS m/z 387 (M^+); HR-FAB-MS m/z 387.1208 [Calcd for $C_{22}H_{17}N_3O_4$, (M^+) 387.1219].

Cinereapyrrole B (2): Dark brown amorphous powder; UV λ_{max} (MeOH) 286 (ϵ 14000) and 230 (ϵ 36000); IR (film) ν_{max} 3400, 1696, 1454 and 1377 cm^{-1} ; 1H - and ^{13}C -NMR (Tables 1, 2); FAB-MS m/z 371 (M^+); HR-FAB-MS m/z 371.1278 [Calcd for $C_{22}H_{17}N_3O_3$, (M^+) 371.1270].

Compound 3: Brown amorphous powder; UV λ_{max} (MeOH) 268 (ϵ 29000) and 224 (ϵ 57000); IR (KBr) ν_{max} 3410, 1700, 1520, 1460, and 1240 cm^{-1} ; 1H - and ^{13}C -NMR (Tables 1, 2); EI-MS m/z 399 (M^+); HR-FAB-MS m/z 399.1184 [Calcd for $C_{23}H_{17}N_3O_4$, (M^+) 399.1219].

Compound 4: Pale yellow amorphous powder; UV λ_{max} (MeOH) 268 (ϵ 22000) and 225 (ϵ 38000); IR (film) ν_{max} 3410, 1690, 1590, 1460, 1360, and 1240 cm^{-1} ; 1H - and ^{13}C -NMR (Tables 1, 2); EI-MS m/z 415 (M^+); HR-FAB-MS m/z 415.1179 [Calcd for $C_{23}H_{17}N_3O_5$, (M^+) 415.1168].

Compound 5: Pale yellow amorphous powder; UV λ_{max} (MeOH) 269 (ϵ 20000) and 225 (ϵ 35000); IR (film) ν_{max} 3410, 1700, 1460, 1360, and 1240 cm^{-1} ; 1H - and ^{13}C -NMR (Tables 1, 2); EI-MS m/z 415 (M^+); HR-FAB-MS m/z 415.1179 [Calcd for $C_{23}H_{17}N_3O_5$, (M^+) 415.1168].

Cytotoxic Activity HeLa cells were seeded onto 96-well microtitre plates at 6×10^3 cells per well, and were pre-incubated for 24 h at 37 °C. The medium was replaced with fresh medium containing different concentrations of each isolated compound. The cells were then incubated at 37 °C for 24 h. After the medium containing the isolated compounds was removed, cell pro-

liferation was determined by fluorometric microculture cytotoxicity assay (FMCA)¹⁰ using a fluorescence platereader. The ratio of the living cells was determined as the fluorescence in the sample wells expressed as a percentage of that in the control wells, and cytotoxic activity was indicated as an IC₅₀ value.

VCR (vincristine) selected multidrug resistant variants of the human epidermoid carcinoma KB cells (KB/VJ-300)¹¹ were a gift from Prof. M. Kuwano (Kyushu University School of Medicine). For the *in vitro* drug treatment experiments, KB cells, 1.1×10^4 cells/ml of the cells were seeded in 195 μ l of culture medium/well in 96-well plates (Corning Glass Works), and incubated for 24 h at 37 °C in a 5% CO₂–95% air atmosphere. The cells were treated in triplicate with 5 μ l of graded concentrations of samples, and were then incubated in a CO₂ incubator at 37 °C for 72 h. Cells viability was determined by the colorimetric assay using MTT.¹²

Acknowledgments This work was partly supported by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan, by a Grant-in-Aid from the Fujisawa Foundation, and by a Grant-in-Aid from the Keimeikai Foundation.

References

- 1) Steglich W., *Pure Appl. Chem.*, **61**, 281–288 (1989).
- 2) Nakatani S., Yamamoto Y., Hayashi M., Komiyama K., Ishibashi M., *Chem. Pharm. Bull.*, **52**, 368–370 (2004).
- 3) Kamata K., Onuki H., Hirota H., Yamamoto Y., Hayashi M., Komiyama K., Sato M., Ishibashi M., *Tetrahedron*, **60**, 9835–9839 (2004).
- 4) Nakatani S., Kamata K., Sato M., Onuki H., Hirota H., Matsumoto J., Ishibashi M., *Tetrahedron Lett.*, **46**, 267–271 (2005).
- 5) Steglich W., Steffan B., Kopanski L., Eckhardt G., *Angew. Chem. Int. Ed. Engl.*, **19**, 459–460 (1980).
- 6) Fröde R., Hinze C., Josten I., Schmidt B., Steffan B., Steglich W., *Tetrahedron Lett.*, **35**, 1689–1690 (1994).
- 7) Hashimoto T., Yasuda A., Akazawa K., Takaoka S., Tori M., Asakawa Y., *Tetrahedron Lett.*, **35**, 2559–2560 (1994).
- 8) Yasuzawa T., Iida T., Yoshida M., Hirayama N., Takahashi M., Shirahata K., Sano H., *J. Antibiot.*, **39**, 1072–1078 (1986).
- 9) Yamori T., Matsunaga A., Sato S., Yamazaki K., Komi A., Ishizu K., Mita I., Edatsugi H., Matsuba Y., Takezawa K., Nakanishi O., Kohno H., Nakajima Y., Komatsu H., Andoh T., Tsuruo T., *Cancer Res.*, **59**, 4042–4049 (1999).
- 10) Larsson R., Kristensen J., Sandberg C., Nygren P., *Int. J. Cancer*, **50**, 177–185 (1992).
- 11) Hayashi M., Koike K., Rho M.-C., Kuwano M., Kishie T., Komiyama K., *Anti-Cancer Drug Des.*, **16**, 255–260 (2001).
- 12) Alley M. C., Scudiero D. A., Monks A., Hursey M. L., Czerwinski M. J., Fine D. L., Abbott B. J., Mayo J. G., Shoemaker R. H., Boyd M. R., *Cancer Res.*, **48**, 589–601 (1998).