Full Paper

12T061A and 12T061C, two new julichrome family compounds, as radical scavengers from *Streptomyces* sp.

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We identified two new radical scavengers, 12T061A (1, C₁₉H₂₀O₇) and 12T061C (2, C₂₀H₂₂O₇), from a culture of the *Streptomyces* sp. Spectroscopic analysis indicated that these compounds are new julichrome family compounds. Compounds 1 and 2 showed radical-scavenging activity with an ED₅₀ of 370 μM and 18 μM, respectively. Moreover, 1 showed tumor cell growth suppressive activity in HepG2 cells, (IC₅₀: 3.6 μM); however, no suppressive activity was shown in 2 (IC₅₀: > 100 μM).

Key Words: DPPH radical scavenger; julichrome; tumor cell growth suppressive activity

Introduction

Free radicals present in the human body cause oxidative stress in various organs and induce inflammatory diseases, while those present in food can cause deterioration of the nutrient factor *via* lipid peroxidation (Inoue et al., 2008; Musiek et al., 2008). Hence, we focused on the identification of a new bioactive compound by assessing radical-scavenging activity (Komoda et al., 2008a, 2008b, 2011). Recently, we identified 12T061C (2) from a culture of a *Streptomyces* sp. This new compound acts as a radical scavenger and we reported its identification recently (Komoda et al., 2014). In our continuing study, we identified another compound, 12T061A (1), from the same culture broth. The chemical structure of compounds 1 and 2 share a similar skeleton.

In our screening program, we used high performance liquid chromatography (HPLC) with an electrochemical detector (ECD) as an initial screening step. ECD can evaluate an oxidation-reduction potential derived from compounds such as antioxidants and radical scavengers. In the next step, we used a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity assay, and identified two new radical scavengers, 12T061A (1) and 12T061C (2), in the culture broth of *Streptomyces* sp. 12T061. Here, we describe the cultivation of the strain, the extraction, isolation, and structural elucidation of the compounds, and the measurement of their radical-scavenging and tumor cell growth suppressive activities.

Materials and Methods

Chemicals. We obtained the chemicals commercially. The deuterated solvents, CD₃OD, acetone-*d*₆, and DMSO-*d*₆, for NMR measurements were from Cambridge Isotope Laboratories (Andover, MA). The yeast extract was obtained from Kyokuto (Tokyo, Japan). Trimethylsilyl diazomethane diethyl ether solution was obtained from Sigma-Aldrich (St. Louis, MO). All the other chemicals were from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

Instruments. Spectroscopic measurements were made using the following instruments: NMR, Jeol (Tokyo, Japan) ECX-400P spectrometer (tetramethylsilane as the internal reference; δ = 0 ppm); HRFABMS, Jeol (Tokyo, Japan) JMS-700; UV-VIS spectra, Hitachi (Tokyo, Japan) U2800A spectrometer; melting point, AS ONE (Osaka, Japan) ATM-01; IR, Perkin Elmer (Yokohama, Japan)

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Spectrum One FT-IR; analytical HPLC, Hitachi (Tokyo, Japan) L-2130 (pump), L-2400 (UV), L-2455 (diode array detector, DAD), and Thermo Scientific (Yokohama, Japan) Esa Coulochem III (electro chemical detector, ECD); preparative HPLC, GL-Science (Tokyo, Japan) PU715 (pump) and UV702 (UV-VIS).

Initial screening for radical-scavenger-producing strain. Each *Streptomyces* sp. strain was inoculated into 5 mL of medium containing glucose 30 g/L, malt extract 2 g/L, and yeast extract 2 g/L at pH 7.3 in a test tube and cultivated at 28°C for four days on a reciprocal shaker. The filtrate of each culture broth was then diluted by the same volume of MeOH and centrifuged (10,000 rpm, 4 min, 4°C). The supernatant was analyzed by HPLC (DAD-ECD) with a gradient system, Capcell Pak C18 MG II column (Shiseido, Tokyo, Japan), $\phi 4.6 \times 50$ mm; 15% MeOH/0.1% trifluoroacetic acid (TFA) (0–2 min) - linear gradient (2–8 min) - 98% MeOH/0.1% TFA (8–17 min), 0.5 mL/min; DAD, 220–600 nm; ECD, 300 mV.

Cultivation of *Streptomyces* sp. 12T061. The 12T061 strain was inoculated into 9.6 L of medium containing glucose 30 g/L, malt extract 2 g/L, and yeast extract 2 g/L at pH 7.3 in a 2-L Erlenmeyer flask and was cultivated at 28°C for 14 days on a rotary shaker (150 rpm).

Isolation of 12T061A (1) and 12T061C (2). The filtrate of a culture broth (9.6 L) of *Streptomyces* sp. 12T061 was acidified to pH 3.0 using HCl and extracted using an equal volume of EtOAc. After evaporation under reduced pressure, the crude extract was purified by silica gel column chromatography. After the column was washed with *n*-hexane, the active compounds were eluted by acetone. The acetone fraction showed DPPH radical-scavenging activity. The radical-scavenging activity was monitored using a colorimetric assay involving DPPH solution. The solution, 2% DPPH in EtOH, was sprayed after development of the thin-layer chromatography (TLC, silica gel, CHCl₃/MeOH = 95/15). Radical-scavenging activity was confirmed by a decrease in the color intensity on a TLC plate. The active fraction was then further purified by Sephadex LH-20 column chromatography (MeOH) and preparative HPLC (75% MeOH/0.1% trifluoroacetic acid (TFA); Capcell Pak C18 MG II column (Shiseido, Tokyo, Japan), $\phi 20 \times 250$ mm; UV, 280 nm) to yield 50 mg of 12T061A (1). We found another active compound in the preparative HPLC. This active compound was subjected to further purification using preparative HPLC (40% CH₃CN/0.1% trifluoroacetic acid (TFA); Capcell Pak C18 MG II column (Shiseido, Tokyo, Japan), $\phi 20 \times 250$ mm; UV, 280 nm) to yield 30 mg of 12T061C (2).

12T061A (1). This compound was a yellow amorphous powder; mp > 300°C (dec.); $[\alpha]_D^{21} + 43^\circ$ (C 0.60, MeOH); HRFABMS m/z [M+Na]⁺ resulted in the following findings: 383.1106, calculated for C₁₉H₂₀O₇Na: 383.1106; UV/VIS $\lambda_{\max}$ (MeOH) nm (ϵ): 226.0 (4700000), 256.5 (4600000). IR $\nu_{\max}$ (ATR) cm⁻¹: 3389, 2975, 2934, 1713, 1627, 1378, 1257, 1120; ¹H NMR (400 MHz, DMSO-*d*₆), δ_H 2.85 (1H, d, J = 18.8 Hz, 2-H $_{\alpha}$), 2.57 (1H, d, J = 18.8 Hz, 2-H $_{\beta}$), 1.14 (3H, s, 3-CH₃), 5.28 (1H, br.s, 3-OH), 3.56 (1H, t, J = 2.0 Hz, 4-H), 7.64 (1H, d, J = 8.1 Hz, 5-H), 7.58 (1H, dd, J = 8.1 and 7.7 Hz, 6-H), 6.88 (1H, d, J = 7.7 Hz, 7-H), 9.88 (1H, s, 8-OH), 15.48 (1H, s, 9-OH),

Fig. 1. Structures of 12T061A (1), and 12T061C (2).

8.78 (1H, s, 10-OH), 5.60 (1H, qd, J = 6.4 and 2.0 Hz, 11-H), 1.21 (3H, d, J = 6.4 Hz, 11-CH₃), 1.65 (3H, s, 13-H); ¹³C NMR (100 MHz, DMSO-*d*₆), δ_C 204.23 (s, C-1), 48.05 (t, C-2), 69.15 (s, C-3), 29.92 (q, 3-CH₃), 47.85 (d, C-4), 118.08 (s, C-4a), 113.30 (d, C-5), 131.68 (d, C-6), 111.33 (d, C-7), 157.43 (s, C-8), 112.67 (s, 8a), 158.26 (s, C-9), 109.61 (s, C-9a), 141.89 (s, C-10), 132.83 (s, C-10a), 69.02 (d, C-11), 19.93 (q, 11-CH₃), 169.11 (s, C-12), 20.73 (q, C-13).

12T061C (2). This compound was a yellow amorphous powder; mp 76–85°C; $[\alpha]_D^{21} + 146^\circ$ (C 0.66, MeOH); HRFABMS m/z [M+H]⁺ found: 375.1440, calculated for C₂₀H₂₃O₇: 375.1444; UV/VIS $\lambda_{\max}$ (MeOH) nm (ϵ): 223 (5300000), 256 (5200000), 410 (1300000); IR $\nu_{\max}$ (ATR) cm⁻¹: 3360, 2971, 1605, 1376, 1237; ¹H NMR (400 MHz, DMSO-*d*₆), δ_H 2.84 (1H, dd, J = 18.3 and 1.6 Hz, 2-H $_{\alpha}$), 2.58 (1H, d, J = 18.3 Hz, 2-H $_{\beta}$), 1.16 (3H, s, 3-CH₃), 5.26 (1H, br.s, 3-OH), 2.82 (1H, br.s, 4-H), 7.48 (1H, d, J = 9.2 Hz, 5-H), 7.34 (1H, d, J = 9.2 Hz, 6-H), 9.45 (1H, s, 7-OH), 3.80 (3H, s, 8-OCH₃), 14.59 (1H, s, 9-OH), 7.15 (1H, s, 10-H), 5.61 (1H, qd, J = 6.1 and 1.5 Hz, 11-H), 1.20 (3H, d, J = 6.1 Hz, 11-CH₃), 1.69 (3H, s, 13-H); ¹³C NMR (100 MHz, DMSO-*d*₆), δ_C 204.53 (s, C-1), 48.84 (t, C-2), 68.90 (s, C-3), 30.57 (q, 3-CH₃), 54.56 (d, C-4), 131.84 (s, C-4a), 123.92 (d, C-5), 123.00 (d, C-6), 147.88 (s, C-7), 143.34 (s, C-8), 61.02 (q, 8-OCH₃), 119.13 (s, 8a), 162.15 (s, C-9), 111.13 (s, C-9a), 119.80 (d, C-10), 132.16 (s, C-10a), 67.77 (d, C-11), 19.19 (q, 11-CH₃), 169.09 (s, C-12), 20.80 (q, C-13).

Trimethylsilyl diazomethane (TMS-CHN₂) treatment of 12T061A (1). Fifty milligrams of 1 was dissolved in 4 mL MeOH. Then 1 mL of 2.0 M trimethylsilyl diazomethane diethyl ether solution was added to a sample MeOH solution and allowed to stand for two days at room temperature. After it was evaporated to dryness, the

Fig. 2. NMR spectral analysis for 12T061A (1), and 12T061C (2).

Fig. 3. Relative stereochemistry of 12T061A (1) and 12T061C (2) obtained by NMR measurement.

reactant was purified by preparative HPLC (50% CH_3CN/H_2O ; Capcell Pak C18 MG II column (Shiseido, Tokyo, Japan), $\phi 20 \times 250$ mm; UV, 254 nm) to yield 20 mg of 9,10-dimethoxy-12T061A (3).

9,10-dimethoxy-12T061A (3). HRFABMS m/z $[M+Na]^+$ found: 411.1422, calculated for $C_{21}H_{24}O_7Na$: 411.1420 1H NMR (400 MHz, acetone- d_6), δ_H 2.81 (1H, d, $J = 17.5$ Hz, 2- H_α), 2.68 (1H, dd, $J = 18.3$ and 1.5 Hz, 2- H_β), 1.23 (3H, s, 3- CH_3), 3.53 (1H, t, $J = 1.9$ Hz, 4-H), 7.59 (1H, dd, $J = 8.4$ and 1.5 Hz, 5-H), 7.58 (1H, dd, $J = 8.4$ and 6.9 Hz, 6-H), 6.91 (1H, dd, $J = 6.9$ and 1.5 Hz, 7-H), 9.98 (1H, s, 8-OH), 4.04 (3H, s, 9-OCH₃), 3.93 (3H, s, 10-OCH₃), 5.81

(1H, qd, $J = 6.5$ and 1.9 Hz, 11-H), 1.23 (3H, d, $J = 6.5$ Hz, 11- CH_3), 1.69 (3H, s, 13-H); ^{13}C NMR (100 MHz, acetone- d_6), δ_C 196.00 (s, C-1), 51.75 (t, C-2), 70.14 (s, C-3), 31.71 (q, 3- CH_3), 50.30 (d, C-4), 129.10 (s, C-4a), 114.15 (d, C-5), 131.30 (d, C-6), 112.31 (d, C-7), 157.80 (s, C-8), 118.38 (s, 8a), 155.64 (s, C-9), 64.91 (q, 9-OCH₃), 121.84 (s, C-9a), 151.33 (s, C-10), 62.04 (q, 10-OCH₃), 133.42 (s, C-10a), 69.52 (d, C-11), 20.43 (q, 11- CH_3), 169.92 (s, C-12), 21.06 (q, C-13).

Trimethylsilyl diazomethane (TMS-CHN₂) treatment of 12T061C (2). Fifteen milligrams of 2 was dissolved in 2 mL MeOH. Then 0.5 mL 2.0 M trimethylsilyl

diazomethane diethyl ether solution was added to a sample MeOH solution and allowed to stand for two hours at room temperature. After it was evaporated to dryness, the reactant was purified by preparative HPLC (35% CH₃CN/0.1% trifluoroacetic acid (TFA); InertSustain (GL-Science, Tokyo, Japan), $\phi 10 \times 250$ mm; UV, 254 nm) to yield 4 mg of 7-methoxy-12T061C (**4**).

7-methoxy-12T061C (4). HRFABMS m/z [M+Na]⁺ found: 411.1420, calculated for C₂₁H₂₄O₇Na: 411.1420; ¹H NMR (400 MHz, CD₃OD), δ_H 2.96 (1H, d, J = 18.4 Hz, 2-H _{α}), 2.66 (1H, dd, J = 18.3 and 1.4 Hz, 2-H _{β}), 1.25 (3H, s, 3-CH₃), 2.90 (1H, t, J = 1.4 Hz, 4-H), 7.59 (1H, d, J = 8.7 Hz, 5-H), 7.55 (1H, d, J = 8.7 Hz, 6-H), 3.98 (3H, s, 7-OCH₃), 3.93 (3H, s, 8-OCH₃), 7.15 (1H, s, 10-H), 5.72 (1H, qd, J = 6.8 and 2.3 Hz, 11-H), 1.28 (3H, d, J = 6.4 Hz, 11-CH₃), 1.74 (3H, s, 13-H); ¹³C NMR (100 MHz, CD₃OD), δ_C 205.78 (s, C-1), 49.91 (t, C-2), 70.82 (s, C-3), 30.51 (q, 3-CH₃), 56.93 (d, C-4), 134.20 (s, C-4a), 125.45 (d, C-5), 120.47 (d, C-6), 151.93 (s, C-7), 57.47 (q, 7-OCH₃), 147.77 (s, C-8), 62.19 (q, 8-OCH₃), 120.81 (s, 8a), 164.35 (s, C-9), 112.79 (s, C-9a), 121.37 (d, C-10), 134.79 (s, C-10a), 69.88 (d, C-11), 19.92 (q, 11-CH₃), 171.71 (s, C-12), 21.10 (q, C-13).

Measurement of DPPH radical-scavenging activity. This assay was performed by a previously reported spectroscopic method (Chen et al., 2005; Washida et al., 2007). An MeOH solution (2 mL) of each sample was mixed with a 0.5 mM DPPH MeOH solution (1 mL) in 0.1 M acetate buffer (pH 5.5, 2 mL). The solution was allowed to stand for 30 min, and the absorbance was measured at 517 nm. In a blank test, the absorbance of MeOH was measured. The DPPH radical-scavenging activity of each sample was recorded against the blank and expressed in percentage units. The ED₅₀ values were taken to be the concentrations required for 50% DPPH radical-scavenging activity.

Tumor cell growth suppressive assay. HepG2 cells were purchased from RIKEN Cell Bank. Following the method of (Kasai et al., 2012), the HepG2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), 30 U/mL penicillin, and 30 μ g/mL streptomycin under 5% CO₂ at 37°C. HepG2 cells were seeded in 96-well plates (2 $\times$ 10⁴ cells/well) and incubated for 24 hours. Then the culture medium was removed and 100 μ L of medium containing each sample was added. After 48 hours, the number of viable cells was determined using a Cell Counting Kit-8 (DOJINDO) and a microplate reader (iMark; Bio-Rad Laboratories, Inc.). Cell viabilities were normalized to OD₄₅₀–OD₆₂₀ against the untreated cells.

Results and Discussions

Around 300 *Streptomyces* sp. strains obtained from soil samples were evaluated in our initial screening program; 21 strains showed a positive peak on the HPLC-ECD analysis. The culture broths of these positive strains were extracted with EtOAc at pH 3.0 and then developed by thin-layer chromatography (TLC, silica gel, CHCl₃/MeOH = 95/15). The radical-scavenging activity of each strain was monitored by the reduction in color intensity of a

Fig. 4. Previously identified Julichrome-type products.

DPPH solution that was sprayed after TLC development. We then identified the *Streptomyces* sp. 12T061 strain which produced radical scavengers in the culture broth. We inoculated this strain into 9.6 L of medium (glucose, 30 g/L; malt extract, 2 g/L; yeast extract, 2 g/L; pH 7.3)

Fig. 5. Trimethylsilyl diazomethane (TMS-CHN₂) treatment of 12T061A (1) and 12T061C (2).

in an Erlenmeyer flask and cultivated it for 14 days at 30°C. The filtrate of the culture broth was acidified to pH 3 and extracted with EtOAc. The crude extract was purified by silica gel column chromatography using an *n*-hexane/acetone system as the eluent. The active fraction was further purified by Sephadex LH-20 chromatography (eluent, MeOH) and preparative HPLC. Finally, radical scavengers 12T061A (**1**, 50 mg) and 12T061C (**2**, 30 mg) were isolated from this culture broth (9.6 L).

The chemical structure of **1** and **2** were analyzed by spectroscopic measurements. Those measurements suggested that **1** and **2** have a similar chemical structure. We have discussed the planar structure and stereochemistry of **2** in a previous paper as a preliminary communication (Komoda et al., 2014). We describe the structure elucidations of **1** and **2** only briefly here.

The result of high-resolution fast atom bombardment mass spectroscopy (HRFABMS) measurements of **1** indicated that the molecular formula of **1** was C₁₉H₂₀O₇. Based on the IR spectrum of this compound, the presence of carbonyl and hydroxyl groups was confirmed. The ¹H nuclear magnetic resonance (NMR) spectrum of **1** (400 MHz, DMSO-*d*₆) showed three aromatic OH signals in the range δ_H 8.5–15.5, three olefinic signals in the range δ_H 6.0–8.0, one hetero connecting signal at δ_H 5.60 (1H, qd, *J* = 6.4 and 2.0 Hz), one broad singlet at δ_H 5.28, one asymmetrical methylene (δ_H 2.85 and 2.57), and three methyl signals (δ_H 1.65, 1.21 and 1.14). ¹³C-NMR (100 MHz, DMSO-*d*₆) and distortionless enhancement by polarization transfer (DEPT) spectra showed one ketone signal at δ_C 204.23, one carbonyl signal at δ_C 169.11, ten olefinic carbon signals in the range δ_C 100–160, two hetero connecting carbons in the range δ_C 60–70, two carbons in the range δ_C 40–50 and three methyl signals in the range δ_C 10–30. The ¹H-¹H correlation spectroscopy (COSY), heteronuclear multiple-quantum coherence (HMQC), and heteronuclear multiple-bond correlation (HMBC) spectra experiments suggested a planar structure of **1** (Fig. 2). In

the ¹H-¹H COSY and HMBC experiments, three aromatic protons (5-H, 6-H, and 7-H) and the three aromatic OH signals (δ_H 8.78, δ_H 9.88, and δ_H 15.48) allowed us to construct the naphthalene system. This naphthalene system is attached to a cyclohexanone ring at C-4a and C-9a. The cyclohexanone ring is substituted with a methyl group and a hydroxyl group at C-3. The position at C-4 was also substituted with a side chain including an acetyl moiety. The HMBC correlation from 9-OH (δ_H 15.48) to C-9a, from 10-OH (δ_H 8.78) to C-4a, and other correlations supported the attachment between the naphthalene system and the cyclohexanone ring at C-4a and C-9a. Together, these findings enabled us to predict the planar structure of **1**. The chemical shifts 8-OH (δ_H 9.88) and 9-OH (δ_H 15.48) suggested these protons formed two hydrogen bonds (Fig. 2). This planar structure suggested that **1** is a new member of the julichrome family of anti-tumor compounds. Several derivatives have been identified in this group (Shaaban et al., 2007; Tsuji and Nagashima, 1970, 1971); however, **1** was shown to be a novel compound.

The relative stereochemistry of **1** was elucidated by using a coupling constant in the ¹H-NMR spectrum and a differential NOE experiment. The NOE observation at 2-H (δ_H 2.57)/3-CH₃ and 3-CH₃/4-H indicated these protons are arranged at the upper side in our model. Likewise, the NOE observation at 11-CH₃/11-H and 11-H/2-H (δ_H 2.85) suggested that these protons are located at the lower side of the cyclohexane ring. The small coupling constant (*J* = 2.0 Hz) in the ¹H-NMR spectrum between 4-H and 11-H indicated that these two protons form a *gauche* arrangement. These data and other NOE observations suggested the relative stereochemistry as shown in Fig. 3. This stereochemistry was consistent with known julichrome group compounds.

We elucidated the chemical structure of **2** as well as **1**. The HRFABMS measurements indicated the molecular formula of **2** to be C₂₀H₂₂O₇. The IR spectrum, ¹H-NMR, and ¹³C-NMR spectrum of **2** were similar to those of **1**.

The ^1H -NMR spectrum of **2** (400 MHz, $\text{DMSO}-d_6$) shows two aromatic OH signals in the range δ_{H} 9.0–15.0, three olefinic signals in the range δ_{H} 7.0–7.5, one hetero connecting signal at δ_{H} 5.61 (1H, qd, $J = 6.1$ and 1.5 Hz), one broad signal at δ_{H} 5.26, one methoxy methyl signal at δ_{H} 3.80, one asymmetrical methylene (δ_{H} 2.84 and 2.58), one broad singlet at δ_{H} 2.82, and three methyl signals (δ_{H} 1.69, 1.20, and 1.16). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) and DEPT spectra showed one ketone signal at δ_{C} 204.53, one carbonyl signal at δ_{C} 169.09, ten olefinic carbon signals in the range δ_{C} 110–165, two hetero connecting carbons in the range δ_{C} 60–70, one methoxy methyl at δ_{C} 61.02, two carbons in the range δ_{C} 40–60, and three methyl signals in the range δ_{C} 10–30. These results and COSY, HMQC, and HMBC data suggested that **2** has a skeleton similar to that of **1**. Then we constructed the planar structure of **2** as shown in Fig. 1. The naphthalene ring attached to the cyclohexane ring and the C-4 side chain were common to both **1** and **2**. The structural difference between **1** and **2** is the substitution pattern on their naphthalene system. Specifically, **1** possesses three hydroxy groups on its naphthalene system, while **2** has two hydroxy groups and one methoxy group on its naphthalene system. The stereochemical data derived from the ^1H - ^1H coupling constants and the NOE differential experiments was also consistent between the two compounds. The absolute stereochemistry of julichrome family derivatives is shown in Fig. 4. Consequently, considering the biosynthetic nature of these compounds, **1** and **2** should have a stereochemistry similar to that of other julichrome family compounds. We suggest the absolute stereochemistry of **1** and **2** to be as shown in Fig. 1.

The results of our DPPH radical assay indicated that **1** and **2** showed radical-scavenging activity. **2**, with ED_{50} 18 μM , had more potent radical-scavenging activity than **1**, with ED_{50} 370 μM . We used two known antioxidative compounds, α -tocopherol (ED_{50} 16 μM) and quercetin (ED_{50} 3 μM), as positive controls in this assay.

Considering the chemical structures of **1** and **2**, we propose that the 7-OH group in **2** should play a more important role in the radical-scavenging activity than does the 10-OH group in **1**. Other OH groups (8-, 9-OH in **1** and 9-OH in **2**) should form hydrogen bonds as shown in Fig. 2. These hydrogen bonds prohibit the proton release from each OH group during the radical-scavenging process. For further investigations, we treated **1** and **2** with trimethylsilyl-diazomethane ($\text{TMS}-\text{CHN}_2$) solution to obtain their methyl ethers. In this treatment, **2** gave 7-methoxy-12T061C (**4**) in two hours, whereas **1** showed no reaction after two hours. Letting the reaction continue, we obtained 9,10-dimethoxy-12T061A (**3**) from **1** after two days. These results suggested that the 7-OH in **2** releases the proton easily. The released proton should play an important role in radical-scavenging activity.

Because **1** and **2** are both new julichrome family compounds, we assessed the tumor cell growth suppressive

activity on these two compounds. In this assay, **1** showed a potent suppressive activity (IC_{50} 3.6 μM) similar to the activity of the positive controls, 5-fluorouracil (IC_{50} 4.0 μM) and doxorubicin (IC_{50} 0.9 μM). However, **2** showed no suppressive activity ($\text{IC}_{50} > 100 \mu\text{M}$).

In this study, we have successfully isolated and characterized 12T061A (**1**) and 12T061C (**2**), two new julichrome family compounds, from a culture of *Streptomyces* sp. 12T061. Compounds **1** and **2** showed DPPH radical-scavenging activity. Moreover, **1** indicated tumor cell growth suppressive activity, while **2** showed no suppressive activity. We are investigating the details of these compounds and other new active compounds. Actually, we have found one other antioxidant compound (12T061B) in the culture. The structural analyses of 12T061B are in progress.

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