

ORIGINAL ARTICLE

Elmenols C-H, new angucycline derivatives isolated from a culture of *Streptomyces* sp. IFM 11490

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Chemical investigations of the ethyl acetate extract of *Streptomyces* sp. IFM 11490 have led to the isolation of six new angucycline metabolites, named elmenols C–H (1–6), along with the previously reported elmonin (7) and elmenols A (8) and B (9). The known LS1924A (10), 6-deoxy-8-methylrabelomycin (11), tetrangulol methyl ether (12) and angucyclinone (13) were additionally identified. The structures of the isolated compounds were elucidated by means of spectroscopic methods including UV, IR, HRESIMS, and 1D and 2D NMR. Compounds 1–6 were evaluated for their abilities to overcome tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) resistance in human gastric adenocarcinoma (AGS) cells. Compounds 5 (10 μM) and 6 (50 μM) in combination with TRAIL showed moderate activity in sensitizing TRAIL-resistant AGS cells.

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INTRODUCTION

Angucyclines are a large group of naturally occurring secondary metabolites produced by actinomycetes.^{1,2} They exhibit a number of biological activities such as antimicrobial, antiviral, antiplatelet and cytotoxic activities.^{3,4} An increasing number of angucycline derivatives with different activities have been isolated from natural sources, predominantly *Streptomyces* sps.^{1,5,6} Their structures are mainly based on the tetracyclic benz[a]anthracene skeleton.^{7,8} In the continuation of our search for new bioactive natural products from actinomycetes,⁹ we collected sea water and soil samples from different areas in Japan. Our laboratory reported the isolation of aotaphenazine¹⁰, sulfotanone¹¹, yoropyrazone¹² and different pyranonaphthaquinone derivatives¹³ from *Streptomyces* sps. These compounds exhibited the ability to overcome tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) resistance in human gastric adenocarcinoma (AGS) cells.

We recently investigated the crude extract of *Streptomyces* sp. IFM 11490 and isolated isobenzofuran derivatives, named elmonin (7) as well as elmenols A (8) and B (9).¹⁴ Further investigations of the culture of *Streptomyces* sp. IFM 11490 resulted in the isolation of new angucycline metabolites named elmenols C–H (1–6; Figure 1). We herein describe the isolation, structural elucidation and evaluation of the TRAIL resistance-overcoming activities of compounds 1–6 in AGS cell lines because we are interested in targeting the TRAIL-related cancer-selective apoptosis-inducing pathway using microbial natural compounds.¹⁵

RESULTS AND DISCUSSION

Streptomyces sp. IFM 11490 was isolated from a soil sample collected from Sapporo in Hokkaido, Japan. The strain was cultured in

Waksman medium¹⁶ at 28 °C for 3 days on a reciprocal shaker. Working up of the crude extract resulted in the isolation of five new compounds (1–5; Figure 1) along with the known LS1924A (10)¹⁷, 6-deoxy-8-methylrabelomycin (11)¹⁸ and tetrangulol methyl ether (12).¹⁹ In addition, previously isolated elmonin (7) and elmenols A (8) and B (9)¹⁴ were detected by the LC-MS analysis. The growth of the same strain on M₂ liquid medium²⁰ under similar conditions led to the isolation of another new compound (6; Figure 1) and the known angucyclinone (10).⁷

Compound 1 was isolated as an optically active brown solid, $[\alpha]_{\text{D}}^{20} = -32.3$ (*c* 0.1, MeOH), with an assigned molecular formula of C₂₁H₂₂O₆ based on (+)-HRESIMS at *m/z* 393.1326 [M+Na]⁺ (calcd for C₂₁H₂₂O₆Na 393.1314). The IR spectrum of 1 suggested absorption bands of OH (3396 cm⁻¹) and carbonyl groups (1671 cm⁻¹). The ¹H NMR and ¹H-¹H-COSY of 1 (Table 1, Supplementary Figures S1 and S3) allowed the construction of two spin systems (Figure 2): H-5'-H-6', ((δ_{H} 7.21 (d, *J* = 8.4 Hz) and 6.97 (d, *J* = 8.4 Hz)) and H-4-H-5-H-6 (δ_{H} 6.59 (d, *J* = 7.8 Hz), 7.29 (t, *J* = 7.8 Hz), and 6.93 (d, *J* = 7.8 Hz)). In addition, the ¹H NMR spectrum showed two methine protons ((δ_{H} 7.52 (brd) and 6.47 (d, *J* = 2.1 Hz)), two aliphatic methylenes (δ_{H} 2.76 (d, *J* = 16.2 Hz)/2.81 (d, *J* = 16.2 Hz) and 3.01 (d, *J* = 16.2 Hz)/3.11 (d, *J* = 16.2 Hz)), two methoxy groups (δ_{H} 3.92 (s) and 3.50 (s)), and one methyl signal (δ_{H} 1.40 (s)). The ¹³C NMR spectrum (Supplementary Figure S2) of 1 aided by HMQC, revealed one carbonyl group (δ_{C} 201.0), five *sp*² methine carbons (δ_{C} 131.9, 131.2, 122.8, 114.6 and 110.1), and seven other *sp*² quaternary carbon atoms (δ_{C} 155.7, 154.5, 144.6, 134.8, 131.9, 125.0 and 125.1). In the aliphatic pattern, two oxymethines (δ_{C} 107.1 and

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This article is dedicated to Professor Satoshi Ōmura in celebration of his 2015 Nobel Prize.

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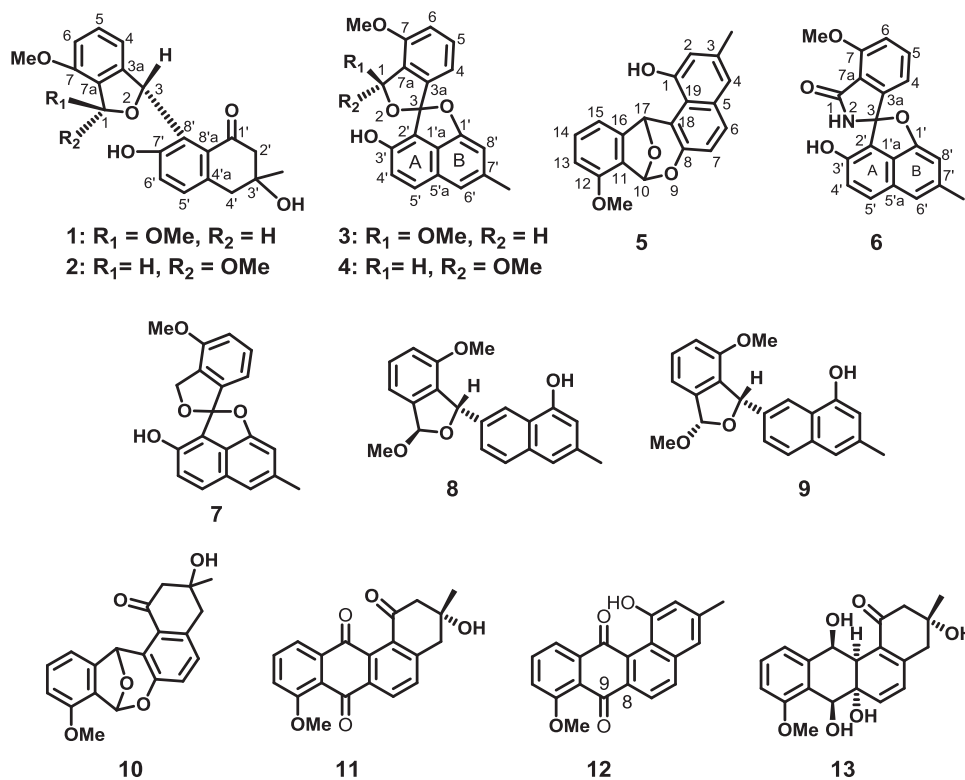


Figure 1 Structures of isolated compounds 1–13.

Table 1 ¹H NMR data (*J* in Hz, 600 MHz) for compounds 1–4 and 6

Position	1 ^a	2 ^a	3 ^b	4 ^b	6 ^b
1	6.47 (1H, d, 2.1)	6.43 (1H, s)	6.34 (1H, s)	6.34 (1H, s)	
3	7.52 (1H, brd)	7.44 (1H, s)			
4	6.59 (1H, d, 7.8)	6.63 (1H, d, 7.6)	6.59 (1H, d, 7.8)	6.65 (1H, d, 7.8)	7.18 (1H, d, 7.8)
5	7.29 (1H, t, 7.8)	7.23 (1H, t, 7.6)	7.39 (1H, t, 7.8)	7.44 (1H, t, 7.8)	7.58 (1H, t, 7.8)
6	6.93 (1H, d, 7.8)	6.86 (1H, d, 7.6)	7.08 (1H, d, 7.8)	7.07 (1H, d, 7.8)	7.32 (1H, d, 7.8)
7					
7a					
2'	2.76 (1H, d, 16.2), 2.81 (1H, d, 16.2)	2.72 (1H, d, 15.0), 2.93 (1H, d, 15.0)			
4'	3.01 (1H, d, 16.2), 3.11 (1H, d, 16.2)	3.03 (1H, d, 16.5), 3.14 (1H, d, 16.5)	7.14 (1H, d, 9.0)	7.14 (1H, d, 9.0)	7.05 (1H, d, 7.8)
5'	7.21 (1H, d, 8.4)	7.13 (1H, d, 8.4)	7.65 (1H, d, 9.0)	7.68 (1H, d, 9.0)	7.56 (1H, d, 7.8)
6'	6.97 (1H, d, 8.4)	6.90 (1H, d, 8.4)	7.10 (1H, s)	7.10 (1H, s)	7.04 (1H, s)
8'			6.52 (1H, s)	6.54 (1H, s)	6.51 (1H, s)
1-OCH ₃	3.50 (3H, s)	3.43 (3H, s)	3.45 (3H, s)	3.56 (3H, s)	
7-OCH ₃	3.92 (3H, s)	3.86 (3H, s)	3.93 (3H, s)	3.96 (3H, s)	3.39 (3H, s)
3'-CH ₃	1.40 (3H, s)	1.38 (3H, s)			
7'-CH ₃			2.44 (3H, s)	2.45 (3H, s)	2.41 (3H, s)
2-NH					9.47 (1H, s)
3'-OH					9.75 (1H, brs)

^aData were measured in CD₃OD.

^bData were measured in acetone-*d*₆.

81.3), one oxygenated tertiary carbon (δ_C 71.2), two methoxy groups (δ_C 54.9 and 53.9), two methylenes (δ_C 54.4 and 44.5) and one methyl carbon (δ_C 27.3) were observed. The isobenzofuran moiety (unit A) in 1 was established from the interpretation of heteronuclear multiple bond correlation (HMBC) spectroscopic data. Long-range HMBC correlations from H-6/C-7a and C-4; H-5/C-7 and C-3a; and H-4/C-7a and C-6 pointed to a 1,2,3-trisubstituted benzene ring system. The HMBC couplings of oxymethines H-1/C-7 and C-3;

H-3/C-7a and C-4 allowed the 1,3-dihydroisobenzofuran ring system to be confidently assigned. The position of two methoxy groups was deduced from the HMBC correlations of MeO-7/C-7 and MeO-1/C-1 (Figure 2). The proposed structure of 3,7-dihydroxy-3-methyl-1-tetralone (unit B) in 1 was confirmed by the HMBC couplings of H-6'/C-8' and C-4'a; H-5'/C-7' and C-8'a; H₂-4'/C-8'a and C-2'; Me-3'/C-4' and C-2'; and H₂-2'/C-8'a and C-4'. It was also supported by comparing its spectral data with the known related (-)-

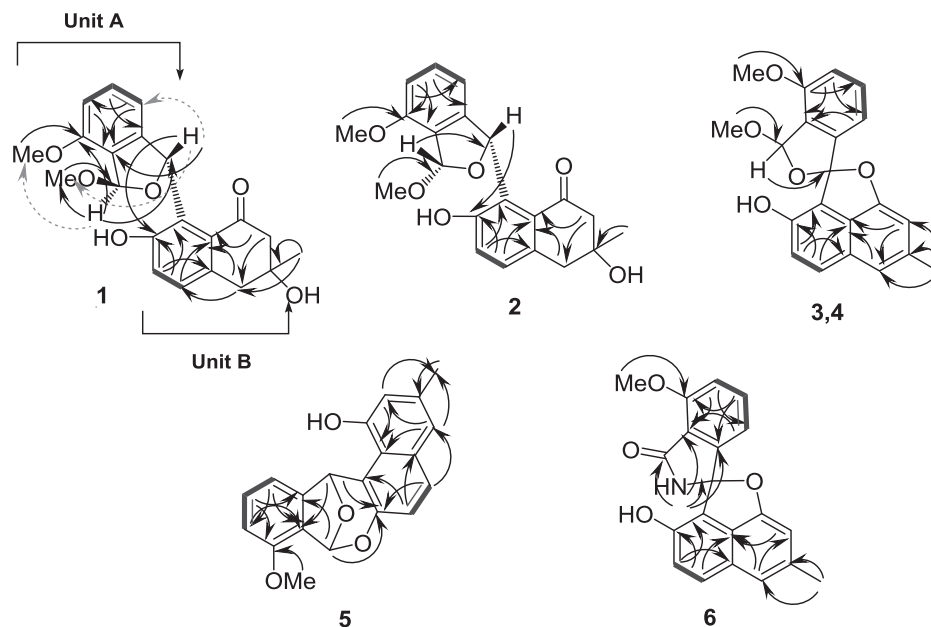


Figure 2 HMBC (—), ^1H - ^1H -COSY (—) and NOESY (---) NMR correlations of compounds **1-6**. A full color version of this figure is available at *The Journal of Antibiotics* journal online.

1,4-dimethoxy-3-(3'-hydroxy-3'-methyl-1'-tetralone)-1(3H)-isobenzofuran.²¹ HMBC correlations from H-3 to C-7' through 3J coupling revealed the connectivity of the 1,3-dihydroisobenzofuran fragment to C-8' of the tetralone moiety. The relative configuration of **1** was assigned by an analysis of the NOESY spectrum and proton coupling constants (Table 1). The NOESY correlation observed between H-3/MeO-1 and H-4; H-1/MeO-7 and the small coupling constant (2.1 Hz) between H-3 and H-1 suggested the relative configuration of C-3 and C-1 (Figure 1). In the case of isobenzofuran derivatives, the coupling constant across the dual homoallylic $^5J_{\text{H,H}}$ is frequently larger for *trans* than for *cis* isomers.²² The structure of **1** was supported by comparisons of the spectral data with emycin E.²³ On the basis of these data, compound **1** was established and named elmenol C (Figure 1).

Compound **2** was also isolated as a brown amorphous solid with positive specific rotation ($[\alpha]_{\text{D}}^{20} +82.1$ in MeOH). The HRESIMS of **2** gave a peak at m/z 393.1316 $[\text{M}+\text{Na}]^+$, which was consistent with the molecular formula $\text{C}_{21}\text{H}_{22}\text{O}_6$, similar to compound **1**. Comparisons of the UV spectra and 1D (Table 1) and 2D NMR data (Figure 2) of **2** and **1** showed that both compounds had the same carbon skeleton and only differed in their stereochemistries. A careful examination of the ^1H NMR data of **2** and **1** revealed differences in the chemical shifts and multiplicity between H-3 (**2**: δ_{H} 7.44 (s); **1**: 7.52 (brd)) and H-1 (**2**: 6.43 (s); **1**: 6.47 (d, $J = 2.1$ Hz)). The observed J -value of ca. 0 Hz between H-1 and H-3 and the absence of a NOESY correlation between H-3 and MeO-1 led to the assignment of H-1 and H-3 in the *cis*-configuration. Thus, compounds **2** and **1** are epimers at C-1. Based on these results, the structure of **2** was assigned to elmenol D. Compounds **1** and **2** are new members of angucyclines and may be a product of a Baeyer–Villiger-style oxidation of tetrangulol methyl ether (**12**).²³

Compounds **3** and **4** were obtained as brown solids. In the HPLC chromatogram, the two compounds appeared as a single peak. However, two sets of signals with 2:1 intensities in the ^1H and ^{13}C NMR spectra proposed that **3** and **4** are a mixture of two inseparable metabolites. The MW of the mixture was 350 Daltons based on (+)-ESIMS. The molecular formula was estimated to be $\text{C}_{21}\text{H}_{18}\text{O}_5$ by

Table 2 ^{13}C NMR data (150 MHz) for elmenols C-F (**1-4**) and H (**6**)

Position	1 ^a	2 ^a	3 ^b	4 ^b	6 ^b
1	107.1	107.6	106.3	106.3	167.9
3	81.3	80.1	120.4	121.0	100.0
3a	144.6	144.5	142.4	142.2	133.0
4	114.6	113.8	115.2	115.21	115.6
5	131.9	130.8	132.9	133.6	132.3
6	110.1	110.3	112.6	113.1	114.8
7	154.5	155.0	156.1	156.0	155.1
7a	125.1	125.0	126.3	125.8	130.5
1'	201.0	201.7	157.4	157.21	156.9
1'a	—	—	128.5	128.8	128.4
2'	54.4	54.5	126.4	126.5	124.9
3'	71.2	71.5	150.3	149.7	148.3
4'	44.5	43.7	122.0	122.3	120.7
4'a	134.8	134.8 ^c	—	—	—
5'	131.2	131.3 ^c	128.3	128.2	126.3
5'a	—	—	118.0	118.4	115.8
6'	122.8	122.8	116.0	116.4	114.6
7'	155.7	155.0	137.0	137.2	132.3
8'	125.0	126.2	104.0	104.4	102.7
8'a	131.9	130.3	—	—	—
1-OCH ₃	53.9	53.5	54.6	55.3	—
7-OCH ₃	54.9	54.8	56.0	56.2	55.8
3'-CH ₃	27.3	27.3	—	—	—
7'-CH ₃	—	—	22.7	23.3	22.4

^aData were measured in CD_3OD .

^bData were measured in acetone- d_6 .

^cValues were obtained from HMBC correlations.

HRESIMS m/z 373.1067 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{21}\text{H}_{18}\text{O}_5\text{Na}$ 373.1052, $\Delta +1.5$ m.m.u.), suggesting that **3** and **4** had the same molecular compositions. The ^1H and COSY NMR spectra (Table 1, Supplementary Figures S10 and S12) of **3** and **4** indicated the presence of an aromatic ABC spin system, two *ortho*-coupled aromatic signals, and two singlet aromatic protons. The aliphatic patterns of **3** and **4**

revealed one oxygenated methine proton, two methoxy groups, and one methyl signal. The ^{13}C NMR data (Table 2) of **3** and **4** showed seven aromatic methines, eleven quaternary carbon atoms, two methoxy groups, and one methyl carbon. An analysis of the 2D NMR spectra of **3** and **4** (Figure 2) revealed that they are closely related to elmonin (**7**).¹⁴ However, **3** and **4** had an additional methoxy group and the oxygenated methylene in elmonin ($\delta_{\text{H/C}}$ 5.30, 5.22/71.9)¹⁴ was replaced by a hemiacetal methine ($\delta_{\text{H/C}}$ 6.34/106.3). HMBC correlations of the methoxy protons to the hemiacetal methine carbon (C-1) revealed that **3** and **4** are a C-1 methoxylated derivative of elmonin. These results indicate that **3** and **4** are isomers with different configurations at C-1 (Figure 1). New compounds **3** and **4** were named elmenols E and F. Compounds **3** and **4** may be formed biosynthetically by oxidative ring cleavage C8/C9 of the precursor **12** followed by rearrangements.

Compound **5** was obtained as a brown solid with positive specific rotation ($[\alpha]_{\text{D}}^{20} +7.5$ in MeOH). The molecular formula of **5** was assigned as $\text{C}_{20}\text{H}_{16}\text{O}_4$ on the basis of the analysis of (+)-HRESIMS data (found m/z 343.0942 $[\text{M}+\text{Na}]^+$, calcd for 343.0963). ^1H NMR and COSY spectra (Supplementary Figures S15 and S17) showed the presence of two coupled spin systems, along with two oxymethines (δ_{H} 7.30 (s) and 6.78 (s)) that appeared as singlets. The first spin system involved two doublets (δ_{H} 6.86 (d, $J=7.8$ Hz) and 7.08 (d, $J=7.8$ Hz)) and one triplet (δ_{H} 7.21 (t, $J=7.8$ Hz)), which was attributed to a 1,2,3-trisubstituted aromatic system. The second spin system consisted of two *ortho*-coupled aromatic protons (δ_{H} 7.49 (d, $J=8.7$ Hz) and 6.82 (d, $J=8.7$ Hz)). In addition, one methoxy group (δ_{H} 3.89 (s)) and one methyl proton (δ_{H} 2.32 (s)) were observed. The ^{13}C NMR spectrum (Supplementary Figure S16) indicated 20 carbon signals, as required by HRESIMS. There were seven sp^2 aromatic methine carbons (δ_{C} 132.6, 129.9, 120.5, 120.9, 113.8, 113.4 and 112.1), nine sp^2 quaternary carbons (δ_{C} 155.7, 154.7, 151.5, 147.9, 134.8, 132.7, 124.6, 120.8 and 120.4), two oxymethine signals (δ_{C} 100.8 and 80.5), one methoxy group (δ_{C} 56.7) and one methyl group (δ_{C} 22.0). In the HMBC spectrum (Supplementary Figure S19), the correlations of H-15/C-11 and C-13; H-14/C-16 and C-12; H-13/C-15 and C-11; and 12-OMe/C-12 supported the trisubstituted aromatic ring bearing a methoxy group at C-12. In addition, long-range HMBC couplings from H-2 and H-4/C-19 and 3-CH₃; H-7 to C-18 and C-5; and H-6 to C-8 and C-4 supported the structure of a tetrasubstituted benzene ring fused with the substituted aryl group. HMBC correlations from 3-CH₃ protons to C-4, C-3 and C-2 fixed the position of the methyl group at C-3. The important HMBC correlations of both oxymethine protons (H-17 to C-11 and C-8; and H-10 to C-16 and C-8) support the structure of **5**. Compound **5** was confirmed by comparisons of NMR data with the isolated and structurally related LS1924A (**10**). New compound **5** was assigned to elmenol G. It is a ring-expanded angucycline with a tetracyclic ring structure, similar to emycin D.²³

Compound **6** was isolated as a white amorphous solid with a positive optical rotation value ($[\alpha]_{\text{D}}^{20} +80.7$ in MeOH). Its molecular formula was $\text{C}_{20}\text{H}_{15}\text{NO}_4$ from the (-)-HRESIMS analysis m/z 332.0924 $[\text{M}-\text{H}]^-$ (calcd for $\text{C}_{20}\text{H}_{14}\text{NO}_4$ 332.0923). The IR spectrum of **6** showed an absorption band due to hydroxy (3380 cm^{-1}) and carbonyl (1679 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra (Supplementary Figures S20 and S20) of **6** were similar to those of compounds **3** and **4**. The major differences in the ^1H NMR spectrum for **6** were the absence of the oxymethine proton and appearance of a sharp singlet NH proton (δ_{H} 9.47 (s)). The ^{13}C NMR spectrum of **6** lacked the oxymethine carbon that appears in **3** and **4** and instead had a carbonyl group (δ_{C} 167.9). In addition, an upfield shift of C-3 and

C-3a were observed for **6**. Long-range HMBC correlations were observed from NH (δ_{H} 9.47 (s)) to C-7a, C-3, C-3a and C-1 revealing the presence of a 1-isoindolinone moiety in **6**. A combination of the above results led to the structural elucidation of elmenol H (**6**). To the best of our knowledge, elmenol H (**6**) is one of the rare angucycline-containing isoindolinone moieties.

We evaluated the bioactivities of elmenols C-H (**1-6**) for their abilities to overcome TRAIL resistance in AGS cells. This cell line has been widely used as a model system to evaluate cancer cell apoptosis and is reported to be refractory to the induction of apoptosis by TRAIL.²⁴ To assess the effects of compounds **1-6** on cell viability in the presence and absence of TRAIL, AGS cells were treated with the indicated agents and subjected to a fluorometric microculture cytotoxicity assay.²⁵ Luteolin (Lut) was used as a positive control at $17.5\text{ }\mu\text{M}$.²⁶ The assay results (Figure 3) showed that compounds **1** and **2** did not show significant difference in the cell viability between the presence and absence of TRAIL (100 ng ml^{-1}), whereas compound **5** at $10\text{ }\mu\text{M}$ and compound **6** at $50\text{ }\mu\text{M}$ exhibited 25 and 30% decreases, respectively, in cell viability in the presence of TRAIL (100 ng ml^{-1}) compared with in the absence of TRAIL. The mixture of elmenols E (**3**) and F (**4**) along with compounds **5** and **6** did not produce any significant reductions in cell viability with TRAIL.

Overall, our search for new bioactive natural products from

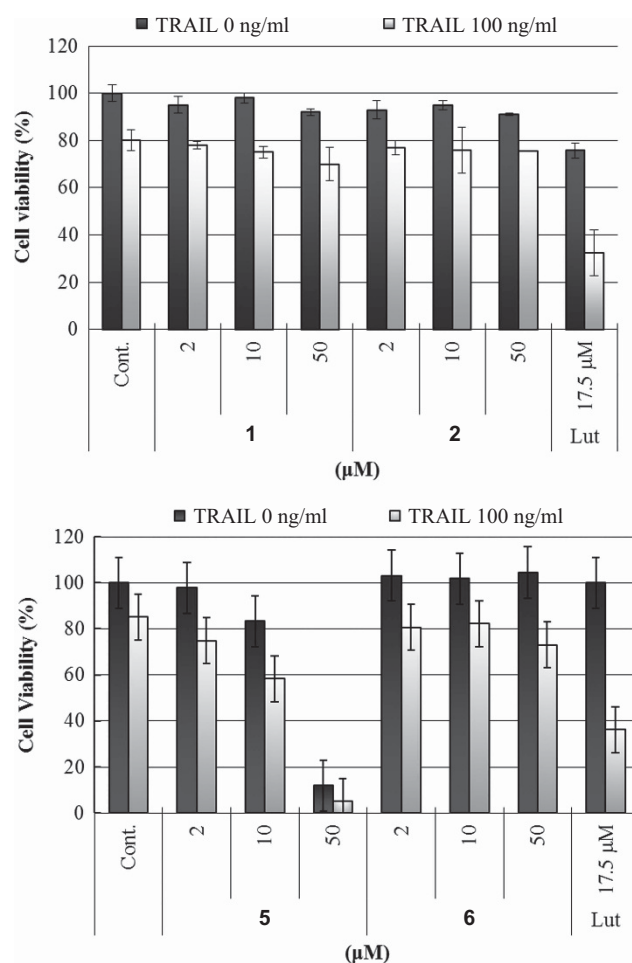


Figure 3 Effect of the isolated compounds **1**, **2**, **5** and **6** on the cell viability of AGS cells in the presence and absence of TRAIL. The standard error bar represents the means ($n=3\pm\text{s.d.}$). A full color version of this figure is available at *The Journal of Antibiotics* journal online.

actinomycetes yielded the terrestrial *Streptomyces* sp. IFM 11490, which produced six new angucycline metabolites named elmenols C-H (1-6). These new structures will enrich the structural diversity of the angucycline family of natural products. Elmenols G (5) and H (6) exhibited moderate TRAIL resistance-overcoming activities in AGS cells.

MATERIALS AND METHODS

General experimental procedures

NMR spectra were recorded on JEOL ECZ600 spectrometers with deuterated solvents, the chemical shifts of which were used as an internal standard. Low-resolution electrospray ionization mass spectra (ESIMS) were obtained on a Shimadzu LCMS-2020 spectrometer. High-resolution electrospray ionization mass spectra (HRESIMS) were recorded on an AccuTOF-T100LP (JEOL) mass spectrometer. IR spectra were measured on ATR (attenuated total reflection) on a JASCO FT-IR 230 spectrophotometer. UV spectra were measured on a Shimadzu UV mini-1240 spectrometer.

Identification of the bacterial strain

The identification of *Streptomyces* sp. IFM 11490 was performed by Professor Tohru Gonoï at the Medical Mycology Research Center, Chiba University, at which a voucher specimen is deposited with the code IFM 11490. This strain was identified on the basis of 16S rRNA gene sequences. A BLAST analysis revealed that the isolate IFM 11490 belonged to the genus *Streptomyces* sp. and had 98.7% similarity with the 16S rRNA of *Streptomyces hyderabadensis*.

Culture media

Waksman liquid medium consisting of glucose (2.0 g/100 ml), meat extract (0.5 g/100 ml), peptone (0.5 g/100 ml), dried yeast (0.3 g/100 ml), NaCl (0.5 g/100 ml) and CaCO₃ (0.3 g/100 ml) was used. The composition of M₂ medium was malt extract (10 g/100 ml), yeast extract (4 g/100 ml) and glucose (4 g/100 ml).

Fermentation

The isolated strain was cultivated from glycerol stock on Waksman agar plates at 28 °C for 3 days. To prepare the seed culture, chunks of a well-grown agar plate were used to inoculate 5 × 500-cm³ Sakaguchi flasks each containing 100 ml of Waksman liquid. Cultures were grown at 28 °C for 3 days with reciprocal shaking at 200 r.p.m. An aliquot of the seed culture (20 ml) was subsequently used to inoculate 32 × 3-L flasks each containing 750 ml of liquid Waksman medium. Fermentation was continued at 28 °C with shaking (200 r.p.m.) for 7 days. The strain was cultured in the same manner on M₂ medium.

Extraction and isolation

The culture broth (24L) of Waksman medium was centrifuged at 6000 r.p.m. for 20 min and then extracted three times with ethyl acetate. The biomass (mycelium) was extracted three times with acetone. After the removal of acetone, the aqueous solution was extracted three times with ethyl acetate. Extracts from the water phase and biomass were combined under reduced pressure. Fractionation of the crude extract (6.90 g) using silica gel PSQ100B column chromatography with a gradient of 0–100% MeOH/CHCl₃ resulted in the generation of five fractions (I-V). Fraction I (293.3 mg) was purified by Sephadex LH-20 (φ 25 × 240 mm) eluted with MeOH, and this was followed by preparative HPLC (YMC-Pack ODS-AM, 10 × 250 mm; eluent, 65% MeOH; flow rate, 2.0 ml min⁻¹; UV detection at 254 nm; YMC Co., Ltd., Kyoto, Japan) to give elmenols C (1, 1.7 mg, t_R 14 min) and D (2, 1.1 mg, t_R 16 min) along with the known LS1924A (10, 2.9 mg). Fraction II (200 mg) was applied to an ODS column (φ 30 × 230 mm) eluted with MeOH to afford four sub-fractions named **Ia** (54.0 mg), **Ib** (44.4 mg), **Ic** (8.0 mg) and **IId** (97.4 mg). Sub-fraction **Ia** was purified by preparative HPLC (YMC-Pack ODS-AM, 10 × 250 mm; eluent, 60% MeOH; flow rate, 2.0 ml min⁻¹; UV detection at 254 nm) to give an elmenol E (3)/F (4) mixture (2.9 mg, t_R 14 min). Compound **II** (26.4 mg) was isolated as a red solid from sub-fraction **Ib**. Fraction **III** (1.20 g) was fractionated using silica gel 60N column

chromatography (φ 30 × 230 mm) eluted with hexane/EtOAc (1:1) to give three sub-fractions named **IIIa** (35.0 mg), **IIIb** (31.8 mg) and **IIIc** (890 mg). Sub-fraction **IIIa** was also purified by HPLC (YMC-Pack ODS-AM, 10 × 250 mm; eluent, 65% MeOH; flow rate, 2.0 ml min⁻¹; UV detection at 254 nm) to afford elmenol G (5, 1.7 mg, t_R 74 min). Compound **12** (4.7 mg) was obtained as a brown solid from sub-fraction **IIIb**. When the strain was cultured in M₂ medium (12L) and extracted under similar conditions, it gave 2.20 g of a crude extract. The extract was applied to silica gel PSQ100B column chromatography using the MeOH/CHCl₃ gradient (0 to 100% MeOH) to give four fractions (VI-IX). Fraction **VII** (666.1 mg) was purified by the ODS column (φ 30 × 230 mm) using H₂O: MeOH (1:1–0:1) as the eluent to yield 3.2 mg of elmenol H (6). Fraction **VIII** (295.1 mg) was purified by preparative HPLC using 80% MeOH in H₂O (YMC-Pack ODS-AM, 10 × 250 mm; flow rate, 2.0 ml min⁻¹) and UV detection at 254 nm to give angucyclinone (**13**).

Cell cultures

AGS cells were derived from the Institute of Development, Aging and Cancer, Tohoku University. Cells were cultured in RPMI-1640 medium (Wako) with 10% fetal bovine serum and maintained in a humidified incubator at 37 °C in 5% CO₂/95% air.

TRAIL resistance test

TRAIL resistance was assessed by comparisons of cell viability in the presence and absence of TRAIL with TRAIL-resistant AGS cell lines. AGS cells were seeded on a 96-well culture plate (6 × 10³ cells per well) in 200 μl of Roswell Park Memorial Institute medium containing 10% fetal bovine serum. Cells were incubated at 37 °C in a 5% CO₂ incubator for 24 h. Test samples with or without TRAIL (100 ng ml⁻¹) at different doses were added to each well. After a 24-h incubation, cells were washed with phosphate-buffered saline, and 200 μl of phosphate-buffered saline containing fluorescein diacetate (10 μg ml⁻¹) was added to each well.²⁵ The plates were then incubated at 37 °C for 1 h, and fluorescence was measured in a 96-well scanning spectrofluorometer at 538 nm with excitation at 485 nm.

Elmenol C (1). Brown solid; [α]_D²⁰—32.3 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 328 (2.4) and 230 (2.7) nm; IR ν_{max} (ATR) ca. 3396, 2357, 1671, 1631, 1485 and 1278 cm⁻¹; ¹H and ¹³C NMR data in Tables 1 and 2; (+)-HRESIMS *m/z* 393.1326 [M+Na]⁺ (calcd for C₂₁H₂₂O₆Na 393.1314).

Elmenol D (2). Brown solid; [α]_D²⁰+82.1 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 328 (3.9) and 230 (4.1) nm; IR ν_{max} (ATR) ca. 3354, 2358, 1671, 1631 and 1484 cm⁻¹; ¹H and ¹³C NMR data in Tables 1 and 2; (+)-HRESIMS *m/z* 393.1316 [M+Na]⁺ (calcd for C₂₁H₂₂O₆Na 393.1314).

Elmenols E (3) and *F* (4). Brown solid; UV (MeOH) λ_{max} (log ε) 342 (3.4), 300 (3.5), 279 (3.9), 246 (4.2) and 218 (4.4) nm; IR ν_{max} (ATR) ca. 3335, 1614, 1488 and 1292 cm⁻¹; ¹H and ¹³C NMR data in Tables 1 and 2; (+)-HRESIMS *m/z* 373.1067 [M+Na]⁺ (calcd for C₂₁H₁₈O₅Na 373.1052).

Elmenol G (5). Brown solid; [α]_D²⁰+7.5 (c 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 382 (3.1), 245 (3.9) and 218 (4.4) nm; IR ν_{max} (ATR) ca. 3350, 1614, 1485 and 1278 cm⁻¹; ¹H NMR (600 MHz, acetone-*d*₆) δ 7.49 (1H, d, J = 8.7 Hz, H-6), 7.30 (1H, s, H-17) 7.21 (1H, t, J = 7.8 Hz, H-14), 7.08 (1H, d, J = 7.8 Hz, H-15) 7.02 (1H, d, J = 1.1 Hz, H-4), 6.86 (1H, d, J = 7.8 Hz, H-13), 6.82 (1H, d, J = 8.7 Hz, H-7), 6.81 (1H, d, J = 1.1 Hz, H-2), 6.78 (1H, s, H-10), 3.89 (3H, s, 12-OCH₃), 2.32 (3H, s, 3-CH₃) p.p.m.; ¹³C NMR (150 MHz, acetone-*d*₆) δ 155.7 (C-12), 154.7 (C-1), 151.5 (C-16), 147.9 (C-8), 134.8 (C-3), 132.7 (C-5), 132.6 (C-14), 129.9 (C-6), 124.6 (C-11), 120.5 (C-7), 120.9 (C-4), 120.8 (C-19), 120.4 (C-18), 113.8 (C-2), 113.4 (C-15), 112.1 (C-13), 100.8 (C-10), 80.5 (C-17), 56.7 (12-OCH₃), 22.0 (3-CH₃) p.p.m.; (+)-HRESIMS *m/z* 343.0942 [M+Na]⁺ (calcd for C₂₀H₁₆O₄Na 343.0963).

Elmenol H (6). White amorphous solid; [α]_D²⁰+80.7 (c 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 383 (3.2), 291 (3.8), 243 (4.4), and 212 (4.7) nm; IR ν_{max} (ATR) ca. 3380, 2920, 1679, and 1279 cm⁻¹; ¹H and ¹³C NMR data in Tables 1 and 2; (-)-HRESIMS *m/z* 332.0924 [M-H]⁻ (calcd for C₂₀H₁₄NO₄ 332.0923).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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- 1 Kharel, M. K. *et al.* Angucyclines: biosynthesis, mode-of-action, new natural products, and synthesis. *Nat. Prod. Rep.* **29**, 264–325 (2012).
- 2 Rohr, J. & Thiericke, R. Angucycline group antibiotics. *Nat. Prod. Rep.* **9**, 103–137 (1992).
- 3 Fan, K. *et al.* Evaluation of the cytotoxic activity of new jadomycin derivatives reveals the potential to improve its selectivity against tumor cells. *J. Antibiot.* **65**, 449–452 (2012).
- 4 Abdelfattah, M. S., Kharel, M. K., Hitron, J. A., Baig, I. & Rohr, J. Moromycins A and B, isolation and structure elucidation of C-glycosylangucycline-type antibiotics from *Streptomyces* sp. KY002. *J. Nat. Prod.* **71**, 1569–1573 (2008).
- 5 Song, Y. *et al.* Cytotoxic and antibacterial angucycline and prodigiosin analogues from the deep-sea derived *Streptomyces* sp. SCSIO 11594. *Mar. Drugs* **13**, 1304–1316 (2015).
- 6 Brötz, E. *et al.* Amycomycins C and D, new angucyclines from *Kitasatospora* sp. *Tetrahedron Lett.* **55**, 5771–5773 (2014).
- 7 Helaly, S. E. *et al.* Warkmycin, a novel angucycline antibiotic produced by *Streptomyces* sp. Acta 2930. *J. Antibiot.* **66**, 669–674 (2013).
- 8 Ma, M. *et al.* Angucyclines and angucyclinones from *Streptomyces* sp. CB01913 featuring C-ring cleavage and expansion. *J. Nat. Prod.* **78**, 2471–2480 (2015).
- 9 Ishibashi, M in *Progress in the Chemistry of Organic Natural Products*, Vol. 99 (eds Kinghorn, A. D., Falk, H. & Kobayashi, J.) 147–198 (Springer, 2014).
- 10 Abdelfattah, M. S., Ishikawa, N., Karmakar, U. K., Yamaku, K. & Ishibashi, M. New phenazine analogues from *Streptomyces* sp. IFM 11694 with TRAIL resistance-overcoming activities. *J. Antibiot.* **69**, 446–450 (2016).
- 11 Abdelfattah, M. S., Ishikawa, N., Utpal, K. & Ishibashi, M. Sulfotanone, a new alkyl sulfonic acid derivative from *Streptomyces* sp. IFM 11694 with TRAIL resistance-overcoming activity. *J. Nat. Med.* **70**, 266–270 (2016).
- 12 Abdelfattah, M. S., Toume, K. & Ishibashi, M. Yoropyrazone, a new naphthopyridazone alkaloid isolated from *Streptomyces* sp. IFM 11307 and evaluation of its TRAIL resistance-overcoming activity. *J. Antibiot.* **65**, 245–248 (2012).
- 13 Abdelfattah, M. S., Toume, K. & Ishibashi, M. New pyranonaphthoquinones and phenazine alkaloid isolated from *Streptomyces* sp. IFM 11307 with TRAIL resistance-overcoming activity. *J. Antibiot.* **64**, 729–734 (2011).
- 14 Yixizhuoma *et al.* Novel cytotoxic isobenzofuran derivatives from *Streptomyces* sp. IFM 11490. *Tetrahedron Lett.* **56**, 6345–6347 (2015).
- 15 Abdelfattah, M. S., Arai, M. A. & Ishibashi, M. Bioactive secondary metabolites with unique aromatic and heterocyclic structures obtained from terrestrial actinomycetes Species. *Chem. Pharm. Bull.* **64**, 668–675 (2016).
- 16 Waksman, S. A. *The Actinomycetes: Classification, Identification and Descriptions of Genera and Species* Vol. 2, 61–292 (Williams & Wilkins Co., Baltimore, MD, USA, 1961).
- 17 Chen, C. *et al.* Isolation and characterization of LS1924A, a new analog of emycins. *J. Antibiot.* **65**, 433–435 (2012).
- 18 Shigihara, Y. *et al.* 6-Deoxy-8-O-methylrabelomycin and 8-O-methyl-rabelomycin from a *Streptomyces* species. *J. Antibiot.* **41**, 1260–1264 (1988).
- 19 Abdelfattah, M., Maskey, R. P., Asolkar, R. N., Grün-Wollny, I. & Laatsch, H. Seitomycin: isolation, structure elucidation and biological activity of a new angucycline antibiotic from a terrestrial Streptomycete. *J. Antibiot.* **56**, 539–542 (2003).
- 20 Kieser, T., Bibb, M. J., Buttner, M. J., Chater, K. F. & Hopwood, D. A. *Practical Streptomyces Genetics* 2nd edn. (The John Innes Center Foundation, Norwich, CT, USA, 2000).
- 21 Zhang, S. M. *et al.* Isolation, stereochemical study, and cytotoxic activity of isobenzofuran derivatives from a marine *Streptomyces* sp. *Chirality* **27**, 82–87 (2015).
- 22 Barfield, M., Spear, R. J. & Sternhell, S. Interproton spin-spin coupling across a dual path in five-membered rings. *J. Am. Chem. Soc.* **93**, 5322–5327 (1971).
- 23 Gerlitz, M., Udvarnoki, G. & Rohr, J. Biosyntheses of novel emycins from the mutant strain *Streptomyces cellulosae* ssp. *griseoincarnatus* 1114–2. *Angew. Chem. Int. Ed.* **34**, 1617–1621 (1995).
- 24 Srivastava, R. K. TRAIL/Apo-2L: mechanisms and clinical applications in cancer. *Neoplasia* **3**, 535–546 (2001).
- 25 Lindhagen, E., Nygren, P. & Larsson, R. The fluorometric microculture cytotoxicity assay. *Nat. Protoc.* **3**, 1364–1369 (2008).
- 26 Horinaka, M. *et al.* Luteolin induces apoptosis via death receptor 5 upregulation in human malignant tumor cells. *Oncogene* **24**, 7180–7189 (2005).

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