Izumiphenazines A−C: Isolation and Structure Elucidation of Phenazine Derivatives from *Streptomyces* sp. IFM 11204

Mohamed S. Abdelfattah,[†,‡] Toume Kazufumi,[†] and Masami Ishibashi*,[†]

Graduate School of Pharmaceutical Sciences, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba 263-8522, Japan, and Chemistry Department, Faculty of Science, Helwan University, Cairo 11795, Egypt

Three new phenazine derivatives, named izumiphenazines A−C (**1**−**3**), and the known phenazine-1,6-dicarboxylic acid (**4**) were isolated from *Streptomyces* sp. IFM 11204. The structures of the isolated compounds were elucidated by means of spectroscopic methods including UV, IR, HRESIMS, and 1D and 2D NMR. Compounds **1**−**3** were evaluated for their activity in overcoming TRAIL (TNF-related apoptosis-inducing ligand) resistance in human gastric adenocarcinoma cells. Compounds **2** (30 μM) and **3** (20 μM) in combination with TRAIL showed synergistic activity in sensitizing TRAIL-resistant AGS cells.

Phenazines are heterocyclic compounds that are produced naturally from different microorganisms, including *Pseudomonas* spp.,[1] *Streptomyces* spp.,[2] *Pelagiobacter variabilis*,[3] *Pantoea agglomerans*,[4] and *Vibrio* sp.[5] The phenazine group of compounds exhibits a broad range of biological activities, such as antibacterial, antimalarial, antitumor, and antiparasitic activities.[6] The first phenazine derivative isolated from streptomycetes was the antibiotic griseolutein.[7] Since then, an increasing number of phenazine derivatives with different activities have been isolated from different *Streptomyces* species (e.g., *griseolutein*, *luteogriseus*, *antibioticus*, and *prunicolor*).

In the course of our screening for bioactive compounds from actinomycetes,[8] we investigated the extract of the *Streptomyces* sp. IFM 11204 because it exhibited relatively polar yellow bands on TLC that turned orange upon spraying with anisaldehyde reagent, dark red with concentrated sulfuric acid, and reddish-brown with Dragendorff's reagent. This and the lack of a color reaction with sodium hydroxide pointed to the phenazine skeleton. We herein report fermentation, isolation, structure elucidation, and biological activity of three new phenazines designated as izumiphenazines A−C (**1**−**3**).

Results and Discussion

The *Streptomyces* sp. IFM 11204 was isolated from a soil sample collected from Izumi forest in Chiba city, Japan. Five-day agar cultures of the strain were used to inoculate 4 × 500 mL Sakaguchi flasks each containing 100 mL of liquid Waksman medium.[9] Fermentation was carried out at 28 °C for 5 days while shaking at 200 rpm. The seed culture (10 mL) was used to inoculate 10 3-L flasks each containing 500 mL of the same medium, which were incubated using similar conditions. After centrifugation, extraction, and evaporation, working up of the crude extract resulted in the isolation of the new izumiphenazines A−C (**1**−**3**) along with the known phenazine-1,6-dicarboxylic acid (**4**).

Compound **4** was isolated as a yellow solid with a molecular weight of 268 based on the negative mode of the ESI mass spectrum. From the deduced molecular formula and the NMR data, compound **4** was easily identified as phenazine-1,6-dicarboxylic acid and confirmed by comparison with the reference data.[10] Izumiphenazine A (**1**) was a red solid. The UV spectrum of **1** (maxima were visible at 451, 384, and 272 nm) resembles the UV spectra of many phenazine derivatives.[11] The IR spectrum of **1**

Table 1. NMR Data for Izumiphenazine A (**1**)[a] (150 and 600 MHz)

position	δ_C	δ_H (*J* in Hz)	HMBC
1	153.5		
2	111.7	7.11 d (7.9)	4, 17a, 1
3	131.3	7.59 t (7.9)	1, 4a,
4	118.0	7.38 d (7.9)	2, C-5a, 17a
4a	142.3		
5a	138.7		
6	130.6		
7	121.2	8.12 s	5a,, 6, 7a, 16b, 18
7a	158.7		
8a	88.7	5.59 dd (6.1, 10.4)	9, 9a
9	71.6	5.34 dd (3.3, 6.1)	8a, 9a, 16a
9a	149.7		
10a	135.9		
11	153.6		
12	111.8	7.32 d (7.7)	10a, 14
13	132.3	7.81 t (7.7)	11, 14a
14	118.4	7.71 d (7.7)	10a, 12, C-15a
14a	139.5		
15a	153.2		
16	33.3	4.19 dd (6.5, 14.8) 3.69 dd (7.2, 14.8)	8a, 9a, 15a, 16a, 16b
16a	40.4	4.80 m	15a, 16, 16b
16b	124.9		
16c	137.7		
17a	132.8		
18	165.7		
1-OH		10.77 s	1, 2, 17a
9-OH		6.33 d (3.3)	8a, 9, 9a
11-OH		10.30 s	10a, 11
18-OH		14.34 br s	

[a] Recorded in DMSO-d_6.

suggested absorption bands of hydroxyl (3370 cm^{-1}) and carbonyl group (1700 cm^{-1}). A molecular formula of $C_{25}H_{16}N_4O_6$ was deduced on the basis of HRESIMS at *m/z* 467.1004 [M − H]$^-$. The ^{1}H NMR spectrum of **1** (Table 1) showed four D$_2$O exchangeable protons [δ_H 14.34 (1H, br s), 10.77 (1H, s), 10.30 (1H, s), and 6.33 (1H, d, *J* = 3.3 Hz)]. The aromatic region of **1** exhibited one sharp singlet [δ_H 8.12 (1H, s)] and two ABC systems [δ_H 7.81 (1H, t, *J* = 7.7 Hz), 7.71 (1H, d, *J* = 7.7 Hz), 7.32 (1H, d, *J* = 7.7 Hz), 7.59 (1H, t, *J* = 7.9 Hz), 7.38 (1H, d, *J* = 7.9 Hz), and 7.11 (1H, d, *J* = 7.9 Hz)]. The aliphatic pattern of **1** revealed two oxygenated methine protons [δ_H δ 5.59 (1H, dd, *J* = 10.4, 6.1 Hz) and δ_H 5.34 (1H, dd, *J* = 6.1, 3.3 Hz)], one methine signal [δ_H 4.80 (1H, m)], and one methylene group [δ_H 4.19 (1H, dd, *J* = 14.8, 6.5 Hz) and 3.69 (1H, dd, *J* = 14.8, 7.2 Hz)]. The ^{13}C NMR spectrum of **1**, aided by heteronuclear multiple quantum coherence (HMQC),

* To whom correspondence should be addressed. Tel: +81-43-290-2913. Fax: +81-43-290-2913. E-mail: mish@p.chiba-u.ac.jp.
† Chiba University.
‡ Helwan University.

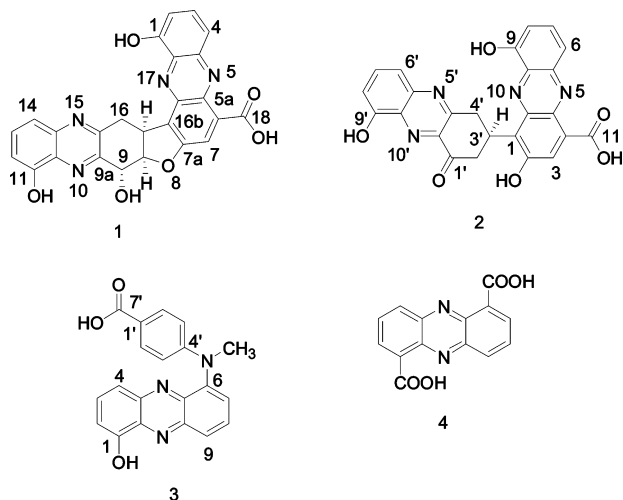**Figure 1.** Structure of izumiphenazines A−C (**1**−**3**) and phenazine-1,6-dicarboxylic acid (**4**).
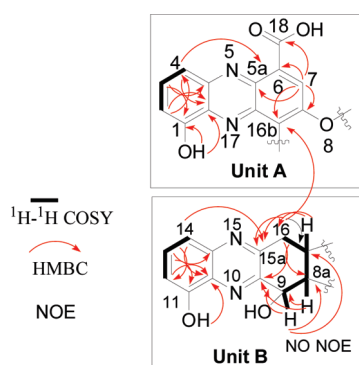

Figure 2. Selected 2D NMR correlations of izumiphenazine A (**1**).

showed one carbonyl (δ_C 165.7) and 20 sp² carbons, three of them being connected to oxygen atoms (δ_C 158.7, 153.6, and 153.5). In the aliphatic region three methine signals (δ_C 88.7, 71.6, and 40.4) and one methylene carbon (δ_C 33.3) were observed.

Analysis of the 2D NMR spectra (¹H−¹H COSY, HMQC, HMBC, and NOE) of izumiphenazine A (**1**) gave two units, A and B, as shown in Figure 2. In unit A, HMBC correlations of H-2 (δ_H 7.38) with C-4 and C-17a; H-3 (δ_H 7.59) with C-1 and C-4a; and H-4 (δ_H 7.11) with C-2 and C-17a suggested the presence of 1,2,3-trisubstituted benzene ring. The ¹H−¹H COSY NMR correlations between H-2−H-3−H-4 supported this assumption. Furthermore, the aromatic proton H-7 (δ_H 8.12) correlated strongly with C-5a, C-16b, and C-18 and weakly with C-6 and C-7a. These correlations revealed a substituted *m*-hydroxybenzoic acid moiety existed in **1**. By considering the presence of a phenazine moiety and due to J^4 HMBC coupling between H-4 (δ_H 7.11) and C-5a, the presence of a 1,6-disubstituted phenazine ring in **1** was confirmed. The proposed structure of unit A was supported by the close similarity of the chemical shifts with the known related metabolite phenacein.[12] In unit B (Figure 2), the long-range HMBC correlations of H-14 (δ_H 7.71) with C-10a, C-12; H-13 (δ_H 7.81) with C-11, C-14a; and H-12 (δ_H 7.32) with C-10a, C-14 also pointed to 1,2,3-trisubstituted benzene ring. The ¹H−¹H COSY couplings between H-12−H-13−H-14 supported this assignment. In addition, the HMBC couplings of H-8a (δ_H 5.59) with C-9, C-9a; H₂-16 (δ_H 4.19, 3.69) with C-8a, C-9a, C-15a, C-16a; H-16a (δ_H 4.83) with C-15a, C-16; and H-9 (δ_H 5.34) with C-8a, C-9a, C-16a revealed the structure of the other ring in unit B. The NOE between the OH-9 (δ_H 6.33) and OH-11 (δ_H 10.30) suggested the presence of a 1,9-disubstituted phenazine ring. These findings revealed that unit B possessed a substituted 1,2,3,4-tetrahydrophenazine-1,9-diol. Due to the HMBC
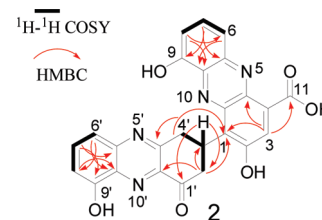

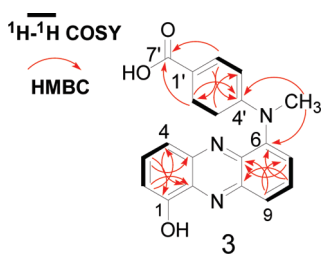**Figure 3.** Selected 2D NMR correlations of izumiphenazine B (**2**).

correlations especially from H₂-16 (δ_H 4.19, 3.69) to C-16b, and by considering the chemical shift of C-8a (δ_C 88.7), the connectivity between units A and B was confirmed and led to the skeleton of **1**. The assignment of the relative configuration of izumiphenazine A (**1**) was established from the ¹H NMR coupling constants and the NOE effect of H-8a (δ 5.59) with H-16a (δ 4.83). Since the NOE was not observed between H-8a (δ 5.59) and H-9 (δ 5.34), the structure of **1** was elucidated.

Izumiphenazine B (**2**) was also a red solid. It has a UV spectrum very similar to that of **1**, indicating a phenazine derivative. The molecular formula of **2** was analyzed as $C_{25}H_{16}N_4O_6$, a formula isomeric with **1**, by HRESIMS (*m/z* 467.0995, [M − H]⁻). Analysis of ¹H NMR data for **2** measured in DMSO-d_6 showed two D₂O exchangeable protons at δ_H 10.24 (1H, br s) and 8.29 (1H, br s) and seven aromatic signals between δ_H 7.88 and 7.12, indicating that **2** belongs to the same class of phenazine as **1**. The ¹H−¹H COSY NMR showed two aromatic spin systems (1,2,3-trisubstituted aromatic rings) as in **1**. In the aliphatic region, two methylene groups [δ_H 3.71 (1H, m, 4′-H$_b$), 3.62 (1H, m, 4′-H$_a$), 2.80 (1H, m, 2′-H$_b$), 2.65 (1H, m, 2′-H$_a$)] and only one methine proton [δ_H 4.92 (1H, m, H-3′)] were observed. When the ¹H NMR data of the aliphatic pattern of **2** were compared with those of **1**, major differences were observed. The two doublet of doublet methines in the proton spectrum of **1** at H-8a (δ_H 5.59) and H-9 (δ_H 5.34) had disappeared, and a new resonance at δ_H 2.80 and 2.65 arising from 2H was observed. Additionally, the D₂O exchangeable proton at δ_H 6.33 in **1** was not observed in **2**. The ¹³C NMR of **2** showed many similarities with the spectra of **1**. The obvious difference was the presence of a carbonyl group (δ_C 204.3) and an extra methylene carbon (δ_C 31.5) in **2**. Several of the relevant carbon chemical shifts were, however, obtained only by interpretation of HMBC and HMQC NMR data. Several correlation patterns derived from HMBC and COSY NMR spectroscopic data of **2** were found to be identical to those of **1**. The HMBC correlations from H₂-2′ (δ_H 2.80, 2.65) to C-1, C-3′, C-10′a; H-3′ (δ_H 4.92) to C-1′, C-2′; and H₂-4′ (δ_H 3.71, 3.62) to C-1, C-4′a allowed the construction of 9′-hydoxy-3′,4′-dihydrophenazine-1(2*H*)-one substituted at the 3′-position. Moreover, the COSY couplings between H-8′−H-7′−H-6′ and H₂-4′−H-3′−H₂-2′ confirmed this assumption (Figure 3). The absolute streochemistry of izumiphenazine B (**2**) was proposed by comparing the CD spectra with that of (3*R*)-3-(4′-biphenyl)tetralin-1-one.[13] Positive Cotton effects at 203 and 249 nm confirmed the 3′*R* absolute configuration of **2**.

Izumiphenazine C (**3**) was also a red solid. The molecular formula of $C_{20}H_{15}N_3O_3$ was determined by HRESIMS *m/z* 344.1044 [M − H]⁻. The ¹H NMR spectra of **3** (Table 2) showed six aromatic protons between δ_H 8.10 and 7.20, pointing also to the presence of a phenazine core. Additionally, a set of aromatic signals [δ_H 7.74 (2H, d, *J* = 8.1 Hz) and 6.74 (2H, d, *J* = 8.1 Hz)] was also present. The HMQC showed the presence of one *N*-methyl group at δ_H 3.54. The ¹³C NMR spectra of **3** revealed signals assigned to one carbonyl carbon (δ_C 168.3), eight quaternary sp² carbons (δ_C 153.8−123.8), and 10 aromatic methine carbons (δ_C 131.0−110.5). In the aliphatic region, one *N*-methyl carbon at δ_C 40.5 was observed. The HMBC correlations of H-2′/H-6′ (δ_H 7.74) with C-7′ and C-4′; H-3′/H-5′ (δ_H 6.74) with C-1′, C-3′, and C-5′; and the *N*-methyl group (δ_H 3.54) with C-4′ and C-6 revealed the existence of *N*-methyl-*p*-aminobenzoic acid linked to the C-6 of the phenazine core (Figure 4).

**Table 2.** NMR Data for Izumiphenazine C (**3**)^a (150 and 600 MHz)

position	δ_C	δ_H (*J* in Hz)	HMBC
1	153.8		
2	110.5	7.20 d (7.4)	4, 10a
3	132.3	7.76 t (7.4)	1, 4a,
4	118.4	7.64 d (7.4)	2, 10a
4a	143.7		
5a	138.0		
6	144.7		
7	127.1	8.10 d (7.8)	5a, 9
8	131.0	7.94 t (7.8)	6, 9a,
9	127.7	7.79 d (7.8)	5a, 7, 10a
9a	144.3		
10a	134.9		
1′	123.8		
2′	130.4	7.74 d (8.1)	4′, 6′, 7′
3′	113.8	6.74 d (8.1)	1′, 5′,
4′	152.2		
5′	113.8	6.74 d (8.1)	1′, 3′
6′	130.4	7.74 d (8.1)	2′, 4′, 7′
7′	168.3		
−NCH₃	40.5	3.54 s	3′, 4′, 6,

^{a} Recorded in DMSO-d_6.

Figure 4. Selected 2D NMR correlations of izumiphenazine C (**3**).

The isolated izumiphenazines A (**1**), B (**2**), and C (**3**), named with regard to their biological origin, "Izumi forest", are new members of this class of natural products. Several phenazine dimers such as phenazostatins A−D[14−16] and esmeraldins A and B[17] were isolated from actinomycetes. To the best of our knowledge, izumiphenazine A (**1**) is the first example of a phenazine dimer connected via a tetrahydrofuran ring.

The isolated compounds **1**−**3** were evaluated for their activity in overcoming TRAIL resistance in AGS cells.[18] This cell line has been widely used as a model system for evaluating cancer cell apoptosis[19] and is reported to be refractory to apoptosis induction by TRAIL.[20] To assess the effects of compounds **1**−**3** on cell viability, in the presence and absence of TRAIL, AGS cells were treated with the indicated agents and subjected to the fluorometric microculture cytotoxicity assay (FMCA) method.[21] We used luteolin (lut) at 18.0 μM as a positive control.[22] The assay results (Figure 5) showed that compounds **3** (20 μM) and **2** (30 μM) exhibited 26% and 30% decreases, respectively, in cell viability in the presence of TRAIL (100 ng/mL) compared with in the absence of TRAIL. On the other hand, treating the cells with compound **1** (40 μM) at 100 ng/mL TRAIL reduced the cell viability to 78%, which was 19% more than the TRAIL alone. These results suggest that izumiphenazines A−C (**1**−**3**) had a synergistic effect in combination with TRAIL against AGS cells.

The resistance of cancer cells toward TRAIL may occur at different points in the TRAIL-induced apoptotic pathways. Understanding the mechanisms of such resistance and developing strategies to overcome it are important for the successful use of TRAIL in cancer therapy.[23] Combined treatment with TRAIL and chemotherapeutic agents, including natural products, can overcome such resistance and sensitize TRAIL-resistant cells to enhance the therapeutic potential of TRAIL against cancer cells. Therefore, a natural product producing synergistic activity with TRAIL would be a new tool for investigating cancer
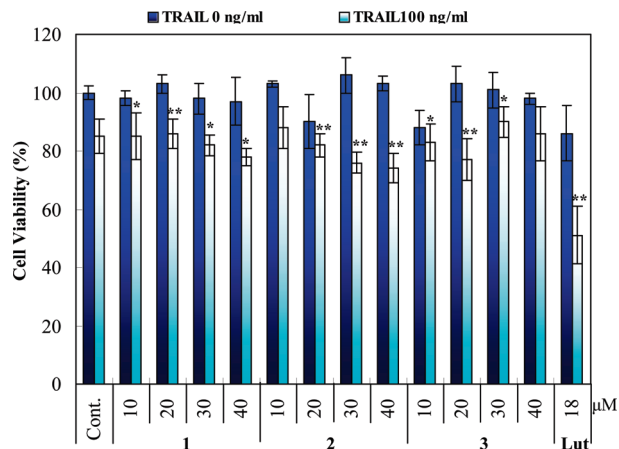
Figure 5. Effect of the isolated compounds **1**−**3** on the cell viability of AGS cells in the presence and absence of TRAIL. The standard error bar represents the means ($n = 3 \pm$ SD). The significance difference was determined by Student's *t*-test (**, $p < 0.01$; *, $p < 0.05$ vs control).

cells.[24] In this study, we report for the first time the synergistic activity of phenazine dimers in sensitizing TRAIL-resistant AGS cells, thereby suggesting their possible use in combination with TRAIL against human gastric adenocarcinoma.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO P-1020 polarimeter. CD spectra were obtained in a JASCO J-720WI spectropolarimeter. IR spectra were recorded on ATR in a JASCO FT-IR 230 spectrophotometer, and UV spectra were obtained on a Shimadzu UV mini-1240 spectrometer. Mass spectra were recorded on an Exactive (Thermo Scientific) mass spectrometer. The NMR data were measured on a JEOL JNM ecp600 spectrometer.

Fermentation. Spores of the strain growing on solid Waksman medium were inoculated into 4 × 500 cm^3 Sakaguchi flasks each containing 100 mL of liquid medium and then incubated at 28 °C for 5 days with rotary shaking at 200 rpm to produce seed culture. The seed culture (10 mL) was then inoculated into each of 10 3-L flasks, each containing 500 mL of liquid Waksman 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), and incubated at 28 °C for 5 days with rotary shaking at 200 rpm.

Extraction and Isolation. Fermentation broth (5 L) was centrifuged at 3500 rpm for 20 min. The resulting mycelial cake was extracted three times with acetone. After removal of acetone, the aqueous solution was extracted three times with EtOAc. The EtOAc-soluble portion was concentrated under reduced pressure. The culture broth was extracted three times with EtOAc. As the TLC of both extracts from the culture filtrate and mycelia showed the same composition, they were combined and concentrated under reduced pressure. The crude extract of *Streptomyces* sp. IFM 11204 (2.1 g) was subjected to Sephadex LH-20 (Φ 25 × 600 mm, MeOH) to give four fractions. Fraction II, containing compounds **1** and **2**, was fractionated by silica gel 60N flash column chromatography (Φ 45 × 250 mm) through a stepwise gradient solvent system consisting of CHCl₃ and MeOH. Compounds **1** (1.8 mg) and **2** (1.4 mg) were finally purified by PTLC (5 plates, 20 × 20 cm, CHCl₃/13% MeOH). Compound **3** (2.6 mg) was isolated from fraction III by PTLC (6 plates, 20 × 20 cm, CHCl₃/18% MeOH) followed by Sephadex LH-20 (Φ 15 × 600 mm, MeOH). Compound **4** (2.0 mg) was precipitated from the acetone solution of fraction IV and also purified by Sephadex LH-20 (Φ 15 × 600 mm, MeOH).

Fluorometric Microculture Cytotoxicity Assay (FMCA). AGS cells were seeded in a 96-well culture plate (6 × 10^3 cells per well) in 200 μL of RPMI medium containing 10% FBS. Cells were incubated at 37 °C in a 5% CO₂ incubator for 24 h. Then the test samples with or without TRAIL (100 ng/mL) at different doses were added to each well. After 24 h incubation, the cells were washed with PBS, and 200 μL of PBS containing fluorescein diacetate (10 μg/mL) was added to

each well. The plates were then incubated at 37 °C for 1 h, and fluorescence was measured in a 96-well scanning spectrofluorometer at 538 nm, following excitation at 485 nm.

Izumiphenazine A (1): red solid; $[\alpha]^{20}{}_D$ −480 (c 0.14, MeOH); UV (MeOH) λ_{max} (log ε) 451 (3.91), 384 (4.19), 272 (4.3) nm; CD (MeOH) λ_{ext} ($\Delta\varepsilon$) 272 (−36.7), 255 (+98.5), 222 (+9.9), 205 (−12.2) nm; IR (ATR) ν_{max} 3370 (br), 2919, 2851, 1701, 1611, 1571, 1405, 1320, 1281, 1203, 1009, 948, 746 cm^{-1}; ^{1}H and ^{13}C data, see Table 1; (−)-HRESIMS m/z 467.1004 [M − H]$^-$ (calcd for $C_{25}H_{16}N_4O_6$, 467.0992).

Izumiphenazine B (2): red solid; $[\alpha]^{20}{}_D$ −350 (c 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 448 (4.18), 383 (4.49), 271 (5.31) nm; CD (MeOH) λ_{ext} ($\Delta\varepsilon$) 288 (−14.2), 249 (+34.1), 203 (+17.2) nm; IR ν_{max} (KBr) 3350 (br), 2921, 280, 1698, 1607, 1567, 1418, 1339, 1159, 1052, 949, 748 cm^{-1}; (−)-HRESIMS m/z 467.0995 [M − H]$^-$ (calcd for $C_{25}H_{16}N_4O_6$, 467.0992); ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.24 (1H, s br, 9′-OH), 8.29 (1H, s br, 2-OH), 7.88 (1H, s br, H-3), 7.79 (1H, t, J = 8.1 Hz, H-7′), 7.70 (1H, d, J = 8.1 Hz, H-6′), 7.65 (1H, t, J = 6.9 Hz, H-7), 7.32 (1H, d, J = 6.9 Hz, H-6), 7.29 (1H, d, J = 8.1 Hz, H-8′), 7.12 (1H, d, J = 6.9 Hz, H-8), 4.92 (1H, m, H-3′), 3.71 (1H, m, 4′-H$_b$), 3.62 (1H, m, 4′-H$_a$), 2.80 (1H, m, 2′-H$_b$), 2.65 (1H, m, 2′-H$_a$); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 204.3 (C$_q$-1′), 165.9 (C$_q$-11), 157.5 (C$_q$-2), 154.1 (C$_q$-9), 153.5 (C$_q$-10′a), 153.2 (C$_q$-9′), 152.5 (C$_q$-4′a), 142.6 (C$_q$-5a), 139.9 (C$_q$-5′a), 139.4 (C$_q$-4a), 136.9 (C$_q$-10a), 135.0 (C$_q$-9′a), 134.3 (C$_q$-4), 132.3 (CH-7′), 132.2 (CH-7), 131.5 (C$_q$-9a), 127.9 (CH-3), 122.9 (C$_q$-1), 118.1 (CH-6′), 117.6 (CH-6), 111.4 (CH-8′), 111.0 (CH-8), 40.4 (CH$_2$-4′), 31.5 (C H$_2$-2′), 27.1 (CH-3′).

Izumiphenazine C (3): red solid; UV λ_{max} (MeOH) 471 (ε 4 107), 370 (ε 9 821), 268 (ε 7 3571) nm; IR ν_{max} (KBr) 3402 (br), 2920, 1682, 1599, 1560, 1517, 1482, 1264, 1176, 1126, 1012, 950, 756 cm^{-1}; ^{1}H and ^{13}C data, see Table 2; (−)-HRESIMS m/z 344.1044 [M − H]$^-$ (calcd for $C_{20}H_{15}N_3O_3$, 344.1035).

Supporting Information Available: 1D and 2D NMR, UV, and CD spectra of the isolated compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Budzikiewicz, H. *FEMS Microbiol. Rev.* **1993**, *10*, 209–228.
(2) (a) Fotso, S.; Santosa, D. A.; Saraswati, R.; Yang, J.; Mahmud, T.; Zabriskie, T. M.; Proteau, P. J. *J. Nat. Prod.* **2010**, *73*, 472–475. (b) Krastel, P.; Zeeck, A.; Gebhardt, K.; Fiedler, H.-P.; Rheinheimer, J. *J. Antibiot.* **2002**, *55*, 801–806. (c) Pusecker, K.; Laatsch, H.; Helmke, E.; Weyland, H. *J. Antibiot.* **1997**, *50*, 479–483.
(3) Imamura, N.; Nishijima, M.; Takadera, T.; Adachi, K.; Sakai, M.; Sano, H. *J. Antibiot.* **1997**, *50*, 8–12.
(4) Giddens, S. R.; Bean, D. C. *Int. J. Antimicrob. Agents* **2007**, *29*, 93–97.
(5) Kodama, K.; Ishii, A.; Kagasaki, T.; Shiozawa, H.; Takahashi, H. *Japanese Patent* JP 08217760, 1996.
(6) Laursen, J. B.; Nielsen, J. *Chem. Rev.* **2004**, *104*, 1663–1686.
(7) Umezawa, H.; Hayano, S.; Maeda, K.; Ogata, Y.; Okami, Y. *Jpn. Med. J.* **1950**, *3*, 111–117.
(8) (a) Aida, W.; Ohtsuki, T.; Li, X.; Ishibashi, M. *Tetrahedron* **2009**, *65*, 369–373. (b) Maekawa, K.; Toume, K.; Ishibashi, M. *J. Antibiot.* **2010**, *63*, 385–388.
(9) Waksman, S. A. *The Actinomycetes: Classification, Identification, and Descriptions of Genera and Species*; Williams & Wilkins Co.: Baltimore, MD, 1961; Vol. 2, pp 61–292.
(10) McDonald, M.; Wilkinson, B.; Van't, L.; Mocek, U.; Lee, S.; Floss, H. G. *J. Am. Chem. Soc.* **1999**, *121*, 5619–5624.
(11) Britton, G. *Biochemistry of Natural Pigments*; Cambridge Univ. Press: Cambridge, UK, 1983; pp 205–206.
(12) Liu, W.-C.; Parker, W. L.; Brandt, S. S.; Atwal, K. S.; Ruby, E. P. *J Antibiot.* **1984**, *37*, 1313–1319.
(13) van Heerden, P. S.; Bezuidenhoudt, B. C. B.; Ferreira, D. *Tetrahedron* **1997**, *53*, 6045–6056.
(14) Kim, W.-G.; Ryoo, I.-J.; Yun, B.-S.; Shin-ya, K.; Seto, H.; Yoo, I.-D. *J. Antibiot.* **1997**, *50*, 715–721.
(15) Kim, W.-G.; Ryoo, I.-J.; Yun, B.-S.; Shin-ya, K.; Seto, H.; Yoo, I.-D. *J. Antibiot.* **1999**, *52*, 758–761.
(16) Maskey, R. P.; Kock, I.; Helmke, E.; Laatsch, H. *Z. Naturforsch. B* **2003**, *58* (7), 692–694.
(17) Keller-Schierlein, W.; Geiger, A.; Zähner, H.; Brandl, M. *Helv. Chim. Acta* **1988**, *71*, 2058–2070.
(18) (a) Ishibashi, M.; Ohtsuki, T. *Med. Res. Rev.* **2008**, *28*, 688–714. (b) Ahmed, F.; Ohtsuki, T.; Aida, W.; Ishibashi, M. *J. Nat. Prod.* **2008**, *71*, 1963–1966.
(19) (a) Liu, J. D.; Lin, S. Y.; Ho, Y. S. *Mol. Carcinog.* **2003**, *37*, 16–24. (b) Jin, C. Y.; Park, C.; Cheong, J.; Choi, B. T.; Lee, T. H.; Lee, J.; Lee, J. D.; Lee, W. H.; Kim, G. Y.; Ryu, C. H.; Choi, Y. H. *Cancer Lett.* **2007**, *257*, 56–64.
(20) Srivastava, R. K. *Neoplasia* **2001**, *3*, 535–546.
(21) Larsson, R.; Kristensen, J.; Sandberg, C.; Nygren, P. *Int. J. Cancer* **1992**, *50*, 177–185.
(22) Horinaka, M.; Yoshida, T.; Shiraishi, T.; Nakata, S.; Wakada, M.; Nakanishi, R.; Nishino, H.; Matsui, H.; Sakai, T. *Oncogene* **2005**, *24*, 7180–7189.
(23) Baritaki, S.; Huerta- Yepez, S.; Sakai, T.; Spandidos, D. A.; Bonavida, B. *Mol. Cancer Ther.* **2007**, *6*, 1387–1399.
(24) Shankar, S.; Srivastava, R. K. *Drug Resist. Updates* **2004**, *7*, 139–156.