Tetracenoquinocin and 5-Iminoaranciamycin from a Sponge-Derived *Streptomyces* sp. Sp080513GE-26

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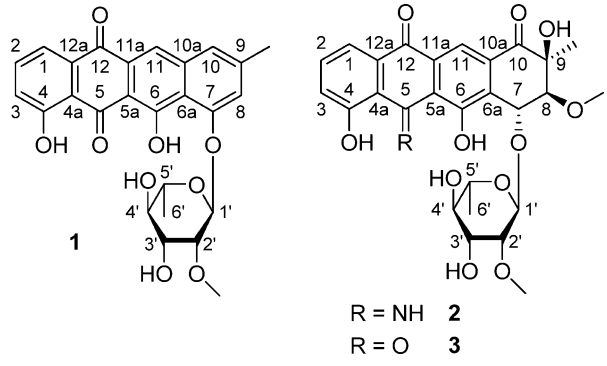
Two new anthracyclines, tetracenoquinocin (**1**) and 5-iminoaranciamycin (**2**), together with the known compounds aranciamycin (**3**) and antibiotic SM 173B were isolated from the culture of *Streptomyces* sp. Sp080513GE-26 associated with a marine sponge, *Haliclona* sp. The structures of **1** and **2** were established on the basis of extensive NMR and MS analyses along with ^{13}C-labeling experiments. The compounds **1**−**3** were evaluated for cytotoxicity against two cancer cell lines.

Marine microorganisms, particularly marine actinomycetes, have attracted considerable attention as one of the most important resources for new biologically active metabolites.[1] For example, new compounds have been isolated from actinomycetes of sponge origin.[2–4] Our group was recently engaged in the isolation of microorganisms, including fungi and actinobacteria, from marine sources. Some of the isolated microorganisms produce novel compounds, namely, the sesquiterpenes JBIR-27, -28,[5] an aspochracin derivative JBIR-15,[6] the glycosyl benzenediols JBIR-37, -38,[7] and the teleocidin JBIR-31.[8] In the present study, we isolated actinobacteria from a marine sponge, *Haliclona* sp., which is inhabited by diverse actinomycetes,[9] and then comprehensively searched for secondary metabolites in the cultures of isolated strains. In the course of our screening program for novel compounds, we succeeded in isolating two anthracyclines, designated as tetracenoquinocin (**1**) and 5-iminoaranciamycin (**2**), from the culture broth of *Streptomyces* sp. Sp080513GE-26. This paper describes the fermentation, isolation, and structure elucidation of **1** and **2** and also briefly describes their biological activity.



Streptomyces sp. Sp080513GE-26 was cultured at 27 °C for 5 days by rotary shaking in 500 mL of the production medium. An aqueous acetone extract of the mycelium collected from the culture broth was partitioned between EtOAc and H_2O. The EtOAc-soluble fraction was separated using medium-pressure liquid chromatography (MPLC) and HPLC to yield **1** as a new anthracycline and two known anthracyclines, aranciamycin (**3**)[10] and antibiotic SM 173B.[11] The supernatant of the fermentation broth was processed using HP-20 resin, MPLC, and HPLC to yield **2** as a new anthracycline. The structures of the new compounds **1** and **2** were elucidated mainly by spectroscopic methods, including 2D NMR techniques.

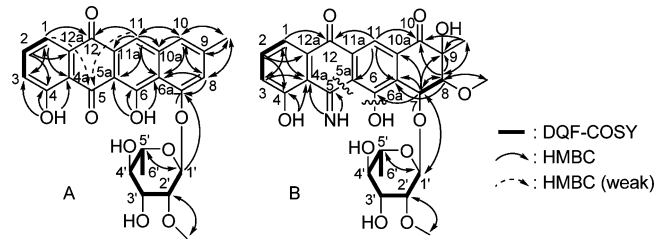


Figure 1. Key correlations observed in 2D NMR spectra of **1** (A) and **2** (B) (the bold lines show $^1H-^1H$ DQF-COSY results, and the arrows show CT-HMBC results).

The molecular formula of tetracenoquinocin (**1**) was established as $C_{26}H_{24}O_9$ from HRESIMS data (*m*/*z* 479.1354). Compound **1** showed UV absorptions at 264 and 487 nm, consistent with a highly conjugated aromatic compound. The IR spectrum of **1** also showed carbonyl (1720 cm^{-1}) and phenolic hydroxy (3430 cm^{-1}) groups, which was also supported by a bathochromic shift in the alkaline condition, indicating the presence of a phenolic hydroxy function in **1**. Additional structural information was obtained by HSQC, HMBC, and DQF-COSY spectra of **1** (Figure 1A). In the HMBC spectrum, the singlet methyl proton 9-CH_3 (δ_H 2.51) was coupled to the aromatic quaternary carbon C-9 (δ_C 143.8) and two aromatic methine carbons, C-8 (δ_C 117.1) and C-10 (δ_C 124.9). Furthermore, the aromatic methine proton H-8 (δ_H 7.18) was coupled to C-6a (δ_C 117.2), C-7 (δ_C 156.1), CH_3-9 (δ_C 22.4), and C-10, and the aromatic methine proton H-10 (δ_H 7.36) was coupled to C-6a, C-8, CH_3-9, C-10a (δ_C 139.3), and C-11 (δ_C 122.3). Additionally, the aromatic methine proton H-11 (δ_H 8.09) was coupled to C-5a (δ_C 109.0), C-6a, C-10, C-10a, C-11a (δ_C 129.0), and C-12 (δ_C 182.0), and the hydrogen-bonded phenolic hydroxy proton 6-OH (δ_H 14.60) was coupled to C-5a, C-6, and C-6a. These results indicated a 3-methylnaphthalene-1,8-diol moiety. In addition, a $^1H-^1H$ spin correlation was observed between two doublet of doublet methine protons H-1 (δ_H 7.85) and H-3 (δ_H 7.26) through the triplet methine proton H-2 (δ_H 7.65), indicating the presence of a 1,2,3-trisubstituted benzene ring moiety. In the HMBC spectrum, H-1 was coupled to C-3 (δ_C 124.6), C-4a (δ_C 117.1), and C-12; H-2 was coupled to C-4 (δ_C 162.7) and C-12a (δ_C 134.6); H-3 was coupled to C-1 (δ_C 120.0) and C-4a; and another phenolic hydroxy proton, 4-OH (δ_H 12.28), was coupled to C-3, C-4, and C-4a. Moreover, the long-range correlations from H-1/11 to C-5 (δ_C 191.3) elucidated a

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Table 1. ^{13}C (125 MHz) and ^{1}H (500 MHz) NMR Spectroscopic Data for Tetracenoquinocin (**1**) and ^{13}C (150 MHz) and ^{1}H (600 MHz) NMR Spectroscopic Data for 5-Iminoaranciamcyin (**2**)

	1[a]		**2**[b]	
position	^{13}C	^{1}H (*J* in Hz)	^{13}C	^{1}H (*J* in Hz)
1	120.0, CH	7.85, dd (7.7, 1.1)	119.3, CH	7.69, dd (7.9, 1.0)
2	136.8, CH	7.65, t (7.7)	134.8, CH	7.67, t (7.9)
3	124.6, CH	7.26, dd (7.7, 1.1)	124.8, CH	7.33, dd (7.9, 1.0)
4	162.7, CH		164.5, C	
4a	117.1, C		114.1, C	
5	191.3, C		162.6, C	
5a	109.0, C		114.5, C	
6	166.6, C		175.8, C	
6a	117.2, C		138.7, C	
7	156.1, C		72.0, CH	5.07, d (2.3)
8	117.1, CH	7.18, s	86.8, CH	3.56, d (2.3)
9	143.8, C		76.5, C	
10	124.9, CH	7.36, s	201.4, C	
10a	139.3, C		134.2, C	
11	122.3, CH	8.09, s	110.8, CH	7.62, s
11a	129.0, C		132.8, C	
12	182.0, C		183.2, C	
12a	134.6, C		134.1, C	
1′	95.6, CH	5.75, d (1.1)	101.0, CH	5.72, d (1.0)
2′	80.2, CH	3.93, dd (3.7, 1.1)	80.8, CH	3.25, m
3′	71.6, CH	4.21, m	71.1, CH	3.40[c]
4′	74.1, CH	3.53, t (9.5)	73.0, CH	3.22, t (9.4)
5′	69.4, CH	3.88, dq (9.5, 6.2)	69.9, CH	3.66, m
6′	17.8, CH_3	1.33, d (6.2)	17.3, CH	1.25, d (6.3)
9-CH_3	22.4, CH_3	2.51, s	23.1, CH_3	1.37, s
8-OCH_3			58.5, CH_3	3.41, s
2′-OCH_3	59.4, CH_3	3.60, s	59.1, CH_3	3.40, s
4-OH		12.28, s		16.04, br s
6-OH		14.60, s		[d]
5-NH				11.77, br s

[a] In $CDCl_3$. [b] In DMSO-d_6. [c] Overlapped. [d] Not observed.

1,10,11-trihydroxy-8-methyltetracene-5,12-dione moiety, indicating the connectivity between a 5-hydroxynaphthalene-1,4-dione and a 3-methylnaphthalene-1,8-diol moiety. The low-field-shifted ^{13}C chemical shift of C-5 (δ_C 191.3) compared with C-12 (δ_C 182.0) also supported the connectivity between these two partial structures. In addition to these resonances ascribed to the tetracene chromophore moiety, **1** showed seven extra ^{13}C resonances. The sequence from the anomeric methine proton H-1′ (δ_H 5.75) to the doublet methyl proton H-6′ (δ_H 1.33) through oxymethine protons H-2′ (δ_H 3.93), H-3′ (δ_H 4.21), H-4′ (δ_H 3.53), and H-5′ (δ_H 3.88) was established by the DQF-COSY spectrum. Long-range coupling between methoxy proton 2′-OCH_3 (δ_H 3.60) and oxymethine carbon C-2′ (δ_C 80.2) showed that this methoxy group is attached at C-2′. The long-range couplings from H-1′ to C-5′ revealed a 3-methoxy-6-methyltetrahydro-2*H*-pyran-2,4,5-triol moiety, indicating a sugar in **1**. Finally, the $^{1}H-^{13}C$ long-range couplings from H-1′ to C-7 certified the connectivity between the sugar and the tetracene chromophore moieties.

5-Iminoaranciamycin (**2**) was obtained as a violet oil. The molecular formula of **2** was established as $C_{27}H_{29}NO_{11}$ by HRESIMS (*m*/*z* 544.1814 $[M + H]^+$). Most of the ^{1}H and ^{13}C NMR data of **2** were similar to those of **1** (Table 1). The same sugar moiety as that of **1** was revealed by 2D NMR analyses. The $^{1}H-^{13}C$ long-range couplings from H-7 (δ_H 5.07) to C-1′ (δ_C 101.0) established the connectivity between the sugar and the anthraquinone moieties (Figure 1B). Major differences in the ^{13}C NMR chemical shifts of 12 carbon signals (C-4a to C-11a) were observed in the chromophore residue. DQF-COSY correlations were detected between oxymethine proton H-7 (δ_H 5.07) and methine proton H-8 (δ_H 3.56). The $^{1}H-^{13}C$ long-range couplings from singlet methyl proton 9-CH_3 (δ_H 1.37) to oxymethine carbons C-8 (δ_C 86.8) and C-9 (δ_C 76.5) and a carbonyl carbon C-10 (δ_C 201.4); from H-7 to aromatic carbons C-6 (δ_C 175.8), C-6a (δ_C 138.7), and C-10a (δ_C 134.2) and oxymethine carbons C-8 and C-9; from H-8 to C-6a, C-7 (δ_C 72.0), 8-OCH_3 (δ_C 58.5), C-9, 9-CH_3 (δ_C 23.1), and C-10; and from aromatic proton H-11 (δ_H 7.62) to C-5a (δ_C 114.5), C-6a, C-10, and C-12 (δ_C 183.2) were observed. In addition, $^{1}H-^{1}H$ spin systems were observed between two methine protons, H-1 (δ_H 7.69) and H-3 (δ_H 7.33), through triplet methine proton H-2 (δ_H 7.67). In the HMBC spectrum, H-1 was coupled to aromatic carbons C-3 (δ_C 124.8), C-4a (δ_C 114.1), and C-12; H-2 was coupled to C-4 (δ_C 164.5) and C-12a (δ_C 134.1); H-3 was coupled to C-1 (δ_C 119.3) and C-4a; phenolic hydroxy proton 4-OH (δ_H 16.04) was coupled to C-4 and C-4a; and imino proton 5-NH (δ_H 11.77) was coupled to C-4a and C-5. By taking into consideration the molecular formula and ^{13}C chemical shift at C-5 (δ_C 162.6), which is upfield shifted compared with the corresponding carbon in **1** (δ_C 191.3), the nitrogen was introduced into the structure of **2** as an imine, as shown in Figure 1B. Because the ^{13}C signal intensity at C-5 in **2** was extremely weak, we attempted to analyze the chromophore moiety of **2**, which was obtained by acid hydrolysis. Acid hydrolysis of **2** unexpectedly gave an aglycone that lacked nitrogen. Therefore, we could not confirm the structure of **2**.

A ^{13}C-labeled acetate-feeding experiment was performed to obtain information about the direct $^{13}C-^{13}C$ connectivity at the unassigned linkages of C-5 and C-5a of **2**. The [1,2-^{13}C]acetate units were localized at the positions C-1/C-12a (^{2}J = 61.7 Hz), C-2/C-3 (^{2}J = 56.2 Hz), C-4/C-4a (^{2}J = 56.1 Hz), C-5/C-5a (^{2}J = 66.2 Hz), C-6/C-6a (^{2}J = 58.5 Hz), C-7/C-8 (^{2}J = 43.8 Hz), C-9/9-CH_3 (^{2}J = 35.9 Hz), C-10a/C-11 (^{2}J = 58.3 Hz), and C-11a/C-12 (^{2}J = 53.9 Hz) (Table 2). Moreover, the signal intensity at C-5 became obvious in the ^{13}C NMR spectrum of ^{13}C-labeled acetate-incorporated **2**. Therefore, the exact ^{13}C assignments and the anthraquinone skeleton of **2** were confirmed by incorporation of ^{13}C-labeled acetate.

The ^{1}H NMR spectrum of **3** isolated from strain Sp080513GE-26 was consistent with the partial NMR data reported for aranciamycin.[12] The complete NMR assignments for **3** were determined using 1D and 2D NMR spectroscopic data (Table S1), confirming the structure of **3** as aranciamycin. Because Tong et al.[13] had

Table 2. ^{13}C (150 MHz) NMR Chemical Shifts and the $^{13}C-^{13}C$ Coupling Constants of [1,2-^{13}C]Acetate-Labeled **2**

position	δ (ppm)	J_{CC} (Hz)
1	119.3	61.7
2	134.8	56.2
3	124.8	56.2
4	164.5	56.1
4a	114.1	56.1
5	162.6	66.2
5a	114.5	66.2
6	175.8	58.5
6a	138.7	58.5
7	72.0	43.8
8	86.8	43.8
9	76.5	35.9
10	201.4	[b]
10a	134.2	58.3
11	110.8	58.3
11a	132.8	53.9
12	183.2	53.9
12a	134.1	61.7
1′	101.0	
2′	80.8	
3′	71.1	
4′	73.0	
5′	69.9	
6′	17.3	
9-CH_3	23.1	35.9
8-OCH_3	58.5	
2′-OCH_3	59.1	

[a] In DMSO-d_6. [b] Signal was singlet, and thus the carbon had no coupling with others.

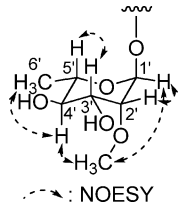

Figure 2. NOESY correlations for sugar component of **1**, **2**, and **3** (the arrows show NOESY results).

previously demonstrated that daunorubicin is converted to 5-iminodaunorubicin with a methanolic ammonia solution, we performed an amination of **3** with methanolic ammonia to confirm the presence of an imino functional group in **2**. The reaction product from **3** was obtained as a violet oil and analyzed by HRESIMS and NMR. The molecular formula, NMR data, and UV absorbance were almost identical to those of **2**, varying by only ±0.7 ppm for ^{13}C NMR data, ±0.1 ppm for ^{1}H NMR data, or ±1.0 nm for UV absorbance. All these findings finally proved **2** to be 5-iminoaranciamycin.

The relative configurations of **1** and **2** were determined by coupling constants and NOESY data. Large coupling constants for the triplet proton H-4′ (J = 9.5 Hz) resulting from diaxial relationships showed that H-3′, H-4′, and H-5′ have axial orientations. The small vicinal coupling constant between the axial proton H-3′ and H-2′ (J = 3.7 Hz) established that H-2′ has an equatorial orientation. Furthermore, the $^{1}J_{C-H}$ (168 Hz) of C-1′ for **1** revealed that H-1′ has an equatorial orientation which shows an α-rhamnopyranoside (anomeric carbons; $^{1}J_{C-H}$ = 166 Hz for methyl α-L-rhamnopyranoside and $^{1}J_{C-H}$ = 158 Hz for methyl β-L-rhamnopyranoside).[14] NOESY correlations of **1** were observed between H-1′/H-2′, H-1′/2′-OCH_3, H-3′/H-5′, H-4′/2′-OCH_3, and H-4′/CH_3-6′ (Figure 2). Thus, the sugar component of **1** was concluded to be 2′-*O*-methyl-α-rhamnose. In the same manner, the coupling constants, a $^{1}J_{C-H}$ (169 Hz) for C-1′, and a NOESY experiment showed that the sugar component of **2** also is 2-*O*-methyl-α-rhamnose.

The absolute configurations for **1** and **2** are proposed on the basis of their relationship to the co-isolated **3**. The specific rotation value ($[\alpha]^{25}_{D}$ +140 (*c* 0.1, MeOH)) of the isolated **3** is similar to the specific rotation value ($[\alpha]^{25}_{D}$ +150 (*c* 0.5, MeOH))[10] reported for aranciamycin, establishing the 7*R*,8*S*,9*S* absolute configuration in addition to the 2-*O*-methyl-α-L-rhamnose.[15] Biosynthetically, the absolute configuration of **2** was assumed to be the same as that of **3**, which was also isolated with **1** and **2**, because the compound was a derivative of aranciamycin by amination at C-5 in the skeleton. By taking into consideration these data, the absolute configurations of **1** and **2** were deduced to be the same as that of aranciamycin.

Cytotoxic activities of **1**−**3** against human cervical carcinoma HeLa cells and human acute myelogenous leukemia LH-60 cells were examined. Aranciamycin showed cytotoxicity with IC_{50} values of 2.7 and 4.1 μM against HeLa and HL-60 cells, respectively, while **1** exhibited weaker cytotoxicities with IC_{50} values of 120 and 210 μM, respectively, and **2** was inactive to these cancer cells (IC_{50} > 200 μM). On comparing the cytotoxic activity of **1**−**3**, it was found that the ketone functional group at C-5 is essential for the cytotoxicity against the cancer cells.

Experimental Section

General Experimental Procedures. Optical rotations were obtained on a Horiba SEPA-300 polarimeter. UV and IR spectra were measured on a Beckman Coulter DU730 UV/vis spectrophotometer and a Horiba FT-720 spectrophotometer, respectively. NMR spectra were taken on a Varian NMR System 500 or 600 NB CL in $CDCl_3$ (δ_C 77.0, δ_H 7.24 ppm) or DMSO-d_6 (δ_C 39.5, δ_H 2.50 ppm), with the residual solvent peak as the internal standard. HRESIMS data were recorded on a Waters LCT-Premier XE mass spectrometer. Normal-phase and reversed-phase MPLC were performed on a Purif-Pack SI-60 (Moritex, Tokyo, Japan) and a Purif-Pack ODS-100 (Moritex), respectively. Analytical reversed-phase HPLC was carried out using an L-column2 ODS (4.6 i.d. × 150 mm; Chemical Evaluation and Research Institute) equipped with a 2996 photodiode array detector (Waters) and a 3100 mass detector (Waters). Preparative reversed-phase HPLC was carried out using an L-column2 ODS (20 i.d. × 150 mm) equipped with a Hitachi High Technologies L-2455 photodiode array detector. Other reagents and solvents were of the highest grade available.

Microorganism. The producing *Streptomyces,* designated as Sp080513GE-26, was isolated from a marine sponge, *Haliclona* sp., collected from Tateyama City (34°59′ N, 139°49′ E), Chiba Prefecture, Japan. The details of the isolation for the strain have been already reported.[9] The basic local alignment search tool search of the 16S rRNA gene sequence (GenBank: AB473554) from Sp080513GE-26 showed that this strain belonged to the genus *Streptomyces*.

Fermentation. *Streptomyces* sp. Sp080513GE-26 was cultivated in 50 mL test tubes each containing 15 mL of a seed medium consisting of starch (Kosokagaku, Tokyo, Japan) 1.0%, polypeptone (Nihon Pharmaceutical, Tokyo, Japan) 1.0%, molasses (Dai-Nippon Meiji Sugar, Tokyo, Japan) 1.0%, and meat extract 1.0% (Extract Ehlrich, Wako Pure Chemical Industry, Osaka, Japan) (pH 7.2). The test tubes were shaken on a reciprocal shaker (355 rpm) at 27 °C for 2 days. Aliquots (2.5 mL) of the broth were transferred to 500 mL baffled Erlenmeyer flasks containing 100 mL of a production medium consisting of starch 2.5%, soybean meal (Nisshin Oillio, Tokyo, Japan) 1.5%, dry yeast (Mitsubishi Tanabe Pharma, Osaka, Japan) 0.2%, $CaCO_3$ (Kozaki Pharmaceutical, Tokyo, Japan) 0.4%, and Seqalife (Marinetech, Tokyo, Japan) 1.75% (pH 6.2), and cultured on a rotary shaker (180 rpm) at 27 °C for 5 days.

Isolation. The fermentation broth (2 L) was separated into the mycelia cake and supernatant by centrifugation. The mycelia cake was extracted with 80% acetone. The extract was evaporated *in vacuo* to remove the acetone, and the aqueous residue was extracted with EtOAc. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The dried residue (1.52 g) was subjected to normal-phase MPLC (Purif-Pack SI-60) with a $CHCl_3$−MeOH linear gradient system (0−30% MeOH), and fractions (including major metabolites) were collected by LC-MS monitoring. As a result, antibiotic SM 173B (1.52 mg) and aranciamycin (3.97 mg) were obtained as the 2% and 10% MeOH eluates, respectively. The 5% MeOH eluate (14.3 mg) was subjected to preparative reversed-phase HPLC, using an L-column2 ODS column (20 i.d. × 150 mm) developed with 80% aqueous MeOH containing

0.1% formic acid (flow rate 10 mL/min), to give tetracenoquinocin (**1**, t_R = 20.6 min, 0.5 mg).

The supernatant of the fermentation broth was applied to a Diaion HP-20 column (Mitsubishi Chemical, Tokyo, Japan), and the column was washed with 20% aqueous MeOH and eluted with 100% MeOH. After evaporation of the 100% MeOH eluent *in vacuo*, the resultant residue (1.41 g) was subjected to reversed-phase MPLC (Purif-Pack ODS-100) with an aqueous MeOH linear gradient system (20−100% MeOH), and fractions (including major metabolites) were collected by LC-MS monitoring. This eluate (67.1 mg) was subjected to preparative reversed-phase HPLC, using an L-column2 ODS column developed with 45% aqueous MeOH containing 0.1% formic acid (flow rate 10 mL/min) to give 5-iminoaranciamycin (**2**, t_R = 18.9 min, 5.1 mg).

Feeding Experiments with Sodium [1,2-^{13}C]Acetate. Aliquots (2.5 mL) of the preliminary cultivation broth were transferred to 500 mL baffled Erlenmeyer flasks containing 100 mL of production medium and cultured on a rotary shaker (180 rpm) at 27 °C. A sterile aqueous solution (1 mL) containing 0.1 g of sodium [1,2-^{13}C]acetate was added to 10 flasks at 2 days after the start of cultivation, and the cultures were then incubated for 5 days.

Conversion of 3 to 2. Aranciamycin (5.0 mg) in 90 μL of MeOH was added to a stirred solution of 180 μL of methanolic ammonia (saturated 0 °C) in an ice bath. The reaction mixture was then evaporated and purified by preparative reversed-phase HPLC, using an L-column2 ODS column developed with 45% aqueous MeOH containing 0.1% formic acid, to give **2** (3.8 mg).

Tetracenoquinocin (1): red oil; $[\alpha]^{25}_D$ −69 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 487 (4.20), 264 (4.73) nm; IR (KBr) ν_{max} 3430, 1720 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$), see Table 1; HRESIMS *m*/*z* 479.1354 $[M - H]^-$ (calcd for $C_{26}H_{23}O_9$, 479.1349).

5-Iminoaranciamycin (2): violet oil; $[\alpha]^{25}_D$ +150 (*c* 0.1, MeOH); UV (MeOH) λ_{max} log ε) 535 (4.00), 288 (4.09), 245 (4.43) nm; IR (KBr) ν_{max} 3330, 1700, 1660 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) and ^{13}C NMR (150 MHz, DMSO-d_6), see Table 1; HRESIMS *m*/*z* 544.1814 $[M + H]^+$ (calcd for $C_{27}H_{30}O_{11}$, 544.1819).

Aranciamycin (3): $[\alpha]^{25}_D$ +140 (*c* 0.1, MeOH); ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$), see Supporting Information.

Cytotoxic Activity. Cytotoxicity against human cervical carcinoma HeLa cells and human acute myelogenous leukemia HL-60 cells was determined by a colorimetric assay, using WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium monosodium salt]. Cells were cultured in DMEM medium (Wako Pure Chemical Industries) for HeLa cells or RPMI medium (Nacali Teaque, Kyoto, Japan) for HL-60 cells supplemented with 10% (v/v) fetal bovine serum (Invitrogen, Carlsbad, CA), penicillin (100 units/mL), and streptomycin (100 μg/mL) at 37 °C in a humidified incubator under a 5% CO_2 atmosphere. The 384-well plates were seeded with aliquots of a 20 μL medium containing 1.0×10^3 cells per well and were incubated overnight before being treated with compounds at various concentrations for 48 h. Plates were incubated for 1 h at 37 °C after the addition of 2 μL of WST-8 reagent solution (Cell Counting Kit; Dojindo, Kumamoto, Japan) per well. The absorption of the formed formazan dye was measured at 450 nm. The vehicle solvent (DMSO) was used as a negative control. Paclitaxel as a positive control showed cytotoxicity against HeLa and HL-60 cells with IC_{50} values of 30 and 160 nM, respectively.

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Supporting Information Available: NMR spectra of **1**, **2**, and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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