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Cytotoxic Angucycline Class Glycosides from the Deep Sea Actinomycete *Streptomyces lusitanus* SCSIO LR32

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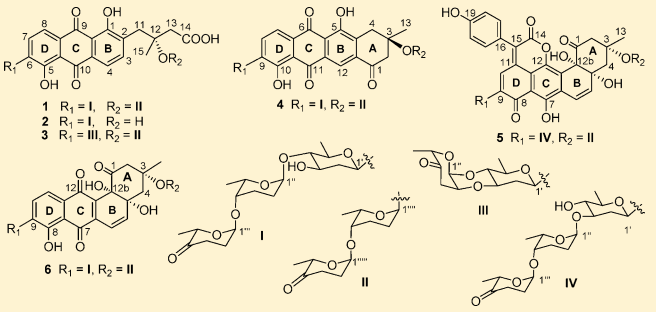
Supporting Information

ABSTRACT: Five new *C*-glycoside angucyclines, named grincamycins B−F (**1**−**5**), and a known angucycline antibiotic, grincamycin (**6**), were isolated from *Streptomyces lusitanus* SCSIO LR32, an actinomycete of deep sea origin. The structures of these compounds were elucidated on the basis of extensive spectroscopic analyses, including MS and 1D and 2D NMR experiments. All compounds except grincamycin F (**5**) exhibited *in vitro* cytotoxicities against the human cancer cell lines HepG2, SW-1990, HeLa, NCI-H460, and MCF-7 and the mouse melanoma cell line B16, with IC_{50} values ranging from 1.1 to 31 μM.



In the past decade, marine actinomycetes have emerged as an exciting new source for the discovery of novel bioactive natural products, especially in the anti-infective and anticancer areas.[1−4] Of the 240 established genera of actinomycetes, 50, including 12 new ones, have been isolated from marine sources.[5] During our efforts to identify new anticancer and anti-infective natural products from marine actinomycetes originating from the South China Sea, we reported the discovery of cytotoxic and antibacterial pseudonocardians and lobophorins from *Pseudonocardia* sp. SCSIO 01299[6] and *Streptomyces* sp. SCSIO 01127,[7] respectively, and the antimalarial marinocarboline and indolactam alkaloids from *Marinactinospora thermotolerans* SCSIO 00652.[8]

The strain SCSIO LR32 was isolated from a South China Sea deep ocean sediment and identified as *Streptomyces lusitanus* based on 16S rDNA sequence analysis. To explore the chemical and biological capabilities of this strain, we fermented the microbe using several different media. Previous work had shown that this strain, when fermented using JNP1 medium, produced predominantly four aromatic amide compounds identified as toluene-2,4-dicarbamic acid dimethyl ester, toluene-2,4-dicarbamic acid diethyl ester, toluene-2,6-dicarbamic acid dimethyl ester, and toluene-2,6-dicarbamic acid diethyl ester.[9] We found that when this strain was fermented using modified-RA medium and the extract was analyzed by HPLC-DAD-UV, an array of secondary metabolites with UV spectra characteristic of the angucycline class of antibiotics was observed.[10,11] In this paper, we report the fermentation, isolation, and structure elucidation of five new angucycline *C*-glycosides, grincamycins B−F (**1**−**5**), from *S. lusitanus* SCSIO LR32 along with the previously reported grincamycin (**6**). Moreover, we report the cytotoxicities of compounds **1**−**6** against the human cancer cell lines HepG2, SW-1990, HeLa, NCI-H460, and MCF-7 and the mouse melanoma cell line B16.

RESULTS AND DISCUSSION

Strain SCSIO LR32 was cultivated on an 8 L scale using modified-RA medium in 2 L shake flasks. Silica gel, Sephadex LH-20, and semipreparative HPLC column chromatography of the fermentation extract yielded **6** as a major product and **1**−**5** as minor metabolites. Compound **6** was detected and isolated as a pale red powder. Positive and negative ESIMS data indicated that **6** has a molecular weight of 938. The ^{1}H and ^{13}C NMR spectroscopic data for **6** (Supporting Information, Table S1) suggested the presence of a typical angucycline and tetrangomycin skeleton,[12,13] with the 3-position *O*-glycosylated and 9-position *C*-glycosylated. The ^{1}H and ^{13}C NMR spectra were characterized by resonances corresponding to five doublet methyl groups, nine methylenes, 14 methines, and two carbonyl moieties arising from sugar residues, subsequently identified as a trisaccharide, consisting of β-olivose, α-rhodinose, and α-cinerulose A, and a disaccharide containing α-rhodinose and

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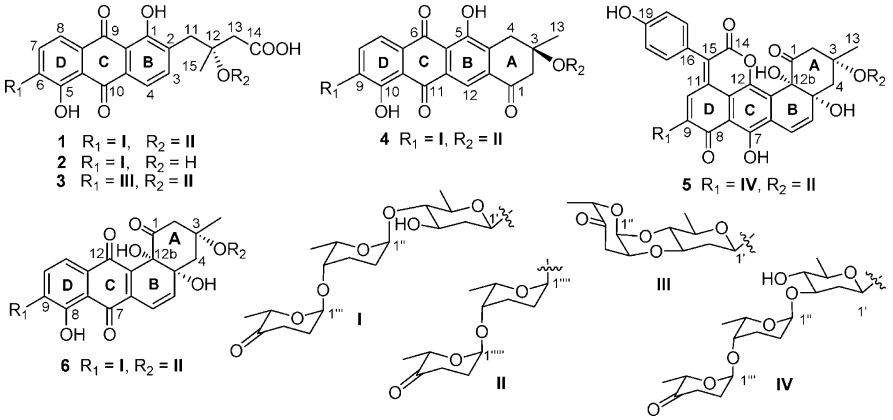
Chart 1



α-cinerulose A through detailed interpretation of both 1D and 2D NMR data. Structural assignments for compound **6** were validated by comparison of the ^{1}H and ^{13}C NMR and MS spectroscopic data with data previously reported for grincamycin, a known angucycline antibiotic from *Streptomyces griseoincarnatus* with inhibitory activity against murine leukemia P388 cells.[14]

Compound **1** was isolated as a red, amorphous solid. Negative mode ESIMS afforded a quasimolecular ion peak at m/z 937 $[M - H]^-$, and subsequent HRESIMS revealed the molecular formula of **1** to be $C_{49}H_{62}O_{18}$. The IR spectrum of **1** displayed characteristic absorptions of hydroxy (3416 cm^{-1}), carbonyl (1731, 1703, and 1628 cm^{-1}), and benzene ring (1604 and 1583 cm^{-1}) functionalities. A full set of 1D (^{1}H and ^{13}C) and 2D (HMQC, HMBC, and NOESY) NMR spectra for **1** was acquired, allowing the complete assignment of its ^{1}H and ^{13}C NMR signals (Table 1). The ^{1}H and ^{13}C NMR spectra were rich in aliphatic signals with additional aromatic signals representative of the 1,5-dihydroxy-2,6-disubstituted anthraquinone skeleton. Two hydrogen-bonded hydroxy groups were suggested by ^{1}H signals at δ_H 12.95 and 12.93. Two pairs of *ortho*-coupled aromatic hydrogens (δ_H 7.56, 7.68, J = 7.5 Hz; δ_H 7.82, 7.56, J = 7.5 Hz) attached at C-3, C-4 and C-7, C-8 on the anthraquinone moiety were apparent on the basis of HMBC correlations as shown in Figure 1. The HMBC correlations from the CH_3-15 to C-11, C-12, C-13 and from CH_2-13 to the carbonyl (C-14) determined the side chain moiety. The ^{13}C NMR spectroscopic data of the side chain (C-11−C-15) were very similar to those of the known vineomycin B_2, which possesses a 12*R* configuration (Supporting Information, Table S2).[15,16] Further HMBC correlations between CH_2-11 and C-1, C-2, C-3 linked this side chain at C-2 on the anthraquinone core to establish the aglycone structure of **1**. Additionally, the ^{1}H and ^{13}C NMR spectra of **1** revealed the presence of both a trisaccharide and a disaccharide identical to those found in **6**. The anomeric methine (CH-1′) of olivose with chemical shifts at δ_H 4.83 and δ_C 70.9 suggested the presence of a *C*-olivoside. Another four acetal carbons at δ_C 99.0 (C-1″), 99.4 (C-1‴), 91.2 (C-1⁗), and 98.8 (C-1⁗′), as well as four doublet methyls, indicated the existence of four deoxysugars *O*-linked to the aglycone of **1**. The $^3J_{C-H}$ coupling from H-1′ to C-5, C-6, C-7 and from H-7 to C-1′ observed in the HMBC spectrum revealed the *C*-glycosidic bond C-6−C-1′ between the aglycone and β-olivose. The position of the *O*-glycosidic bond C-12−*O*−C-1⁗ between the aglycone and disaccharide was deduced on the basis of the HMBC correlation of H-1⁗/C-12. The connections of C-4′−*O*−C-1″ and C-4″−*O*−C-1‴ in the trisaccharide were determined on the basis of HMBC correlations of H-4′/C-1″, H-1″/C-4′, H-4″/C-1‴, and H-1‴/C-4″, respectively. The additional HMBC correlations of H-4⁗/C-1⁗′ and of H-1⁗′/C-4⁗ established the linkage C-4⁗−*O*−C-1⁗′ between α-rhodinose and α-cinerulose A in the disaccharide. The relative configuration of these sugar residues was confirmed by NOESY experiments (Figure 2). Because angucyclines with the same tetracyclic core structure as grincamycin (**6**) have been shown to rearrange to tricyclic compounds similar to compound **1** under acidic conditions,[15,17] these two metabolites likely share the same absolute configurations. The absolute configuration for **6** was established by comparison with the tetrahydro derivative of P-1894B.[14,18] Therefore, **1** is proposed to have a 12*R* configuration and to be composed of D-olivose, L-rhodinose, and L-cinerulose A sugars. This new compound was accordingly named grincamycin B.

Compound **2** was isolated as a red, amorphous solid. Its molecular formula, $C_{37}H_{44}O_{14}$, was determined by HRESIMS. Particularly striking was the signal at m/z 711.2649 $[M - H]^-$, which is less than that of **1** by a $C_{12}H_{18}O_4$ fragment. Comparisons of the ^{1}H and ^{13}C NMR spectra for **2** with those obtained with **1** revealed an absence of sugar resonances in **2**. Only three anomeric methines at δ_H 4.75 and δ_C 71.0 (CH-1′), at 4.99 and 99.1 (CH-1″), and at 5.08 and 99.5 (CH-1‴) were observed. The ^{13}C NMR signal of the oxygen-bearing carbon C-12 was shifted upfield, from δ_C 77.2 in **1** to δ_C 71.5 in **2**, suggesting that the C-12 is not *O*-glycosylated. Furthermore, the ^{1}H and ^{13}C NMR data of **2** could be thoroughly assigned by comparing with spectroscopic data for grincamycin B (**1**) and previously reported congeners.[19,20] Consequently, the structure of **2** was solved and named grincamycin C.

Compound **3** was obtained as a brown-yellow, amorphous solid, and its molecular formula determined to be $C_{43}H_{50}O_{16}$ on the basis of HRESIMS. Similarities in the ^{1}H and ^{13}C NMR spectroscopic data for **3** and **1** indicated a close structural relationship between the two compounds. Compounds **3** and **1** were found to be structurally differentiated only by the identity of the C-6 glycoside. In a fashion similar to that observed with compounds **1** and **2**, a characteristic anomeric methine at δ_H 4.97 (br d, J = 10.5 Hz) and δ_C 71.5 suggested the presence of β-olivose in **3**. The COSY coupling of H-1″/H-2″/H-3″ revealed a fragment of C-1″−C-2″−C-3″ with the aid of HMQC data (Figure 3). Further long-range couplings from H-3″ to the C-4″ carbonyl, from H-1″ to an oxygen-bearing methine (C-5″), and from H_3-6″ to C-5″ and C-4″ in the HMBC

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Table 1. Summary of ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Spectroscopic Data for Grincamycins B−D (1−3) in $CDCl_3$

	grincamycin B (1)[a]		grincamycin C (2)[b]		grincamycin D (3)[c]	
position	δ_C type	δ_H mult. (J in Hz)	δ_C type	δ_H mult. (J in Hz)	δ_C type	δ_H mult. (J in Hz)
1	161.5, C		161.1, C		161.5, C	
2	134.7, C		134.6, C		134.7, C	
3	139.5, CH	7.56, d (7.5)	139.5, CH	7.50, d (7.0)	139.5, CH	7.65, d (7.5)
4	118.2, CH	7.68, d (7.5)	118.8, CH	7.56, d (7.0)	118.5, CH	7.72, d (7.5)
4a	131.5, C		131.4, C		131.9, C	
5	158.8, C		158.8, C		158.8, C	
6	137.7, C		138.4, C		137.7, C	
7	133.1, CH	7.82, d (7.5)	133.0, CH	7.73, d (7.0)	133.3, CH	7.88, d (7.5)
8	118.2, CH	7.56, d (7.5)	119.3, CH	7.50, d (7.0)	119.4, CH	7.80, d (7.5)
8a	131.4, C		131.4, C		131.7, C	
9	187.7, C		187.5, C		188.1, C	
9a	115.1, C		115.3, C		115.5, C	
10	187.7, C		187.6, C		188.0, C	
10a	115.1, C		115.1, C		115.4, C	
11	38.3, CH_2	3.12, d (13.5)	38.5, CH_2	3.00, br d	38.4, CH_2	3.22, d (13.5)
		3.08, d (13.5)		2.85, br d		3.14, d (13.5)
12	77.2, C		71.5, C		77.3, C	
13	44.2, CH_2	2.60, d (15.0)	44.0, CH_2	2.50[d,e]	44.7, CH_2	2.69, d (15.0)
		2.72, d (15.0)				2.76, d (15.0)
14	174.1, C		174.5, C		174.3, C	
15	23.1, CH_3	1.43, s	29.7, CH_3	1.25[d,e]	23.1, CH_3	1.42[d,e]
1-OH		12.95, br s		12.82, br s		13.10, br s
5-OH		12.93, br s		12.82, br s		13.04, br s
1′	70.9, CH	4.83, d (11.2)	71.0, CH	4.75, br s	71.5, CH	4.97, br d (10.5)
2′	38.3, CH_2	2.55, m; 1.40, m	38.5, CH_2	2.36, m; 1.30, m	36.6, CH_2	2.46, m; 1.51, m
3′	71.4, CH	3.81, m	71.1, CH	3.80, br s	76.9, CH	3.81, ddd (11.6, 9.0, 4.5)
4′	88.6, CH	3.07, t (9.0)	88.9, CH	3.06, t (8.0)	74.4, CH	3.50, t (9.0)
5′	74.5, CH	3.53, m	74.5, CH	3.51, m	74.5, CH	3.57, m
6′	18.4, CH_3	1.35, d, (6.5)	18.6, CH_3	1.29[d,e]	17.5, CH_3	1.40[d,e]
1″	99.0, CH	4.97, br s	99.1, CH	4.99, br s	91.4, CH	5.18, d (2.5)
2″	24.1, CH_2	2.08, m; 1.65, m	24.5, CH_2	2.10, m; 1.67, m	71.1, CH	4.35, dd (5.4. 2.5)
3″	24.6, CH_2	2.06, m; 1.90, m	25.3, CH_2	2.10, m; 1.91, m	39.5, CH_2	2.67, m
4″	74.4, CH	3.52, br s	74.7, CH	3.68, br s	207.7, C	
5″	67.9, CH	4.21, q (6.5)	67.8, CH	4.22, q (6.7)	77.7, CH	4.75, q (6.7)
6″	16.9, CH_3	1.25[d,e]	16.9, CH_3	1.27[d,e]	16.1, CH_3	1.40[d,e]
1‴	99.4, CH	5.04, t (5.2)	99.5, CH	5.08, t (5.0)		
2‴	28.3, CH_2	2.30, m; 2.06, m	28.3, CH_2	2.47;[d,e] 2.10, m		
3‴	33.4, CH_2	2.46, m	33.5, CH_2	2.48[d,e]		
4‴	210.8, C		210.6, C			
5‴	70.5, CH	4.25, q (6.5)	71.1, CH	4.32, q (6.8)		
6‴	14.7, CH_3	1.20[d,e]	14.9, CH_3	1.25[d,e]		
1⁗	91.2, CH	5.17, br s			91.5, CH	5.24, br s
2⁗	24.5, CH_2	1.90, m; 1.48, m			25.5, CH_2	2.00, m; 1.40, m
3⁗	24.1, CH_2	2.10, m; 1.80, m			24.8, CH_2	2.07, m; 1.89, m
4⁗	76.1, CH	3.68, br d			74.8, CH	3.62, br s
5⁗	66.5, CH	3.95, q (6.8)			66.9, CH	4.04, q (7.5)
6⁗	16.9, CH_3	1.27[d,e]			16.9, CH_3	1.12, d (7.5)
1‴″	98.8, CH	4.99, t (5.3)			98.7, CH	5.04, t (5.3)
2‴″	28.2, CH_2	2.30, m; 2.06, m			28.4, CH_2	2.32, m; 2.05, m
3‴″	33.4, CH_2	2.46, m			33.5, CH_2	2.46, m
4‴″	210.9, C				210.9, C	
5‴″	71.0, CH	4.28, q (6.5)			71.1, CH	4.29, q (6.5)
6‴″	14.7, CH_3	1.20[d,e]			14.8, CH_3	1.25, d (6.5)

[a]The signals were assigned with the aid of HMQC and HMBC data. [b]The signals were assigned by comparison with those of grincamycin B (1) and with data from related fridamycin D.[19] [c]The signals were assigned with the aid of ^{1}H−^{1}H COSY, HMQC, and HMBC data. [d,e]These signals under the same superscript and within the same column were overlapped.

spectrum of 3 established a cinerulose B unit. This cinerulose B was linked to β-olivose through the *O*-glycosidic bond C-1″−*O*−C-4′, which was confirmed by an HMBC correlation of H-1″/C-4′. To satisfy the molecular formula, another linkage,

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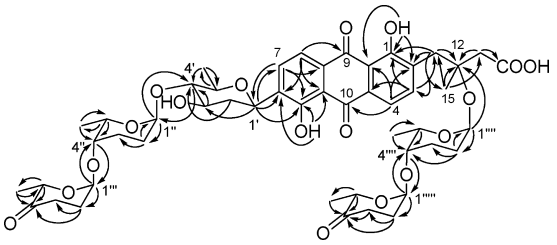

Figure 1. HMBC correlations of grincamycin B (**1**).

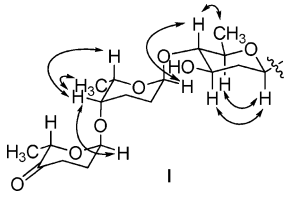

Figure 2. Key NOESY correlation (double-headed arrow) of the α-cinerulose A-(1→4)-α-rhodinosyl (1→4)-β-olivosyl unit (**I**) in grincamycins B, C, and E (**1**, **2**, and **4**) and grincamycin (**6**).

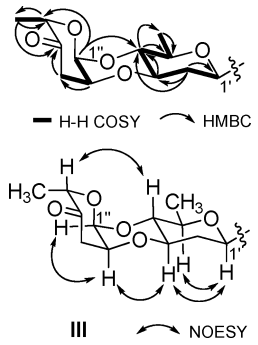

Figure 3. 2D NMR (COSY, HMBC, NOESY) correlations of the α-cinerulose B-(1→4, 2→3)-β-olivosyl unit (**III**) in grincamycin D (**3**).

C-2″−*O*−C-3′, was proposed to complete the planar structure elucidation of **3**. The small coupling constant (J = 2.5 Hz) indicated an ax-eq orientation between H-1″ and H-2″. The NOESY correlations of H-1″/H-2″/H-3′/H-1′/H-5′ and of H-5″/H-4′ permitted elucidation of the disaccharide relative configuration as shown in Figure 3. The ^{1}H and ^{13}C NMR spectroscopic data for the α-cinerulose B-(1→4, 2→3)-β-olivosyl unit were in good agreement with the data previously reported for compounds with the same disaccharide.[21,22] Compound **3** was named grincamycin D.

Compound **4** was obtained as a yellowish powder. ESIMS run in both negative and positive ion modes afforded pseudomolecular ion peaks at m/z 919.5 $[M - H]^-$ and 943.4 $[M + Na]^+$, respectively, indicating a molecular weight of 920 for **4**. Subsequent HRESIMS established the molecular formula of **4** as $C_{49}H_{60}O_{17}$. Notably, compound **4** was found to have a linear tetracyclic anthraquinone aglycone, as deduced from 1D (^{1}H and ^{13}C) and 2D (COSY, HMQC, and HMBC) NMR experiments (Table 2, Figure 4); this structural trait differs from the angucycline aglycone of grincamycin (**6**). In the downfield sp^2-region of the ^{13}C NMR spectrum, two carbonyls at δ_C 187.2 (C-11) and 187.5 (C-6), as well as 12 carbons typical of an anthraquinone backbone, were noted. The carbonyl moiety of ring A was shifted from δ_C 204.7 to δ_C 195.8 relative to the analogous moiety in **6**, indicating that this carbonyl is conjugated with the anthraquinone. Further HMBC correlations involving rings A and B confirmed the structure of the tetracyclic anthraquinone aglycone as shown in Figure 4. Additionally, ^{1}H and ^{13}C NMR resonances attributable to the saccharide moieties of **4** were very similar to those previously noted for **6**, suggesting that both compounds share the same sugar moieties. The *C*-glycosyl bond between C-9 and C-1′ was established by the HMBC correlations from H-1′ to C-9, C-10 and from H-8 to C-1′. The connection of C-3−*O*−C-1‴″ was determined by the HMBC correlation of H-1‴″/C-3. Furthermore, we found that **6** could be converted into **4** under UV irradiation (Supporting Information, Figure S35).[23] The transformation yield is about 50% after UV irradiation for 24 h. This photochemical transformation established the 3*R* configuration of **4** as well as the configurations of the sugars. Thus, the structure of **4** was named grincamycin E.

Compound **5** was isolated as a dark red powder. HRESIMS of **5** revealed a prominent peak at m/z 1069.4076 $[M - H]^-$, establishing the molecular formula as $C_{57}H_{66}O_{20}$. From the characteristic absorption bands at 222, 302, and 511 nm in the UV spectrum, as well as the established molecular formula, **5** was determined to be a member of the angucycline class of antibiotics. The aglycone in **5** was found to be identical to that of urdamycin C on the basis of comparisons of the ^{1}H and ^{13}C NMR data for **5** and those previously reported for urdamycin C, an enlarged chromophore (Supporting Information, Table S3), possessing an angular tetracyclic ring system and the unique structural element (*p*-OH-phenyl)-pyranone.[24−26] The ^{1}H and ^{13}C NMR spectra of **5** also revealed a set of sugar signals that were nearly identical to those of the disaccharide and trisaccharide in **1**. One key difference was that the ^{13}C NMR signals of C-3′ (δ_C 76.0) and C-4′ (δ_C 75.1) on β-olivose were different (C-3′: δ_C 71.4, C-4′: δ_C 88.6 in **1**), thus defining the C-3′−*O*−C-1″ system as the glycosidic linkage connecting β-olivose and α-rhodinose.[24] The CH-3′ and CH-1″ correlated to each other in the HMBC and NOESY spectra, thus verifying this linkage (Figures 5 and 6). This new compound was named grincamycin F.

Of the many angucycline antibiotics discovered thus far, only two distinct mechanisms for angucycline core formation have been reported. Feeding experiments revealed that two angucycline antibiotics, PD117198 and BE-7585A, are biosynthesized through a linear fused tetracyclic anthracyclinone intermediate.[27,28] Most angucycline antibiotics have been shown by feeding experiments to be biosynthesized in a straightforward manner to directly form the angucycline core through a decaketide intermediate; examples include urdamycin A,[26] vineomycin A_1,[16] and PD116740.[29] However, the angucycline antibiotics suffer rearrangements to afford linear derivatives upon exposure to light, heat, or acids.[15,17] For instance, aquayamycin can be rearranged into a linear tetracyclic product with light, and a tricyclic product can be formed upon acidic treatment in methanol.[23] Because grincamycin (**6**) and aquayamycin have identical aglycones, **6** might be expected to undergo similar rearrangements. Compounds **1**−**3** belong to a category of nonclassically rearranged angucycline *C*-glycosides. The aglycones of **1**−**3** share a side chain (C-11−C-15) that derives from cleavage of the C-1/C-12b bond of ring A in **6** and was concluded to be identical with that of fridamycins A−D and vineomycin B_2.[17] Although the minor metabolites **1**−**5** were simultaneously detected from the fermentation of strain SCSIO LR32, compounds **1**−**4** may actually result from rearrangement processes that occur during fermentation.

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Table 2. Summary of ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Spectroscopic Data for Grincamycins E (4) and F (5)

	grincamycin E (4)[a]		grincamycin F (5)[b]	
position	δ_C type	δ_H mult. (J in Hz)	δ_C type	δ_H mult. (J in Hz)
1	195.8 C		206.8 C	
2	50.6, CH_2	2.62, d (16.0)	51.9, CH_2	3.27, dd (13.0, 1.7)
		2.89, d (16.0)		2.74, d (13.0)
3	75.7, C		76.8−77.3, C[c]	
4	33.4, CH_2	3.61, d (18.6)	43.7, CH_2	1.73[d,e,f,g]
		2.75, d (18.6)		2.07[d,e,f,g]
4a	137.8, C		78.1, C	
5	160.9, C		136.8, CH	6.14, d (10.0)
5a	117.0, C			
6	187.5, C		118.7, CH	7.08, d (10.0)
6a	131.5, C		124.7, C	
7	119.4, CH	7.71, d (8.0)	156.6, C	
7a			111.7, C	
8	133.3, CH	7.80, d (8.0)	186.3, C	
9	138.5, C		144.2, C	
10	158.7, C		133.9, CH	7.77, s
10a	115.3, C			
11	187.2, C		133.3, C	
11a	130.6, C		115.8, C	
12	116.1, CH	8.26, s	140.5, C	
12a	136.9, C		127.5, C	
12b			80.2, C	
13	25.3, CH_3	1.42, s	28.2, CH_3	1.24[d,e,f,g]
14			159.3, C	
15			131.6, C	
16			122.3, C	
17, 21			133.3, CH	7.33, d (8.0)
18, 20			115.3, CH	6.94, d (8.0)
19			159.0, C	
5-OH		12.95, br s		
7-OH				13.12, br s
10-OH		12.94, br s		
1′	70.8, CH	4.79, br d (12.0)	71.0, CH	4.70, d (11.0)
2′	38.2, CH_2	2.41, m; 1.35, m	39.1, CH_2	2.38, m; 1.36, m
3′	70.9, CH	3.73, m	76.0, CH	3.70, m
4′	88.2, CH	2.99, t (8.5)	75.1, CH	2.98, t (8.6)
5′	74.4, CH	3.48, m	72.2, CH	3.66, m
6′	18.1, CH_3	1.26[d,e,f,g]	17.6, CH_3	1.23[d,e,f,g]
1″	99.3, CH	4.89, br s	98.1, CH	4.94, br s
2″	24.1, CH_2	2.05, m; 1.60, m	24.1, CH_2	2.20, m; 1.70[d,e,f,g]
3″	25.2, CH_2	2.00, m; 1.91, m	24.6, CH_2	2.10;[d,e,f,g] 1.90, m
4″	74.5, CH	3.40, m	75.0, CH	3.47, m
5″	67.8, CH	4.12, q	66.4, CH	3.80, m
6″	16.9, CH_3	1.07[d,e,f,g]	16.9, CH_3	1.25[d,e,f,g]
1‴	98.9, CH	4.99, t (5.5)	99.8, CH	5.09, t (5.0)
2‴	28.1, CH_2	2.30, m; 2.02, m	28.2, CH_2	2.35, m; 2.07
3‴	33.3, CH_2	2.40, m	33.5, CH_2	2.34, m
4‴	211.5, C		211.6, C	
5‴	70.8, CH	4.23, q (7.0)	70.8, CH	4.29[d,e,f,g]
6‴	14.4, CH_3	1.17, d (7.0)	14.6, CH_3	1.20[d,e,f,g]
1⁗	91.1, CH	5.05, br s	92.1, CH	5.04, br s
2⁗	25.1, CH_2	2.10, m; 1.50, m	24.5, CH_2	2.02, m; 1.48, m
3⁗	23.9, CH_2	2.20, m; 1.80, m	24.1, CH_2	2.10, m; 1.70[d,e,f,g]
4⁗	74.4, CH	3.63, m	76.0, CH	3.50, m
5⁗′	66.7, CH	4.12, br s	65.9, CH	3.70, br s
6⁗	16.3, CH_3	1.30[d,e,f,g]	16.9, CH_3	1.27[d,e,f,g]
1⁗″	98.7, CH	4.96, t (5.0)	99.0, CH	5.08, t (5.0)
2⁗″	28.0, CH_2	2.15, m; 1.88, m	28.2, CH_2	2.35, m; 2.07, m
3⁗″	33.3, CH_2	2.36, m	33.5, CH_2	2.46, m
4⁗″	211.5, C		211.6, C	
5⁗″	70.7, CH	4.21, q (6.5)	70.9, CH	4.19[d,e,f,g]
6⁗″	14.5, CH_3	1.06[d,e,f,g]	14.6, CH_3	1.20[d,e,f,g]

[a]Recorded in $CDCl_3/CD_3OD$; the signals were assigned with the aid of $^{1}H-^{1}H$ COSY, HMQC, and HMBC data. [b]Recorded in $CDCl_3/CD_3OD$; the signals were assigned with the aid of HMQC and HMBC data. [c]Obscured by solvent. [d,e,f,g]These signals under the same superscript and within the same column were overlapped.

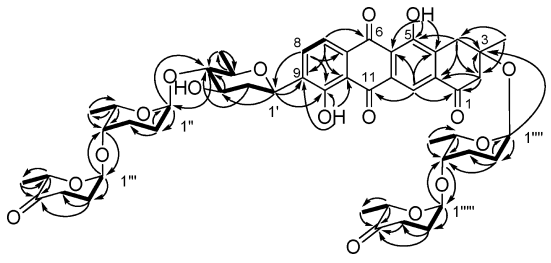

Figure 4. COSY (bold) and HMBC (arrow) correlations for grincamycin E (4).

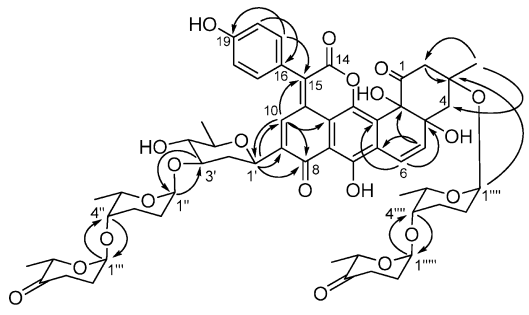

Figure 5. Key HMBC correlations for grincamycin F (5).

Compounds 1−6 were tested for their *in vitro* cytotoxic activities against the human hepatoma cell line HepG2, human pancreatic cell line SW-1990, human cervical cancer cell line HeLa, human lung cancer cell line NCI-H460, human breast cancer cell line MCF-7, and the mouse melanoma cell line B16 using previously described MTT-based methods.[30] As depicted in Table 3, the known grincamycin (6) was the most effective compound evaluated. It displayed antiproliferative activities with the selected panel of cells; IC_{50} values ranged from 1.1 to 11 μM. Using a similar approach, the new grincamycins B−E (1−4) showed cytotoxicity toward these cell lines with IC_{50} values ranging from 2.1 to 31 μM. It is worth noting that grincamycin F (5) differs from 6 primarily in the structure of its enlarged aglycone, which contains a six-membered lactone ring and a hydroxybenzene in addition to the typical angucycline

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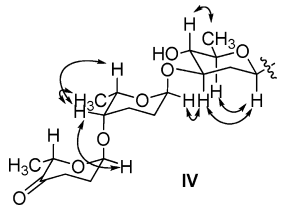

IV

Figure 6. Key NOESY correlations of the α-cinerulose A-(1→4)-α-rhodinosyl (1→3)-β-olivosyl unit (**IV**) in grincamycin F (**5**).

Table 3. *In Vitro* Cytotoxic Activities (IC_{50}, μM) of Compounds 1−6 against Six Cancer Cell Lines (n = 3)

	B16	HepG2	SW-1990	HeLa	NCI-H460	MCF-7
1	2.1	8.5	11	6.4	[a]	12
2		31	31			11
3	9.7	9.7	22	12	30	6.1
4	5.4	11	16	11		8.7
5						19
6	1.1	5.3	6.4	5.3	11	2.1
5-FU[b]	33	34	45	43	29	35
doxorubicin[b]	1.7	7.1		5.3	11	6.9

[a] IC_{50} > 100 μM. [b] 5-Fluorouracil (5-FU) and doxorubicin were both used as positive controls.

four-ring system. These results suggest that the enlarged aglycone of **5** eliminates cytotoxicity (IC_{50} > 100 μM).

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were determined with an MCP 300 (Anton Paar) polarimeter at 25 °C. UV spectra were recorded on an U-2910 spectrometer (Hitachi). IR spectra were obtained on a 5DX-FTIR spectrophotometer (Nicolet). NMR spectra were recorded with an Avance 500 spectrometer (Bruker) at 500 MHz for ^{1}H nucleus and 125 MHz for ^{13}C nucleus in $CDCl_3$ or $CDCl_3/CD_3OD$. Chemical shifts (δ) are given with reference to TMS. Coupling constants (J) are given in Hz. ESIMS spectra were detected with an Esquire 3000 plus spectrometer (Bruker). HRESIMS data were acquired with a Xevo G2 TOF mass spectrometer (Waters) and a microTOF-QII mass spectrometer (Bruker). Column chromatography (CC) was performed using silica gel (100−200 mesh; Qingdao Marine Chemicals) and Sephadex LH-20 (Amersham Pharmacia). HPLC was performed with a 210 solvent delivery module equipped with a 335 photodiode array detector (Varian) and using a Prodigy ODS (2) column (150 × 4.6 mm, 5 μm; Phenomenex).

Bacterial Materials. The strain SCSIO LR32 was isolated using modified ISP4-medium from a sediment sample collected in the South China Sea at E 119°57.260′ and N 20°59.877′, 3370 m below sea level. It was identified as *Streptomyces lusitanus* using 16S rDNA sequence analysis.[9] The DNA sequence has been deposited in GenBank (accession no. JQ315184). The strain has been preserved at the RNAM Center for Marine Microbiology, South China Sea Institute of Oceanology, Chinese Academy of Sciences.

Fermentation and Extraction. The strain was maintained on modified ISP-4 medium plates. A spore and mycelial suspension was inoculated into each of the 250 mL Erlenmeyer flasks containing 50 mL of modified AM2 medium. The flasks were incubated at 28 °C on a rotary shaker (200 rpm) for two days, and then each of the seed cultures was aseptically transferred to 2 L Erlenmeyer flasks containing 450 mL of modified RA medium per flask. The flasks were then incubated at 28 °C on a rotary shaker (200 rpm) for eight days. After fermentation, the culture (8 L) was centrifuged to yield supernatant and a mycelial cake. The supernatant was extracted with equal volumes of butanone three times, and then samples were evaporated to dryness. The mycelial cake was extracted with 2 L of acetone three times, and the solvent was then evaporated to dryness. The two organic extracts were finally combined to give 8.3 g of residue.

JNP1 medium: 2.0% soluble starch, 0.2% fish meal, 0.2% trehalose, 0.3% beef extract, 3.0% sea salt, pH 7.0−7.4.

Modified AM2 medium: 1.0% soybean flour, 2.0% soluble starch, 0.5% yeast extract, 0.2% peptone, 0.2% $CaCO_3$, 0.4% sea salt, pH 7.2−7.4.

Modified RA medium. 1.0% glucose, 2.0% soluble starch, 1.0% malt meal, 1.0% maltose, 0.5% corn steep liquor, 3.0% sea salt, trace element solution (0.1 mL/1 L), pH 7.2−7.4

Isolation. The extract was subjected to silica gel CC using gradient elution with a $CHCl_3$−MeOH mixture (100:0, 99:1, 98:2, 95:5, 90:10, 80:20, v/v) to give six fractions (Fr.1−Fr.6), correspondingly. Fr.2 was applied to ODS CC eluting with a linear gradient of 20% to 100% MeOH over 80 min by medium-pressure chromatography (MPLC). The subfractions were analyzed by HPLC and grouped into four fractions (Fr.2-1−Fr.2-4). Fr.2-1 was purified by Sephadex LH-20 CC eluting with $CHCl_3$−MeOH (1:1) to give **6** (49 mg) and Fr.2-1-2. The latter was chromatographed on silica gel CC repeatedly, eluting with $CHCl_3$−MeOH (98:2), to afford **5** (18 mg). Fr.2-3 was isolated by silica gel CC repeatedly, eluting with $CHCl_3$−MeOH (98:2), and then the subfraction was further purified by Sephadex LH-20 CC eluting with $CHCl_3$−MeOH (1:1) to give **2** (5 mg). Fr.2-4 was subjected to silica gel CC using gradient elution with a petroleum ether−EtOAc mixture (100:0, 98:2, 95:5, 90:10, 80:20, 50:50, v/v) to give six fractions (Fr.2-4-1−Fr.2-4-6). Fr.2-4-2 was further separated by silica gel CC eluting with $CHCl_3$−MeOH (97:3) to obtain **4** (12 mg). Fr.2-4-3 was subjected to ODS CC eluting with a linear gradient of 20% to 100% MeOH over 80 min by MPLC to give **1** (10 mg) and **3** (13 mg).

*Grincamycin B (**1**):* red solid; $[\alpha]^{25}_D$ −18 (*c* 0.40, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 230 (4.86), 258 (4.65), 294 (4.11), 442 (4.36) nm; IR (KBr) ν_{max} 3416, 2976, 2936, 1731, 1703, 1628, 1604, 1583, 1431, 1375, 1260 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Table 1; (−)-ESIMS *m*/*z* 937.7 $[M - H]^-$; (−)-HRESIMS *m*/*z* 937.3853 $[M - H]^-$ (calcd for $C_{49}H_{61}O_{18}$, 937.3863).

*Grincamycin C (**2**):* red, amorphous solid; $[\alpha]^{25}_D$ −41 (*c* 0.73, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 230 (4.85), 258 (4.64), 294 (4.13), 442 (4.36) nm; IR (KBr) ν_{max} 3421, 2925, 2854, 1732, 1627, 1605, 1581, 1431, 1374, 1260 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Table 1; (+)-ESIMS *m*/*z* 735.0 $[M + Na]^+$, (−)-ESIMS *m*/*z* 711.6 $[M - H]^-$, 1462.0 $[2M + K - H]^-$; (−)-HRESIMS *m*/*z* 711.2649 $[M - H]^-$ (calcd for $C_{37}H_{43}O_{14}$, 711.2658).

*Grincamycin D (**3**):* brown-yellow, amorphous solid; $[\alpha]^{25}_D$ +70 (*c* 0.55, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 230 (4.86), 258 (4.64), 294 (4.14), 442 (4.35) nm; IR (KBr) ν_{max} 3433, 2978, 2936, 2885, 1734, 1628, 1605, 1583, 1431, 1374, 1259 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Table 1; (+)-ESIMS *m*/*z* 845.2 $[M + Na]^+$, (−)-ESIMS *m*/*z* 821.3 $[M - H]^-$; (−)-HRESIMS *m*/*z* 821.3002 $[M - H]^-$ (calcd for $C_{43}H_{49}O_{16}$, 821.3026).

*Grincamycin E (**4**):* yellowish powder; $[\alpha]^{25}_D$ −112 (*c* 0.17, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 214 (5.18), 262 (4.48), 442 (4.45) nm; IR (KBr) ν_{max} 3404, 2975, 2934, 2855, 1732, 1700, 1630, 1608, 1577, 1423, 1316, 1240 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Table 2; (+)-ESIMS *m*/*z* 943.4 $[M + Na]^+$, 959.3 $[M + K]^+$, (−)-ESIMS *m*/*z* 919.5 $[M - H]^-$, 951.6 $[M + MeOH - H]^-$; (−)-HRESIMS *m*/*z* 919.3763 $[M - H]^-$ (calcd for $C_{49}H_{59}O_{17}$, 919.3758).

*Grincamycin F (**5**):* dark red powder; $[\alpha]^{25}_D$ +110 (*c* 0.09, $CHCl_3$−MeOH, 9:1); UV (MeOH) λ_{max} (log ε) 222 (5.16), 302 (4.77), 511 (4.74) nm; IR (KBr) ν_{max} 3420, 2970, 2924, 2853, 1729, 1638, 1604, 1445, 1282 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Table 2; (+)-ESIMS *m*/*z* 1093.6 $[M + Na]^+$, 1125.4 $[M + Na + MeOH]^+$, (−)-ESIMS *m*/*z* 1069.7 $[M - H]^-$; (−)-HRESIMS *m*/*z* 1069.4076 $[M - H]^-$ (calcd for $C_{57}H_{65}O_{20}$, 1069.4075).

*Grincamycin (**6**):* pale red powder; $[\alpha]^{25}_D$ −61 (*c* 0.33, $CHCl_3$) [lit. $[\alpha]^{22}_D$ −48 (*c* 0.1, $CHCl_3$)];[14] ^{1}H and ^{13}C NMR spectroscopic data, see Table S1.

Photochemical Transformation of 6 into 4. A solution of grincamycin in MeOH (2.0 mg/mL) was irradiated under $UV_{254\ and\ 365}$ for 24 h at room temperature. During irradiation, MeOH was

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occasionally supplied to maintain the volume. The product was analyzed by HPLC with a Prodigy ODS (2) column (150 × 4.6 mm, 5 μm; Phenomenex). The solvent system comprised solvent A (0.1% AcOH/15%MeCN in H_2O) and solvent B (0.1%AcOH/85%MeCN in H_2O). Elution was performed using a linear gradient of 20% to 100% solvent B over the course of 20 min, followed by holding at 100% solvent B for 15 min. The flow rate was 1.0 mL/min, and UV detection was at 440 nm. Grincamycin (**6**) (1 mg/mL) and grincamycin E (**4**) (1 mg/mL) were used as reference compounds.

Cytotoxicity Assay. The cell growth inhibitory activities of compounds **1**−**6** against the human cell lines HepG2, SW-1990, HeLa, NCI-H460, and MCF-7 and the mouse cell line B16 were determined using the previously published MTT method.[30] Briefly, the cancer cell lines were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum in a humidified atmosphere of 5% CO_2 at 37 °C. Then 100 μL of cell suspension was plated in 96-well plates to a final concentration of 2 × 10^3 cells per well and incubated for 12 h. Following incubation, 50 μL of the test compound solutions (in DMSO) at various concentrations was added to each well. After the exposure to compounds **1**−**6** for 48 h, 50 μL of MTT solution (1 mg/mL in PBS) was added to each well, and the plates were incubated for 4 h at 37 °C. Then, 200 μL of DMSO was added in each well. The absorbance caused by formazan crystallization was read at 550 nm using a microplate reader (model 550, Bio-Rad). The calculation of cell viability used the following formula: cell viability (%) = (the average $A_{550\ nm}$ of the treated group/the average $A_{550\ nm}$ of the untreated group) × 100.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

HRESIMS and 1D and 2D NMR spectra of compounds **1**−**6**. HPLC analysis of the conversion of **6** into **4** by UV irradiation. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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