Bioactive Diterpene Derivatives from the Marine Sponge *Spongionella* sp.

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Three new compounds of the rare classes trisnorditerpenes, bisnorditerpenes, and norditerpenes, gracilins J−L (**1**, **2**, **6**), and a new diterpene, 3′-norspongiolactone (**8**), were isolated from the extract of the marine sponge *Spongionella* sp. using NMR- and bioassay-guided fractionation, in addition to three known gracilins (**3**−**5**) as well as the known diterpenoid tetrahydroaplysulphurin-1 (**7**). The structures were elucidated using NMR spectroscopic techniques and mass spectrometric analysis. The structure of gracilin H (**3**) was confirmed by single-crystal X-ray analysis. All compounds were tested for their cytotoxicity and for their potential to inhibit EGF-R tyrosine kinase.

Marine sponges have drawn attention for their ability to produce an immense number and variety of bioactive secondary metabolites.[1] These metabolites may play a role in warding off predators, and they may also deter fouling organisms.[2] In connection with our longstanding interest in the chemistry of marine sponges, we had the occasion to examine the organic extract of the marine sponge *Spongionella* sp. obtained from the U.S. National Cancer Institute's Open Repository Program. Isolations were carried out using column chromatographic techniques, including HPLC, and were guided by NMR and cytotoxicity screening to yield four new compounds. Gracilins J (**1**) and K (**2**) are new representatives of the rare classes bisnorditerpenes and trisnorditerpenes, respectively, and were obtained in addition to the known gracilins H (**3**) and I (**4**). The known norditerpene gracilin A (**5**) was isolated together with its new 12-hydroxy derivative, gracilin L (**6**). In addition to the known tetrahydroaplysulphurin-1 (**7**), the new diterpenoid 3′-norspongiolactone (**8**) was likewise obtained. The structures were elucidated by a combination of NMR techniques, high-resolution mass spectrometry, and comparison with the published spectral data of related compounds.

Previous chemical investigations of species from the genus *Spongionella* have revealed the presence of diterpenes, nor- and bisnorditerpenes,[3−9] furanosesterterpenes,[10,11] and polyhydroxylated sterols.[12−14] Among these compounds, the gracilins isolated from *Spongionella gracilis* were considered as an interesting group of compounds from the structural point of view, due to the presence of the quite unusual diacetoxyhexahydrodifuro[2,3-*b*;3′,2′-*d*]furan-2-one moiety in the structure of some of them.[9]

In 2006, the cytotoxic activity of gracilins B, C, and G-I against a diverse panel of 12 human cancer cell lines representative of different tumor types including lung, colon, breast, ovary, pancreas, prostate, cervix, melanoma, and leukemia was reported.[9] All five compounds showed cytotoxic activity with GI_{50} values in the micromolar range. Moreover, gracilin B exhibited broad-spectrum inhibition of cell adhesion to different extracellular matrix (ECM) proteins. These effects, though specific, are not restricted to a particular integrin subfamily, and therefore, gracilin B could putatively interfere with the survival, growth, migration, and invasion of a wide diversity of tumor cell types.[9] Gracilin A was also reported to be a potent phospholipase A_2 (PLA_2) inhibitor with a 69% inactivation efficiency.[15]

In this study, the isolated compounds were tested for their cytotoxicity and for their potential to inhibit tyrosine kinases. Many investigators have found that overexpression of receptor protein tyrosine kinases (RPTKs), such as epidermal growth factor receptor (EGF-R),[16] insulin-like growth factor I,[17] and fibroblast growth factor receptor,[18] is associated with different types of human tumors. Of the 30 RPTKs that have been implicated in human cancers, the deregulation of the EGF-R system seems to be the most prevalent.[19−22] As overexpressed EGF-R signaling plays a role in carcinogenesis, inhibiting this process is a rational target for anticancer drug development.

Results and Discussion

The ^{13}C NMR and MS data of **1** indicated the molecular formula $C_{24}H_{32}O_{10}$ and, thus, nine degrees of unsaturation. The presence of three deshielded methyl groups at δ_H 1.99 (3H, s) and 2.05 (6H, s) in the ^1H NMR spectrum and three carbonyls at δ_C 169.7 (qC), 169.8 (qC), and 170.9 (qC) in the ^{13}C NMR spectrum (Table 1) indicated the presence of three acetate groups. The ^1H NMR and the ^{13}C NMR spectra also revealed the presence of a trisubstituted double bond [(δ_H 6.53, 1H, ddt, J = 1.5, 7, and 16 Hz) and (δ_C 127.1, qC; 144.9, CH)] and a lactone group [δ_C 167.1, qC]. The conjugation between the double bond and the lactone carbonyl group was deduced from the HMBC correlation between the carbon at δ_C 167.1 (qC) and the proton at δ_H 6.53 (1H, ddt, J = 1.5, 7, and 16 Hz). This analysis brings the total number of unsaturations in the structure to five, and thus, four rings had to be incorporated into the structure of **1**. The presence of three downfield resonances in the ^{13}C NMR spectrum between δ_C 100 and 120 and their corresponding resonances in the ^1H NMR spectrum between δ_H 5.90 and 6.20 indicated the presence of three acetal groups. Given the fact that **1** has 10 oxygen atoms, of which eight have been used, it can be inferred that the three acetal carbons are linked together through two oxygen bridges, and one of them, C-15, has to be directly attached to the lactone oxygen. This assumption was corroborated by the HMBC correlations between C-14 and H-15, C-15 and H-16, and C-16 and H-13 (Table 1). The 3-substituted 4,5-diacetoxyhexahydrodifuro[2,3-*b*;3′,2′-*d*]furan-2-one moiety was then deduced from the COSY correlations between H-10 and H-11, H-11 and H-16, and H-15 and H-10 (Figure 1) as well as the HMBC correlations between C-9 and H-10, C-9 and H-11, C-10 and H-12,

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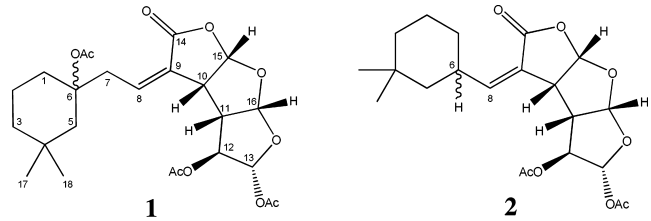
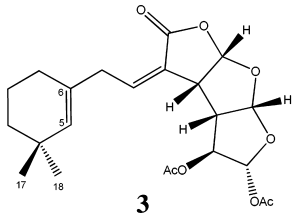
C-11 and H-13, and C-14 and H-10 (Table 1). Searching the Marinlit[23] database for compounds containing this substructure indicated that **1** is related to the known gracilins B−D, G, H (**3**), and I (**4**). This was confirmed by comparison of the spectroscopic data of **1** with those reported.[5,7,9]



1

2



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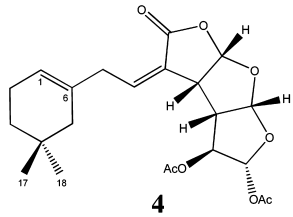

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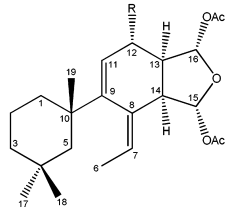
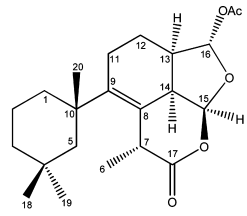


5, R = H
6, R = OH



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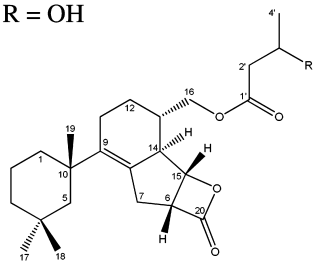

8, R = H
9, R = CH_3

The COSY and HMBC correlations (Figure 1 and Table 1) confirmed that the remaining resonances observed in the ^{1}H and ^{13}C NMR spectra account for 3,3-dimethylcyclohexyl acetate. Finally, the connection between the latter and the 4,5-diacetoxyhexahydrodifuro[2,3-*b*;3′,2′-*d*]furan-2-one moiety was deduced from the COSY correlation between H_2-7 and H-8 as well as the HMBC correlations between C-6 and H-8, and C-9 and H_2-7. Dereplication proved that **1** is a new bisnorditerpene to which the name gracilin J was given.

The relative configuration of gracilin J (**1**) was established by the analysis of the NOESY spectrum. Correlations between H-10, H-11, H-15, and H-16 (Figure 1) revealed that all these protons are present on the same face of the molecule. However, it was not possible to deduce the configuration at C-12 and C-13. Previously, for gracilins G−I, the configuration at these chiral centers was based on the calculated coupling constants.[9] However, in this study, additional support for this assignment was obtained from the X-ray crystal structure of the related compound, gracilin H (**3**) (Figure 2). The relative configuration of the cyclohexyl moiety cannot be related to the core part of the molecule owing to free rotation about C-6−C-7 and C-7−C-8. The observed correlations in the NOESY spectrum are consistent with a chair conformation of the cyclohexane ring (Figure 1). Finally, the *Z* configuration of the double bond was confirmed on the basis of the NOESY correlation observed between H-8 and H-10.

The ^{13}C NMR and MS data of **2** indicated the molecular formula $C_{21}H_{28}O_8$ and, thus, eight degrees of unsaturation. The close similarity of its ^{1}H and ^{13}C NMR spectra (see Table 1) to those of **1** indicated that both structures are related. Further analysis of the ^{1}H and ^{13}C NMR spectra revealed that **2** lacks the methylene group at position 7 and that the acetate group attached to C-6 has been replaced by a proton (δ_H 3.72, m). This loss was confirmed by the mass spectrum, which showed that compound **2** is 72 mass units less than compound **1**. Further confirmation was derived from the correlation between H-6 and H_2-1, H_2-5, and H-8 in the COSY spectrum and the correlations between C-1 and H-8 as well as C-5 and H-8 in the HMBC spectrum. The remaining COSY and HMBC correlations observed for the 3,3-dimethylcyclohexyl moiety and the 3-substituted 4,5-diacetoxyhexahydrodifuro[2,3-*b*;3′,2′-*d*]furan-2-one moiety in **2** were reminiscent of those observed for **1** (Table 1). Compound **2** is proved to be a new trisnorditerpene, for which the name gracilin K was suggested.

As described above for **1**, the relative configuration of the 4,5-diacetoxyhexahydrodifuro[2,3-*b*;3′,2′-*d*]furan-2-one moiety in **2** was determined by the NOESY spectrum, but it was not possible to deduce the relative configuration at C-6.

Compounds **3** and **4** were obtained as colorless crystals of an inseparable mixture of two stereoisomers in a 4:3 ratio, as indicated by the analysis of the ^{1}H NMR spectrum. Careful comparison of the ^{13}C NMR spectroscopic data of this mixture with those of **1** and **2** revealed the presence of a 3-substituted 4,5-diacetoxyhexahydrodifuro[2,3-*b*;3′,2′-*d*]furan-2-one moiety and an extra 18 carbon atoms. After all carbons had been assigned to their directly bonded protons, aided by an HSQC spectrum, it was possible to construct the two different substituted six-membered rings of **3** and **4** by interpretation of the COSY NMR spectrum. The presence of the two six-membered rings as two separate spin systems was later confirmed by carrying out different 1D-TOCSY experiments. Dereplication using the MarinLit[23] database proved that **3** and **4** are the known gracilins H and I, respectively.

Additionally, the structure of **3** was studied by single-crystal X-ray diffraction. Colorless chips of **3** were recrystallized from MeOH, and the single-crystal analysis revealed that one molecule of **3** makes up the asymmetric unit of the chiral, orthorhombic, unit cell (Figure 2). Although the NMR data indicate the presence of two stereoisomers with double bonds in the C-5═C-6 or C-1═C-6 positions coexisting in solution in a 4:3 ratio, the X-ray crystal structure could not resolve these (assuming both to be present) and was modeled with the double bond in the C-5═C-6 position, with a resulting C-5−C-6 bond distance of 1.366(6) Å. Attempts to model the double bond as being disordered over the C-5═C-6 and C-1═C-6 positions with a multiple-site model were not successful.

It should be noted that the C-1−C-6 separation of 1.425(6) Å in **3** is short for a carbon−carbon single bond. However, this bond length could be affected by the positional disorder of C-3 and its attached H atoms (modeled as being split over two sets of sites in a 0.524(19):0.476(19) ratio) and C-2 (not modeled as disordered, but possessing a rather anisotropic displacement ellipsoid). Thus, it is not clear if the X-ray structure of **3** represents the unresolved cocrystallization of both stereoisomers or the preferential crystallization of the C-5═C-6 double-bond stereoisomer. The three five-membered rings **A** (C-9/C-10/C-14/C-15/O-2), **B** (C-10/C-11/C-15/C-16/O-3), and **C** (C-11/C-12/C-13/C-16/O-4) in **3** are all approximately planar, and the dihedral angles between their mean planes are **A/B** = 61.63(16)°, **B/C** = 66.37(16)°, and **A/C** = 53.65(16)°, which correlates with the highly folded conformation of the molecule in the solid state. The absolute structure of **3** could not be established, and the relative configurations of the stereogenic centers in the molecule were determined as C-10 *S**, C-11 *R**, C-12

Table 1. NMR Spectroscopic Data (400 MHz, $CDCl_3$) for Gracilins J (**1**) and K (**2**)

	gracilin J (**1**)			gracilin K (**2**)		
position	δ_C, mult.	δ_H, mult. (*J* in Hz)	HMBC[a]	δ_C, mult.	δ_H, mult. (*J* in Hz)	HMBC[a]
1	35.8, CH_2	2.10, m	3, 5, 7	32.1, CH_2	1.66, m	2, 6, 8
		1.20, m			1.08, m	
2	18.4, CH_2	1.61, m	1, 3	21.7, CH_2	1.51, m	1, 3
		1.45, m				
3	38.7, CH_2	1.36, m	5, 17, 18	38.7, CH_2	1.33, m	17, 18
		1.05, m			1.17, m	
4	30.8, qC		3, 5, 17, 18	30.5, qC		3, 5, 17, 18
5	45.3, CH_2	2.24, m	1, 7, 17, 18	45.0, CH_2	1.33, m	6, 8, 17, 18
		1.10, m			0.97, m	
6	83.4, qC		1, 5, 7, 8	32.9, CH	3.72, m	1, 5, 8
7	37.8, CH_2	3.53, ddd (1.2, 7.0, 16.0)	1, 5, 8			
		3.28, ddd (1.2, 7.0, 16.0)				
8	144.9, CH	6.53, ddt (1.5, 7.0, 16.0)	7, 10	155.9, CH	6.28, dd (2.0, 10.0)	5, 6, 10
9	127.1, qC		7, 8, 10, 11	123.4, qC		8, 10, 11
10	45.9, CH	3.91, ddd (1.5, 6.0, 12.0)	7, 8, 11, 12, 15, 16	45.7, CH	3.83, ddd (1.5, 6.0, 12.0)	8, 12, 14
11	51.9, CH	3.10, q (6.0)	10, 12, 13, 15, 16	52.0, CH	3.72, q (6.0)	12, 13, 15
12	78.8, CH	5.03, s	10, 11, 13, 16	78.6, CH	5.13, s	10, 11, 13, 16
13	100.6, CH	6.19, s	11, 12, 16	101.0, CH	6.17, s	11, 12, 16
14	167.1, qC		7, 8, 10, 15	166.9, qC		8, 10, 15
15	106.5, CH	5.99, d (6.0)	10, 11, 16	106.5, CH	5.98, d (6.0)	10, 11, 16
16	114.2, CH	6.12, d (6.0)	11, 13, 15	114.3, CH	6.12, d (6.0)	11, 13, 15
17	33.9, CH_3	0.84, s	3, 5, 18	33.4, CH_3	0.85, s	3, 5, 18
18	25.9, CH_3	0.92, s	3, 5, 17	24.6, CH_3	0.87, s	3, 5, 17
6-OAc	170.9, qC	1.99, s				
	22.5, CH_3					
12-OAc	169.8, qC			170.1, qC		
	20.9, CH_3	2.05, s	12	21.0, CH_3	2.07, s	12
13-OAc	169.7, qC			169.9, qC		
	20.9, CH_3	2.05, s	13	21.0, CH_3	2.06, s	13

[a] HMBC correlations are from carbon stated to the indicated proton(s).

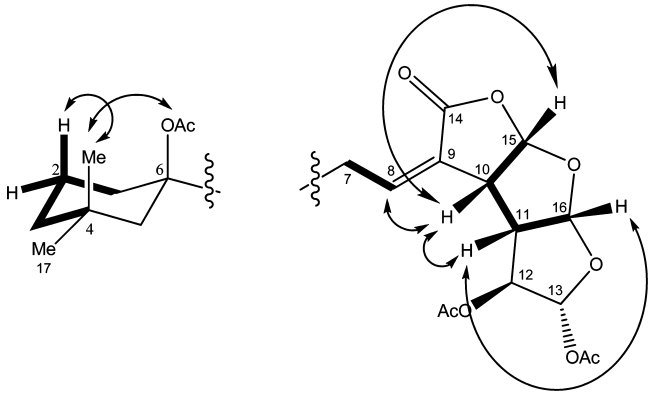

Figure 1. Key COSY (—) and NOESY (↔) correlations of compound **1** and the conformation of the six-membered ring.

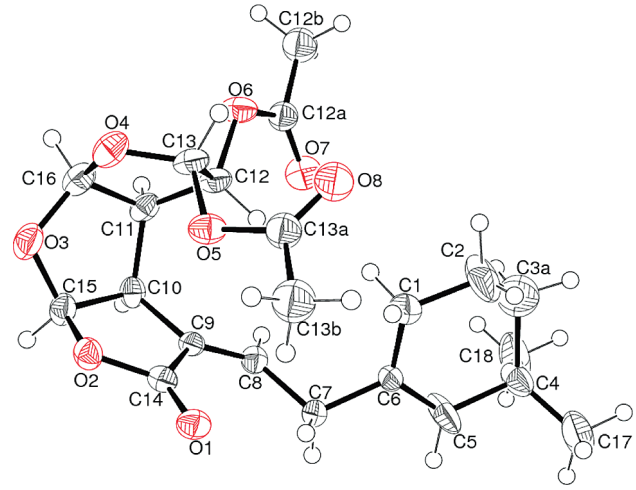

Figure 2. Molecular structure of **3**, with the non-hydrogen atoms represented by 50% displacement ellipsoids. Only one orientation of C-3 is shown.

*R**, C-13 *S**, C-15 *R**, and C-16 *S**, which correspond to those of the equivalent atoms in the known gracilins B and C. This is supported by the fact that the complete series of gracilins all show a strong positive optical rotation. Apart from two possible weak intermolecular C−H···O contacts, with H···O separations of 2.46 and 2.53 Å (see Supporting Information), the packing of the molecules in the crystal must be controlled by van der Waals forces.

The ^{13}C NMR and MS data of **6** indicated the molecular formula $C_{23}H_{34}O_6$ and, thus, one additional oxygen atom compared to the known gracilin A (**5**). The close similarity of its ^1H and ^{13}C NMR spectra (see Table 2) to those of **5** readily established that compound **6** is the 12-hydroxy derivative of gracilin A. This was corroborated by the chemical shift values observed for C-12 (δ_C 68.3) and H-12 (δ_H 4.14) as well as the correlations from H-12 to both H-11 and H-13 observed in the COSY spectrum and the correlations from C-9, C-11, C-13, C-14, and C-16 to H-12 in the HMBC spectrum. The connection of the 1,3,3-trimethylcyclohexyl moiety and the hexahydroisobenzofuran moiety in **6** was readily established by the HMBC correlations from C-10 to H-11 and from C-9 to H_2-1 and H_3-19 (Table 2). Thus, **6** was identified as a new natural product to which the name gracilin L was suggested.

The configurations at C-10, C-13, C-14, C-15, and C-16 of **6** are coincident with those of 9,11-dihydrogracilin A, whose relative configuration was determined by X-ray analysis of its 8-keto derivative.[24] To determine the configuration at C-12, molecular mechanics simulations were carried out on **6** with 12 α-OH and 12 *β*-OH (Figure 3). The proximity between H-12 and H-16 was confirmed by a strong NOE correlation from H-12 to H-16, indicating that the 12-OH is in the α configuration (Figure 3). This fact was also corroborated by the absence of an NOE correlation between H-12 and H-14, which would only be possible for a 12 *β*-OH.

The ^{13}C NMR and MS data of **8** indicated the molecular formula $C_{24}H_{36}O_4$. Its ^1H and ^{13}C NMR data (see Table 2)

Table 2. NMR Spectroscopic Data (400 MHz, $CDCl_3$) for Gracilin L (**6**) and Nor-spongiolactone (**8**)

	gracilin L (**6**)			nor-spongiolactone (**8**)		
position	δ_C, mult.	δ_H, mult. (*J* in Hz)	HMBC[a]	δ_C, mult.	δ_H, mult. (*J* in Hz)	HMBC[a]
1	38.4, CH_2	1.56, m	2, 3, 19	38.3, CH_2	1.80, m 0.99, m	2, 3
2	19.5, CH_2	1.48, m	1, 3	20.9, CH_2	1.45, m	1, 3
3	39.2, CH_2	1.36, m 1.04, m	1, 2, 17, 18	39.6, CH_2	1.22, m 1.08, m	2, 17, 18
4	31.3, qC		3, 5, 17, 18	31.4, qC		3, 5, 17, 18
5	50.7, CH_2	1.45, m 1.32, m	1, 3, 17, 18, 19	49.9, CH_2	1.90, m 1.03, m	17, 18, 19
6	17.7, CH_3	1.62, d (7.0)	7	54.1, CH	3.88 ddd (6.0, 11.2, 16.0)	7, 14, 15
7	121.6, CH	5.43, q (7.0)	6, 14	30.4, CH_2	3.02, dd (16.0, 11.0) 2.68, dd (16.0, 6.0)	6, 14, 15
8	133.0, qC		6, 11, 14, 15	133.5, qC		6, 7, 11, 13, 14, 15
9	155.7, qC		1, 6, 7, 11, 12, 14, 19	139.0, qC		5, 7, 12, 19
10	39.3, qC		1, 2, 5, 11, 19	39.2, qC		1, 2, 5, 19
11	128.7, CH	5.92, d (4.0)	12, 13	26.6, CH_2	2.02, m 1.78, m	12, 13
12	68.3, CH	4.14, dd (4.0, 5.5)	11, 13, 14, 16	26.2, CH_2	2.28, m	11, 13, 14, 16
13	56.4, CH	2.70, ddd (2.2, 5.0, 9.0)	11, 12, 14, 15, 16	36.6, CH	1.63, m	11, 12, 14, 15, 16
14	54.2, CH	3.07, dd (1.2, 9.0)	7, 11, 12, 13, 15, 16	50.2, CH	2.39, d (11.0)	7, 12, 15, 16
15	100.9, CH	6.36, s	13, 14, 16	80.1, CH	4.71, dd (6.0, 3.8)	6, 7, 13, 14
16	105.2, CH	5.99, d (2.0)	12, 13, 15	67.8, CH_2	4.12, dd (11.2, 6.5) 4.02, dd (11.2, 6.5)	12, 13, 14, 2′
17	35.7, CH_3	0.87, s	3, 5, 18	26.0, CH_3	0.76, s	3, 5
18	27.8, CH_3	0.98, s	3, 5, 17	32.9, CH_3	0.83, s	3, 5
19	26.2, CH_3	1.24, s	1, 5	30.4, CH_3	0.95, s	1, 5
20				172.2, qC		6, 7, 15
1′				173.7, qC		13, 16, 2′, 3′, 4′
2′				36.1, CH_2	2.26, m 1.28, m	16, 3′, 4′
3′				18.4, CH_2	1.61, m 0.82, m	2′, 4′
4′				13.7, CH_3	0.92, t	2′, 3′
15-OAc	171.1, qC					
	21.5, CH_3	2.05, s	15			
16-OAc	170.1, qC					
	21.5, CH_3	2.07, s	16			

[a] HMBC correlations are from carbon stated to the indicated proton(s).

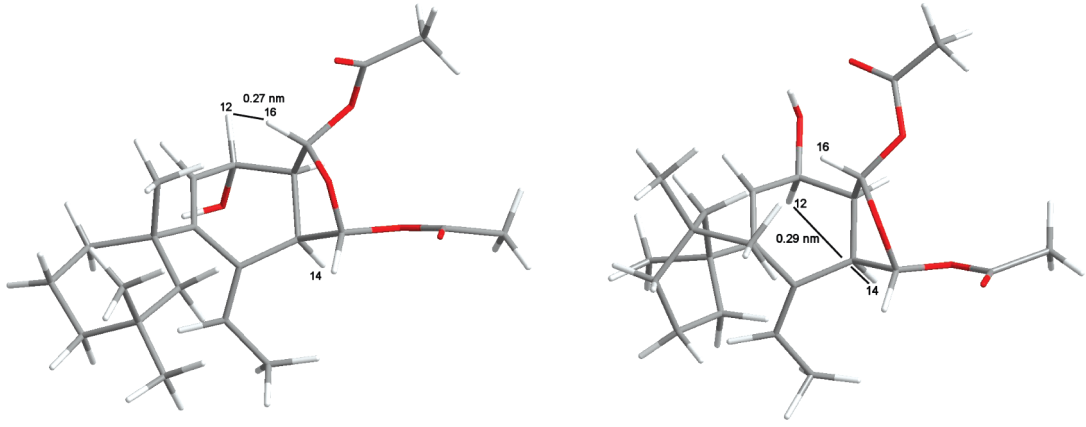

Figure 3. Molecular mechanics models of the 12 α-OH (left) and 12 *β*-OH (right) indicating that the observed NOE H-12 to H-16 is consistent only with the 12 α-OH stereochemistry.

suggested a close structural similarity to the known spongiolactone (**9**),[8] with the main difference of lacking one methyl and one methine signal, which were replaced by an additional methylene signal. Thus, compound **8** was identified as the C-3′-demethyl congener of spongiolactone (**9**), representing a new natural product, for which the name 3′-norspongiolactone was suggested. This was further confirmed by the COSY correlations (Figure 4) and the HMBC correlations (Table 2).

Since the spectroscopic data obtained for the diterpene part of **8** were virtually identical to those reported for **9**, it was assumed that the relative configuration of both compounds is identical, which is consistent with the observed NOE data (Figure 4).

This study, therefore, adds three new gracilins to the 10 known analogues and one new spongiolactone to the only other known representative of this rare family.

Cytotoxicity. The isolated compounds **1**−**8** were evaluated for their cytotoxicity against K562 human chronic myelogenous leukemia cells and the normal human peripheral blood mononuclear cells (PBMC). All compounds showed cytotoxic activity against the leukemia cell line, with IC_{50} values ranging from 0.6 to 15 μM

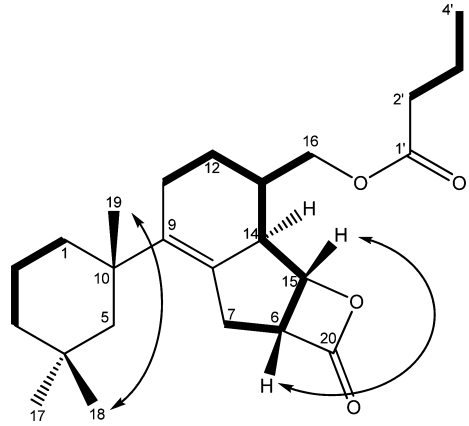

Figure 4. Key COSY (—) and NOESY (↔) correlations of compound **8**.

Table 3. Cytotoxicity of Compounds **1**−**8** against K562 and PBMC Cells

	IC_{50} values (μM)[a]	
compound	K562	PBMC
1	15 ± 1	30 ± 10
2	8.5 ± 0.5	9 ± 1
3/4	4.5 ± 0.5	6.5 ± 1.5
5	0.6 ± 0.2	0.8 ± 0.4
6	2.65 ± 0.05	3.0 ± 0.5
7	2.3 ± 0.2	4.5 ± 0.7
8	12 ± 1	30 ± 10

[a] The results are means ± standard deviation of three independent replicates.

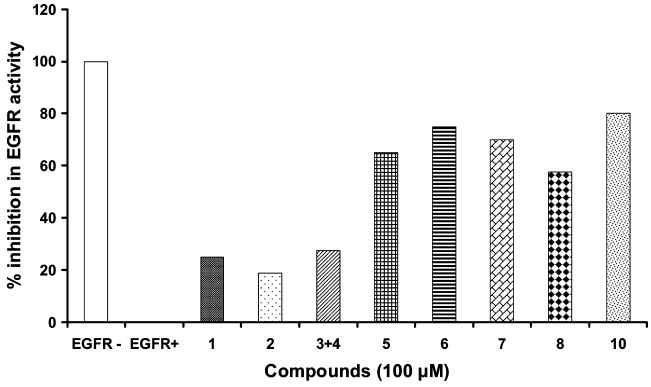

Figure 5. EGF-R inhibitory activity after incubation with 100 μM of each test compound.

(Table 3), while they showed similar or slightly less toxicity toward the normal PBMC cells, with IC_{50} values ranging from 0.8 to 30 μM (Table 3). Compound **5** was the most toxic, while **1** was the least potent; however the latter showed modest selectivity toward the leukemia cell line.

Tyrosine Kinase Inhibition. The inhibitory properties of the isolated compounds toward the protein tyrosine kinase, EGF-R, were investigated using an ELISA-based *in vitro* assay kit.[25] All compounds proved to be active at 100 μM (Figure 5), with gracilin L (**6**) being the most potent (75% inhibition) and, thus, almost as active as the positive control, genistein, which showed 80% inhibition, while gracilin K (**2**) was the least potent (19% inhibition).

Experimental Section

General Experimental Procedures. Optical rotations were recorded using a Perkin-Elmer 343 polarimeter. UV and IR spectra were measured on a Perkin-Elmer Lambda 25 UV/vis spectrometer and a Thermo Nicolet IR 100 FT/IR spectrometer, respectively. ^{1}H, ^{13}C, and all 2D NMR experiments were acquired on a Varian Unity INOVA 400 MHz spectrometer. Low-resolution electrospray mass spectra were obtained using a Perseptive Biosystems Mariner LC-MS, and high-resolution mass data were obtained on a Finnigan MAT 900 XLT. HPLC separations were carried out using a Phenomenex reversed-phase (C18, 250 × 10 mm, L × i.d.) column connected to an Agilent 1100 series binary pump and monitored using an Agilent photodiode array detector. Detection was carried out at 210, 236, 254, 280, and 360 nm. Molecular dynamics calculations were carried out in ChemBio3D Ultra ver. 10 for 10 000 steps, 300 K, and 2 fs time interval using the MM2 force field.

Animal Material. A sample of *Spongionella* sp. (phylum Porifera, class Demospongiae, order Dendroceratida, family Dictyodendrillidae) was collected in September 2002 from West Angaur, the Philippines, at a depth of 10 m, by Coral Reef Foundation scientists under contract with the U.S. NCI. Collected material was stored at −20 °C until used. Voucher specimens are stored at the Smithsonian Institution, USA, under 0CDN 8588-T.

Extraction and Isolation. The frozen sample was ground with dry ice (CO_2) and extracted with deionized water at 4 °C by stirring for about 2 h. The aqueous extract was freeze-dried, and the residue was then successively extracted with MeOH−CH_2Cl_2 (1:1), followed by MeOH (100%). The organic extracts were combined and concentrated under vacuum. The crude organic extract was stored at −5 °C until used.

Fractionation of the crude extract [5 g] was performed using a modified Kupchan scheme,[26] which results in four crude fractions: hexane (FH) [0.55 g], CH_2Cl_2 (FD) [0.15 g], MeOH/H_2O (FM) [0.19 g], and *sec*-BuOH (WB) [1.2 g]. Cytotoxicity screening indicated that FH and FD were the only fractions worth fractionating further.

The combined hexane (FH) and CH_2Cl_2 (FD) fractions were loaded on a Sephadex LH-20 column equilibrated with MeOH/CH_2Cl_2 (1:1), and two fractions were collected. The second fraction (0.39 g) was purified by reversed-phase HPLC using a gradient of MeCN in H_2O as eluent (50−100% over 50 min, 100% for 10 min) at a flow rate of 1.25 mL/min to afford the pure compounds **1** [9 mg], **2** [11 mg], **3** and **4** as a mixture [115 mg], **5** [10 mg], **6** [17 mg], **7** [23 mg], and **8** [5 mg].

Gracilin J (1): colorless oil; $[\alpha]^{20}_D$ +120 (*c* 0.1, CH_3OH); UV (MeCN) λ_{max} ($\log_\epsilon$) 226 (3.74) nm; IR (film) ν_{max} 1742, 1649, 1119 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 498.2332 [M + NH_4]$^+$ (calcd for $C_{24}H_{36}NO_{10}$, 498.2334).

Gracilin K (2): colorless oil; $[\alpha]^{20}_D$ +150 (*c* 0.1, CH_3OH); UV (MeCN) λ_{max} ($\log_\epsilon$) 230 (4.14) nm; IR (film) ν_{max} 1739, 1651, 1156 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 426.2119 [M + NH_4]$^+$ (calcd for $C_{21}H_{32}NO_8$, 426.2122).

Gracilins H (3) and I (4): colorless crystals (MeOH); mp 120.4 °C; $[\alpha]^{20}_D$ +280 (*c* 0.1, CH_3OH); UV (MeCN) λ_{max} ($\log_\epsilon$) 224 (3.91) nm; IR (film) ν_{max} 1740, 1645, 1123 cm^{-1}; HRESIMS *m*/*z* 438.2121 [M + NH_4]$^+$ (calculated for $C_{22}H_{32}NO_8$, 438.2122).

Gracilin L (6): colorless oil; $[\alpha]^{20}_D$ +170 (*c* 0.1, CH_3OH); UV (MeCN) λ_{max} ($\log_\epsilon$) 222 (3.92) nm; IR (film) ν_{max} 3348, 1650, 1642, 1010 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 2; HRESIMS *m*/*z* 424.2701 [M + NH_4]$^+$ (calcd for $C_{23}H_{38}NO_6$, 424.2694).

3′-norspongiolactone (8): colorless oil; $[\alpha]^{20}_D$ +22 (*c* 0.1, CH_3OH); UV (MeCN) λ_{max} ($\log_\epsilon$) 198 (3.26) nm; IR (film) ν_{max}1630, 1658, 1768 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 2; HRESIMS *m*/*z* 406.2945 [M + NH_4]$^+$ (calcd for $C_{24}H_{40}NO_4$, 406.2952).

X-ray Structure Determination of 3: $C_{22}H_{28}O_8$, M_r = 420.44, orthorhombic, space group $P2_12_12_1$ (No. 19), a = 10.6853(4) Å, b = 11.4061(5) Å, c = 17.0613(6) Å, V = 2079.39(14) Å^3, Z = 4, ρ_{calc} = 1.343 g cm^{-3}, Mo Kα radiation (λ = 0.71073 Å). A colorless chip (0.11 × 0.08 × 0.04 mm) was mounted on a Nonius KappaCCD diffractometer, and data were collected at 120(2) K. A total of 11 794 scanned intensities were merged (R_{Int} = 0.052) to 2694 unique data [2202 with $I > 2\sigma(I)$] with $2\theta < 55°$. All the non-hydrogen atoms in the structure were located by direct methods with SHELXS-97.[27] The structural model was refined against $|F|^2$ with SHELXL-97.[27] The hydrogen atoms were placed geometrically (C−H = 0.95−1.00 Å) and refined as riding with $U_{iso}(H) = 1.2U_{eq}(C)$ or $1.5U_{eq}$(methyl C). The methyl groups were allowed to rotate, but not to tip, to best fit the electron density. Final residuals of $R(F) = 0.066$ [$I > 2\sigma(I)$] and $wR(F^2) = 0.137$ (all data) were obtained. Anomalous dispersion was negligible, and the absolute structure of **3** could not be established from the present experiment; therefore Friedel opposites were merged before refinement.

Cytotoxicity Assay. K562 human chronic myelogenous leukemia cells and human peripheral blood mononuclear cells (PBMC) were used in these experiments. PBMCs were isolated from the whole blood of healthy donors using Ficoll-Paque PREMIUM. The cell lines were cultured in RPMI 1640 medium (BioWhittaker) supplemented with 10% heat-inactivated fetal calf serum (FCS) and 1% antibiotic−antimycotic (BioWhittaker) at 37 °C, in a 5% CO_2, humidified atmosphere. The cytotoxicity of **1**−**8** against normal and leukemic cells was measured using the CellTiter-Glo luminescent cell viability assay (Promega, Madison, WI), based on ATP bioluminescence as a marker of cell viability.[28] Briefly, cells were suspended in the culture medium to a concentration 1×10^6 cells/mL and incubated with different concentrations of each compound for 24 h at 37 °C, in a 5% CO_2, humidified atmosphere. Cell culture plates were then equilibrated to room temperature for 30 min before cell lysis was initiated by adding an equal volume of CellTiter-Glo reagent to each well. Plates were then incubated for 10 min at room temperature. The luminescence produced by the luciferase-catalyzed luciferin plus ATP reaction was detected using a Berthold Orion microplate Luminometer. The data were normalized to the control group. Cytotoxicity of compounds was calculated as the percentage decrease in luminescence in comparison with the control. Three independent experiments were carried out for each compound concentration, and IC_{50} values were determined on semilogarithmic curves.

Tyrosine Kinase Inhibition. The isolated compounds were screened for their tyrosine kinase inhibitory activity using an ELISA-based *in vitro* assay kit from Sigma (cat. no. PTK101). The assay used a protein tyrosine kinase (PTK)-specific polymer substrate (PGT)-coated multiwell plate. The substrate contains multiple tyrosine residues that can be phosphorylated by wide range of PTKs. The phosphorylation reaction is initiated by the addition of the epidermal growth factor receptor (EGF-R) (4 units) in the tyrosine kinase reaction buffer that contains Mg^{2+}, Mn^{2+}, and ATP. Compounds were incubated with EGF-R at a final concentration of 100 μM before the addition of EGF-R to the substrate. The phosphorylated polymer substrate was then probed with a purified phosphotyrosine specific monoclonal antibody conjugated to horseradish peroxidase (HRP). Color was developed with HRP chromogenic substrate and quantified using an ELISA reader. The intensity of light measured by an ELISA reader at 492 nm reflects the relative amount of tyrosine kinase activity in the reaction. The absolute amount of tyrosine kinase activity in each reaction was then derived from the EGF-R standard curve. Compounds were dissolved in DMSO and diluted with the reaction buffer so that the final concentration of DMSO in the reaction is 0.05%. Each compound was tested twice, and the average reading was recorded. Similar reactions containing 0.05% DMSO with or without EGF-R were used as positive and negative control, respectively. Compounds' activities were compared with that of 100 μM genistein (Sigma-Aldrich, cat. no. G6649).

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Supporting Information Available: NMR spectra of **1**−**8** including ^1H, ^{13}C, DEPT 135, COSY, HSQC, and HMBC in $CDCl_3$, as well as the detailed crystal data for **3** are available free of charge via the Internet at http://pubs.acs.org. Crystallographic data for **3** including atomic positional and displacement parameters, all geometrical data, and full software details have been deposited in CIF format with the Cambridge Crystallographic Data Centre (deposition number CCDC 702621). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44(0) 1223-336033 or e-mail: deposit@ccds.cam.ac.uk].

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