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Anti-inflammatory Cembranoids from the Soft Corals *Sinularia querciformis* and *Sinularia granosa*

Yi Lu,[†] Chiung-Yao Huang,[†] Yu-Fang Lin,[†] Zhi-Hong Wen,[†] Jui-Hsin Su,[†] Yao-Haur Kuo,[‡] Michael Y. Chiang,[§] and Jyh-Horng Sheu*[,†,⊥]

Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Kaohsiung 804, Taiwan, Republic of China, National Research Institute of Chinese Medicine, Taipei 112, Taiwan, Republic of China, Department of Chemistry, National Sun Yat-sen University, Kaohsiung 804, Taiwan, Republic of China, and Asia-Pacific Ocean Research Center, National Sun Yat-sen University, Kaohsiung 804, Taiwan, Republic of China

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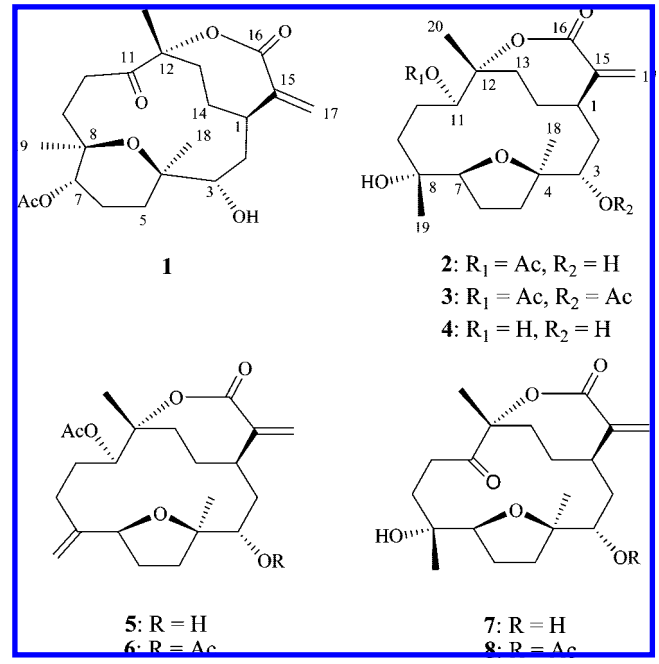
Four new cembranoids, querciformolides A−D (**1**−**4**), along with two known cembranoids, **7** and **8**, have been isolated from the soft coral *Sinularia querciformis*. Furthermore, chemical investigation of *Sinularia granosa* has afforded three new cembranoids, querciformolide B (**2**) and granosolides A (**5**) and B (**6**). The structures of the new metabolites were elucidated on the basis of extensive spectroscopic methods, and that of **2** was further confirmed by X-ray diffraction analysis. The absolute configurations of **1** and **2** were determined by a modified Mosher's method. Among these metabolites, **2**−**6** are rarely found cembranoids possessing a tetrahydrofuran moiety with a 4,7-ether linkage; in addition, **1** is the first ε-lactone cembrane found that possesses a tetrahydropyran moiety with a 4,8-ether linkage. None of these compounds were found to be cytotoxic toward a limited panel of cancer cell lines. However, compounds **3**, **7**, and **8** significantly inhibited the accumulation of the pro-inflammatory iNOS and COX-2 proteins in LPS-stimulated RAW264.7 macrophage cells.

Soft corals of the *Sinularia* genus are a rich source of terpenoids, steroids, and others.[1] We have previously discovered a series of sesquiterpenoids,[2−4] norcembranoids,[5] cembranoids,[6] steroids,[7−9] and other metabolites[10] from Formosan soft corals of this genus. Our recent investigation of bioactive natural products from two Formosan soft corals, *Sinularia querciformis* (Pratt, 1903, family Alcyoniidae) and *S. granosa* (Tixier-Durivault, 1970, family Alcyoniidae), has led to the isolation of six new cembranoids (**1**−**6**), along with two known metabolites, **7** and **8**.[11] New metabolites querciformolides A−D (**1**−**4**) were isolated from *S. querciformis* and querciformolide B (**2**), and granosolides A and B (**5** and **6**) were obtained from *S. granosa*. The relative structures of **1**−**6** were established by detailed spectroscopic analysis, and the relative structure of **2** was further proven by X-ray diffraction analysis. The absolute stereochemistries of **1** and **2** were determined by a modified Mosher's method.[12,13] Metabolites **1**−**8** were found to be noncytotoxic (IC_{50}'s >20 μg/mL) to a limited panel of human tumor cell lines, including cervical epitheloid (HeLa), laryngeal (Hep 2), medulloblastoma (Daoy), and breast (MCF-7) carcinoma cells. As it has been demonstrated that massive production of nitric oxide and prostaglandins via the pro-inflammatory proteins inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) plays an important pathophysiological role in the development of inflammatory diseases,[14] evaluation of the ability of **1**−**8** to inhibit the expression of iNOS and COX-2 in LPS (lipopolysaccharide)-stimulated RAW264.7 macrophage cells was carried out to search for bioactive substances from marine organisms. The results revealed that compounds **3**, **7**, and **8** possess significant anti-inflammatory activity.

Results and Discussion

The tissues of soft corals *S. querciformis* and *S. granosa* were homogenized and extracted exhaustively with EtOH. The crude products of the EtOH extracts of both soft corals were triturated with *n*-hexane followed by EtOAc. Each EtOAc-soluble fraction was then concentrated under reduced pressure, and the residues were repeatedly purified by chromatography to yield metabolites **1**−**4**, **7**, and **8** from *S. querciformis* and **2**, **5**, and **6** from *S. granosa* (see Experimental Section).



Querciformolide A was isolated as a white powder; its molecular formula was established as $C_{22}H_{32}O_7$ by HRESIMS (*m*/*z* 431.2044 $[M + Na]^+$), implying seven degrees of unsaturation. IR absorptions were observed at 3471, 1732, and 1715 cm^{-1}, suggesting the presence of hydroxy and carbonyl groups in **1**. In the ^{13}C NMR and DEPT spectra (Table 1), signals of four methyls (including one acetate methyl), seven sp^3 methylenes, one sp^2 methylene, three sp^3 methines (including two oxymethines), three sp^3 quaternary carbons, and four sp^2 quaternary carbons (including one ketone and two ester carbonyls) were observed. From signals (Tables 1 and 2)

* To whom correspondence should be addressed. Tel: 886-7-5252000, ext. 5030. Fax: 886-7-5255020. E-mail: sheu@mail.nsysu.edu.tw.
† Department of Marine Biotechnology and Resources, National Sun Yat-sen University.
‡ National Research Institute of Chinese Medicine.
§ Department of Chemistry, National Sun Yat-sen University.
⊥ Asia-Pacific Ocean Research Center, National Sun Yat-sen University.

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Table 1. ^{13}C NMR Data for Compounds **1**−**7**

C#	**1**[a]	**2**[b]	**3**[a]	**4**[a]	**5**[a]	**6**[a]	**7**[b]
1	32.6 (CH)	32.5 (CH)	32.3 (CH)	33.1 (CH)	32.0 (CH)	31.8 (CH)	32.5 (CH)
2	38.2 (CH_2)	37.3 (CH_2)	33.6 (CH_2)	37.2 (CH_2)	38.0 (CH_2)	34.1 (CH_2)	38.2 (CH_2)
3	75.2 (CH)	75.3 (CH)	74.3 (CH)	75.1 (CH)	74.2 (CH)	75.4 (CH)	74.6 (CH)
4	77.5 (C)	87.4 (C)	86.0 (C)	87.3 (C)	86.1 (C)	84.7 (C)	87.0 (C)
5	34.5 (CH2)	37.1 (CH_2)	36.7 (CH_2)	37.1 (CH_2)	37.6 (CH_2)	37.5 (CH_2)	37.3 (CH_2)
6	21.6 (CH_2)	25.4 (CH_2)	25.2 (CH_2)	25.5 (CH_2)	28.7 (CH_2)	28.6 (CH_2)	25.6 (CH_2)
7	77.6 (CH)	84.1 (CH)	84.2 (CH)	85.2 (CH)	81.3 (CH)	81.8 (CH)	84.7 (CH)
8	75.8 (C)	74.4 (C)	74.3 (C)	74.6 (C)	150.5 (C)	149.9 (C)	74.0 (C)
9	34.1 (CH_2)	39.8 (CH_2)	39.8 (CH_2)	40.2 (CH_2)	31.7 (CH_2)	31.6 (CH_2)	34.3 (CH2)
10	29.4 (CH_2)	26.0 (CH_2)	25.9 (CH_2)	27.1 (CH_2)	33.8 (CH_2)	33.8 (CH_2)	34.1 (CH_2)
11	210.5 (C)	74.1 (CH)	74.1 (CH)	73.1 (CH)	74.4 (CH)	74.4 (CH)	211.2 (C)
12	91.2 (C)	87.9 (C)	88.0 (C)	91.4 (C)	87.4 (C)	87.4 (C)	91.6 (C)
13	34.3 (CH_2)	33.5 (CH_2)	33.4 (CH_2)	33.2 (CH_2)	33.4 (CH_2)	33.3 (CH_2)	34.3 (CH_2)
14	31.1 (CH_2)	29.3 (CH_2)	28.9 (CH_2)	29.4 (CH_2)	29.2 (CH_2)	28.9 (CH_2)	30.7(CH_2)
15	144.8 (C)	144.2 (C)	143.9 (C)	144.2 (C)	144.7 (C)	144.4 (C)	144.6 (C)
16	168.4 (C)	168.9 (C)	168.9 (C)	169.5 (C)	169.2 (C)	169.1 (C)	168.5 (C)
17	124.9 (CH_2)	124.1 (CH_2)	124.1 (CH_2)	124.2 (CH_2)	123.7 (CH_2)	123.7 (CH_2)	124.7 (CH_2)
18	18.8 (CH_3)	15.1 (CH_3)	16.4 (CH_3)	15.8 (CH_3)	17.9 (CH_3)	19.5 (CH_3)	17.0 (CH_3)
19	20.3 (CH_3)	19.1 (CH_3)	19.1 (CH_3)	19.0 (CH_3)	114.6 (CH_2)	115.0 (CH_2)	23.5 (CH_3)
20	29.5 (CH_3)	24.1 (CH_3)	24.1 (CH_3)	23.3 (CH_3)	23.8 (CH_3)	23.8 (CH_3)	29.5 (CH_3)
Ac-1	21.2 (CH_3)	21.1 (CH_3)	21.1 (CH_3)		21.2 (CH_3)	21.3 (CH_3)	
	170.2 (C)	171.1 (C)	171.2 (C)		170.8 (C)	170.9 (C)	
Ac-2			21.2 (CH_3)			21.2 (CH_3)	
			170.1 (C)			170.3 (C)	

[a] Spectra recorded at 125 MHz in $CDCl_3$. [b] Spectra recorded at 100 MHz in $CDCl_3$.

Table 2. ^1H NMR Data for Compounds **1**−**7**

	1[a]	**2**[b]	**3**[a]	**4**[a]	**5**[a]	**6**[a]	**7**[b]
1	3.14 ddd (11.0,11.0, 6.0)[c]	3.32 m	3.31 m	3.03 m	3.47 m	3.47 m	3.08 ddd (11.2,11.2, 6.0)
2	1.95 m; 1.54 m	1.89 m; 1.82 m	1.84 m; 1.76 m	1.86m; 1.74 m	1.84 m	1.80 m	1.97 m; 1.63 m
3	3.43 d (10.0)	3.80 dd (11.2, 4.8)	5.00 dd (11.5, 5.0)	3.77 dd (11.5, 4.5)	3.70 dd (11.0, 4.5)	4.94 dd (7.0, 2.0)	3.58 dd (11.6, 2.4)
5	1.82 m; 1.58 m	1.89 m; 1.60 m	1.76 m; 1.44 m	1.86 m; 1.58 m	2.16 m; 1.81 m	1.88 m; 1.76 m	1.83 m; 1.68 m
6	1.90 m; 1.76 m	2.04 m	2.00 m	2.06 m	1.96 m	1.94 m	1.96 m; 1.86 m
7	4.70 dd (11.5, 4.0)	4.06 dd (8.8, 5.6)	4.05 m	4.05 dd (7.5, 6.0)	4.37 dd (7.5, 7.0)	4.37 dd (7.5, 7.0)	3.95 dd (7.2, 7.2)
9	2.36 dd (14.5, 8.5);	1.55 m;	1.76 m;	2.11 m;	2.41 m;	2.43 m;	2.24ddd (14.0, 10.0, 3.2);
	1.59 m	1.73 m	1.52 m	1.74 m	1.86 m	1.86 m	1.64 m
10	3.36 dd (19.5, 8.0);	1.62 m;	1.59 m;	1.43 m	1.90 m;	1.90 m;	3.63 ddd (20.8, 10.0, 3.2);
	2.19 dd (19.5, 10.0)	1.51 m	1.50 m		1.46 m	1.46 m	2.63 ddd (21.2, 8.0, 3.2)
11		6.38 d (10.8)	6.37 d (11.0)	4.80 dd (6.5, 6.5)	6.30 d (9.0)	6.27 d (9.0)	
13	2.43 dd (15.0, 6.5); 1.82 m	1.98 m; 1.88 m	1.95 m; 1.87 m	1.98 m; 1.88 m	1.92 m ; 1.84 m	1.92 m; 1.84 m	2.36 dd (15.6, 5.6); 1.88 m
14	2.06 m;	2.44 dddd (12.4, 12.4, 6.0, 6.0)	2.46 dddd (12.5, 12.5, 6.0, 6.0)	2.37 dddd (13.0, 13.0, 6.5, 6.5)	2.47 m;	2.51 m;	2.07 m;
	1.04 ddd (12.0, 12.0, 6.5)	1.12 m	1.19 m	1.09 m	1.02 m	1.00 m	1.12 m
17	6.34 s; 5.49 s	6.29 s; 5.50 s	6.27 s; 5.45 s	6.28 s; 5.51 s	6.25 s; 5.47 s	6.24 s; 5.42 s	6.33 s; 5.50 s
18	1.13 s	1.22 s	1.26 s	1.08s	1.25 s	1.32 s	1.07 s
19	1.25 s	1.18 s	1.16 s	1.14 s	5.17 s; 5.01 s	5.19 s; 5.03 s	1.14 s
20	1.46 s	1.39 s	1.36 s	1.33 s	1.32 s	1.32 s	1.45 s
Ac-1	2.05 s	2.16 s	2.13 s		2.11 s	2.11 s	
Ac-2			2.06 s			2.07 s	

[a] Spectra recorded at 500 MHz in $CDCl_3$. [b] Spectra recorded at 400 MHz in $CDCl_3$. [c] *J* values (in Hz) in parentheses.

appearing at δ_C 210.5 (C), 168.4 (C), 144.8 (C), 124.9 (CH_2), 91.2 (C), 34.3 (CH_2), 32.6 (CH) and 31.1 (CH_2) and δ_H 6.34 (1H, s), 5.49 (1H, s), and 3.14 (1H, ddd, J = 11.0, 11.0, 6.0 Hz), **1** was assigned as a cembrane possessing one seven-membered ε-acyl-α-methylenelactone functional group by comparing the very similar NMR data of **1** and those of **7** and **8** (Tables 1 and 2).[11] From the $^1H-^1H$ COSY spectrum of **1**, it was possible to identify three different structural units, which were assembled with the assistance of an HMBC experiment (Figure 1). Key HMBC correlations of H-3 to C-4; H_2-5 to C-3 and C-4; H-7 to C-8 and C-9; H_2-9, H_2-10, and H_2-13 to C-11 (ketocarbonyl carbon); H_2-17 to C-1, C-15, and C-16; H_3-18 to C-3, C-4, and C-5; H_3-19 to C-7, C-8, and C-9; and H_3-20 to C-11, C-12, and C-13 permitted the connection of the carbon skeleton. Furthermore, the acetoxy group positioned at C-7 was confirmed from the HMBC correlations of H-7 (δ 4.70) and protons of an acetate methyl (δ 2.05) to the ester carbonyl carbon at δ 170.2 (C). Thus, **1** was revealed as a cembranoid possessing a ε-lactone ring, on the basis of the above analysis. A hydroxy group was positioned at C-3, as both of the (*S*)- and (*R*)-MTPA esters of **1**, prepared for the determination of absolute structure (discussed later), showed significant differences in the chemical shifts of H-3 and C-3 in comparison with those of **1**. In considering the degrees of unsaturation and molecular formula, an ether linkage was placed between C-4 and C-8. On the basis of the above analysis, the gross structure of **1** was established.

The relative structure of **1** was elucidated by the analysis of NOE correlations, as shown in Figure 2. It was found that H-1 (δ 3.14, ddd, J = 11.0, 11.0, 6.0 Hz) showed NOE interactions with H_3-18 (δ 1.13, s); therefore, assuming the α-orientation of H-1, H_3-18 should also be positioned on the α-face. One of the methylene protons at C-10 (δ 3.36, dd, J = 19.5, 8.0 Hz) exhibited NOE correlations with H-1 and was characterized as H-10α, while the other (δ 2.19, dd, J = 19.5, 10.0 Hz) was assigned as H-10β. NOE correlations observed between H-10α and H_3-19, and H_3-19 and H_3-18, reflected the α-orientation of the methyl group at C-8. Also, H_3-20 was found to interact with H-10β, revealing the β-orientation of the methyl substituent at C-12. Furthermore, NOE correlations observed between H_3-18 and H-5α and between H-5β and both H-3 and H-7 assigned the α-orientations of both hydroxy and acetoxy groups. On the basis of the above findings and other detailed

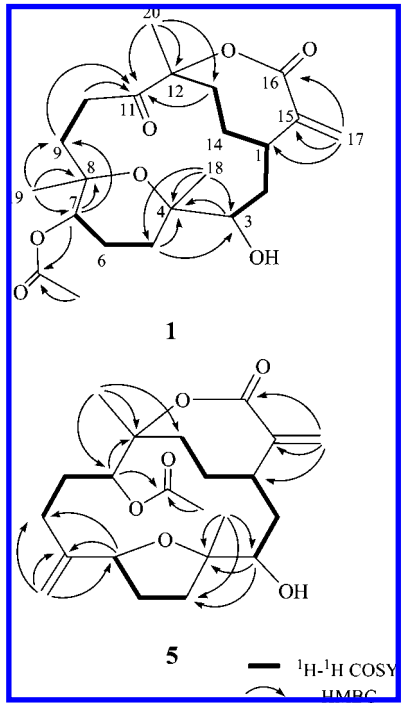

Figure 1. ^{1}H−^{1}H COSY and HMBC correlations for **1** and **5**.

NOE correlations (Figure 2), the relative structure of querciformolide A (**1**) was determined. Furthermore, in order to resolve the absolute structure of **1**, we determined the absolute configuration at C-3 using a modified Mosher's method.[12,13] The (*S*)- and (*R*)-2-methoxy-2-trifluoromethyl-2-phenylacetic (MTPA) esters of **1** (**1a** and **1b**, respectively) were prepared using the corresponding *R*-(−)- and *S*-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chlorides, respectively. The determination of $\Delta\delta$ values ($\delta_S - \delta_R$) for the protons neighboring C-3 led to the assignment of the *S* configuration at C-3, as shown in Figure 3.

The HRESIMS spectrum of querciformolide B (**2**) exhibited a molecular ion peak at *m/z* 433.2204 ([M + Na]$^+$), consistent with the molecular formula $C_{22}H_{34}O_7$ and implying six degrees of unsaturation. The IR absorptions of **2** showed the presence of hydroxy (3445 cm^{-1}) and ester carbonyl (1738 and 1718 cm^{-1}) groups. Comparison of the NMR data (Tables 1 and 2) of **2** with those of known compound **7** revealed that the structures of the compounds are very similar, with the difference that the ketocarbonyl carbon (C-11) of **7** was replaced by an acetoxy group-bearing methine carbon. Moreover, the relative structure of **2** was fully established by single-crystal X-ray diffraction anaylsis (Figure 4).[15] The absolute configuration of **2** was also determined by the use of a modified Mosher's method.[11,12] The (*S*)- and (*R*)-MTPA esters of **2** (**2a** and **2b**, respectively) were prepared using the corresponding *R*-(−)- and *S*-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chlorides, respectively. The determination of the chemical shift differences ($\delta_S - \delta_R$) for the protons neighboring C-3 led to the assignment of the *S* configuration at C-3 in **2** (Figure 3).

The molecular formula $C_{24}H_{36}O_8$ of querciformolide C (**3**) was established from the HRESIMS spectrum. By comparison of the NMR data of **3** with those of **2**, it was found that the ^{1}H and ^{13}C NMR data of **3** were very similar to those of **2**, with the difference that **3** contains one more acetyl group relative to **2**. The chemical shift of H-3 in **2** (δ 3.80) was shifted downfield (δ 5.00) in **3**, suggesting that **3** is the 3-acetyl derivative of **2**. This was further supported by acetylation of **2** with acetic anhydride in pyridine to yield **3**. Querciformolide D (**4**) was found to be more polar than compounds **1**−**3**. The HRESIMS of **4** established a molecular formula of $C_{20}H_{32}O_6$. The ^{1}H and ^{13}C NMR spectral data of **4** were similar to those of **2**, the difference being that an acetoxy group

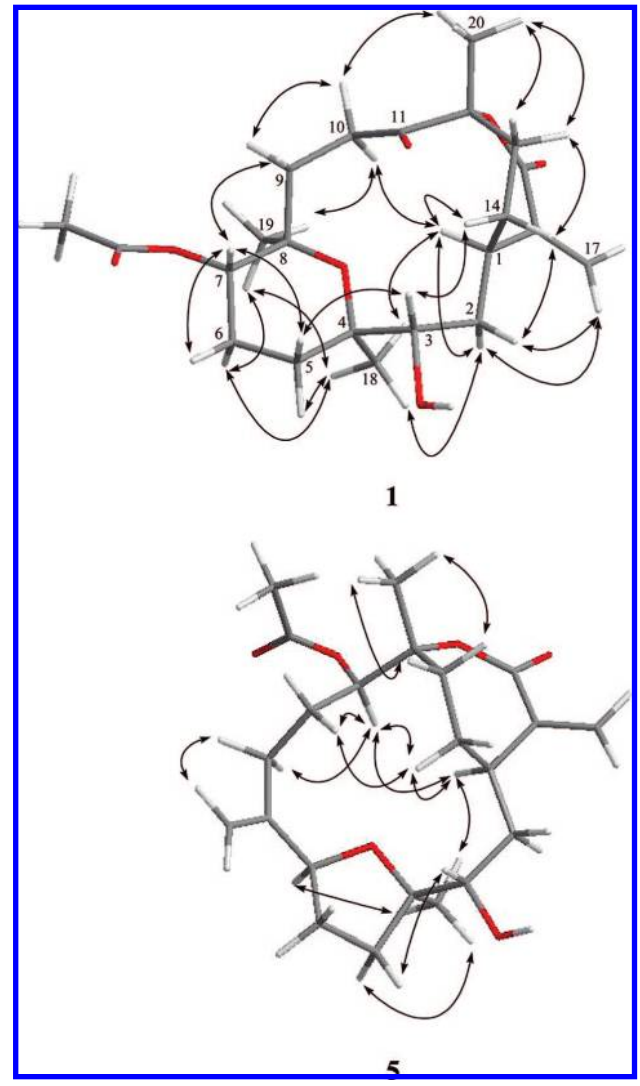

Figure 2. Selective NOE correlations for **1** and **5**.

attached at C-11 in **2** was replaced by a hydroxy group in **4**. We observed further that acetylation of **4** gave a product that was found to be identical with **3** by comparison of the physical and spectroscopic data. Thus, the structure of **4** was determined unambiguously.

Granosolide A (**5**) was found to have the molecular formula $C_{22}H_{32}O_6$, as established from the HRESIMS and NMR data and, thus, had seven degrees of unsaturation. Similar to compounds **2**−**4**, the IR spectrum of **5** indicated the presence of hydroxy (3460 cm^{-1}) and carbonyl (1739 and 1712 cm^{-1}) groups. The ^{1}H and ^{13}C NMR spectroscopic data of **5** were found to be similar to those of **2**−**4**. By comparison of the NMR data of **5** with those of **2** (Tables 1 and 2), it was found that the signals of a 1,1-disubstituted double bond (δ_H 5.17 and 5.01, each 1H; δ_C 150.5 and 114.6, C-8/C-19) of **5** were replaced by those of a methyl group (δ_C 19.1; δ_H 1.18, C-19) and a quaternary oxygenated carbon (δ 74.4, C-8) in **2**. Thus, **5** might be the dehydration derivative of **2**. Furthermore, the planar structure of **5**, including the positions of the above-mentioned double bond and the hydroxy group, was determined by ^{1}H−^{1}H COSY and HMBC correlations (Figure 1). Careful analysis of the NOESY spectrum of **5** allowed the determination of the structure of **5** as shown in Figure 2. Metabolite **6** was found to have the molecular formula $C_{24}H_{34}O_7$, as revealed from its HRESIMS (*m/z* 457.2205 [M + Na]$^+$). Comparison of the ^{1}H and ^{13}C NMR spectroscopic data of **6** with those of **5** revealed **6** as the acetyl derivative of **5**. It was found that H-3 of **5** resonated upfield (δ 3.70, dd, J = 11.0,

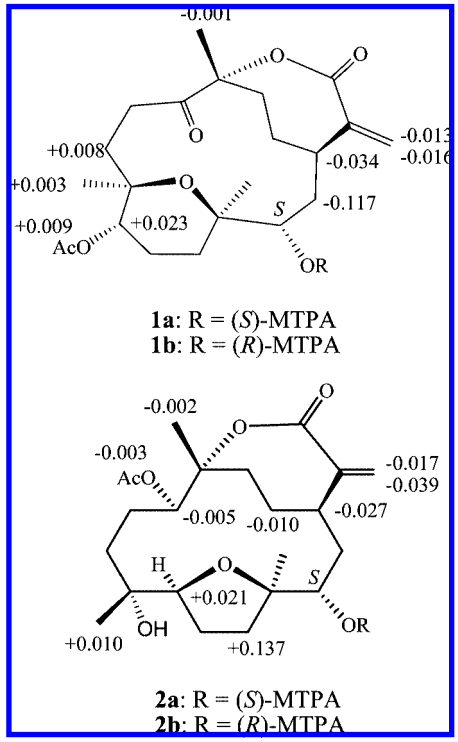

Figure 3. ^{1}H NMR chemical shift differences $\Delta\delta$ ($\delta_S - \delta_R$) in ppm for the MTPA esters of **1** and **2**.

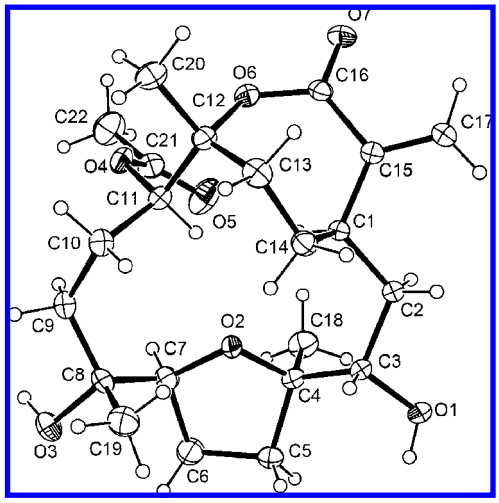

Figure 4. Molecular structure of **2** based on X-ray analysis.

4.5 Hz) relative to **6** (δ 4.94, dd, J = 7.0, 2.0 Hz). Thus, the hydroxy group at C-3 of **5** was replaced by an acetoxy group in **6**. Furthermore, acetylation of **5** gave a product that was found to be identical to **6** by comparison of their physical (mp and specific optical rotation) and spectroscopic (IR, ^{1}H and ^{13}C NMR) data. Thus, **6** was determined to be the 3-acetyl derivative of **5**.

In the previous reports, several cembrane-related compounds were identified from the soft corals *S. querciformis*[15] and *S. granosa*.[16,17] However, in the present study cembranoids possessing an ε-lactone moiety were isolated for the first time from the above two corals. Also, it is worth noting that metabolite **1** is the first ε-lactone cembrane derivative found that possesses a tetrahydropyran moiety with a 4,8-ether linkage, and **2**−**6** are cembranoids possessing a tetrahydrofuran moiety with the rarely found 4,7-ether linkage.[11] The cytotoxicity of **1**−**8** against four human cancer cell lines, HeLa (cervical epitheloid carcinoma), Hep 2 (laryngeal carcinoma), Daoy (medulloblastoma), and MCF-7 (breast carcinoma), was assayed. It was found that all eight metabolites were inactive (ED_{50}'s <20 μg/mL) toward these cancer cell lines. Furthermore, the *in vitro* anti-inflammatory effect of cembranoids **1**−**8** was tested. In this assay, the up-regulation of the pro-inflammatory iNOS and COX-2 proteins of LPS-stimulated RAW264.7 macrophage cells was evaluated using immunoblot analysis. At a concentration of 10 μM, compounds **3** and **8** were found to significantly reduce the levels of iNOS to 23.7 ± 2.3% and 20.3 ± 3.9% relative to the control cells stimulated with LPS only. Furthermore, at the same concentration, metabolites **7** and **8** could more significantly reduce COX-2 expression (17.4 ± 2.9% and 14.0 ± 4.1%, respectively) by LPS treatment. Thus, compounds **3**, **7**, and **8** might be useful anti-inflammatory agents, **8** being the most promising, as it showed potent activity for inhibiting the expression of both iNOS and COX-2 proteins.

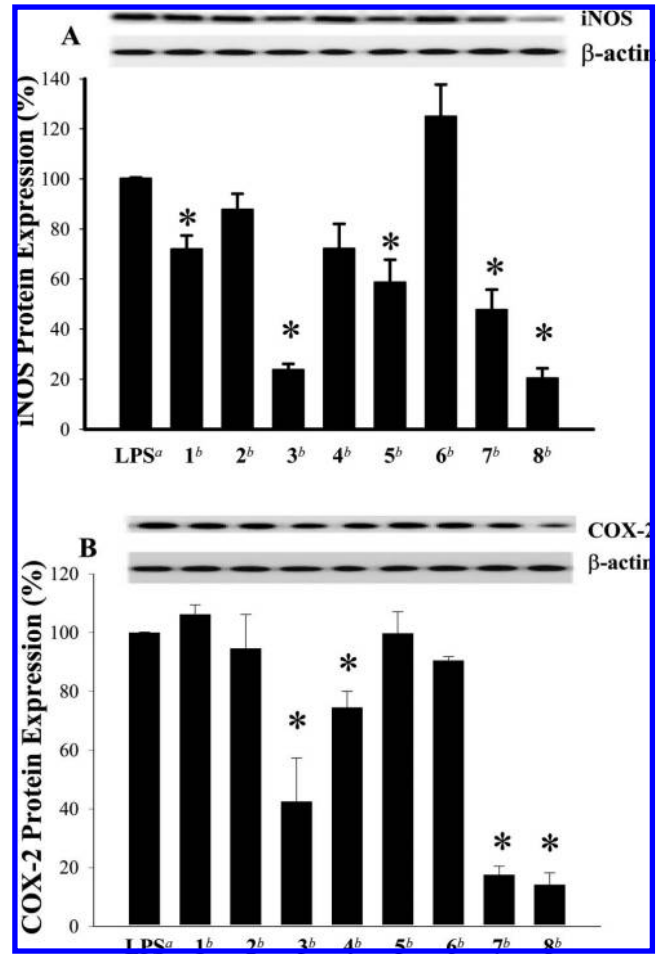

Figure 5. Effect of compounds **1**−**8** on iNOS and COX-2 protein expression of RAW264.7 macrophage cells by immunoblot analysis. (A) Immunoblots of iNOS and β-actin; (B) immunoblots of COX-2 and β-actin. The values are mean ± SEM (n = 6). Relative intensity of the LPS alone stimulated group was taken as 100%. Under the same experimental conditions CAPE (caffeic acid phenylethyl ester, 10 μM) reduced the levels of iNOS and COX-2 to 2.5 ± 3.7% and 67.2 ± 13.4%, respectively. *Significantly different from LPS alone stimulated group (*P < 0.05). [a]Stimulated with LPS. [b]Stimulated with LPS in the presence of **1**−**8** (10 μM).

Experimental Section

General Experimental Procedures. Melting points were determined using a Fisher-Johns melting point apparatus. Optical rotations were measured on a Jasco P-1020 polarimeter. Ultraviolet spectra were recorded on a Jasco V-650 spectrophotometer. IR spectra were recorded on a Jasco FT/IR-4100 infrared spectrophotometer. The NMR spectra were recorded on a Varian 400MR FT-NMR (or Varian Unity INOVA

500 FT-NMR) instrument at 400 MHz (or 500 MHz) for ^{1}H and 100 MHz (or 125 MHz) for ^{13}C in $CDCl_3$ using TMS as internal standard. LRMS and HRMS were obtained by ESI on a Bruker APEX II mass spectrometer. Silica gel (Merck, 230−400 mesh) was used for column chromatography. Precoated silica gel plates (Merck, Kieselgel 60 F-254, 0.2 mm) were used for analytical TLC. High-performance liquid chromatography was performed on a Hitachi L-7100 HPLC apparatus with a Merck Hibar Si-60 column (250 × 21 mm, 7 μm).

Animal Material. *Sinularia querciformis* (specimen no. 20040112-7) and *S. granosa* (specimen no. 20040112-2) were collected by hand using scuba off the coast of Pingtung, located on the southernmost tip of Taiwan in January 2004, at a depth of 5−10 m, and stored in a freezer until extraction. Two voucher samples were deposited at the Department of Marine Biotechnology and Resources, National Sun Yat-sen University.

Extraction and Separation. Sliced tissues of the soft coral *S. querciformis* (0.9 kg, wet wt) were exhaustively extracted with EtOH (1 L × 6). The combined EtOH extract was filtered and concentrated under reduced pressure. The residue was partitioned between EtOAc and H_2O, and the EtOAc extract (9.4 g) was subjected to column chromatography on silica gel and eluted with EtOAc in *n*-hexane (0−100%, stepwise) to yield 15 fractions. Fraction 9, eluted with *n*-hexane−EtOAc (1:1), was further purified over silica gel using *n*-hexane−acetone (4:1) to afford three subfractions. Subfraction 2 was separated by normal-phase HPLC using *n*-hexane−acetone (7:1) to afford **1** (9.2 mg), and subfraction 3 was also separated by normal-phase HPLC using *n*-hexane−acetone (5:1) to afford **3** (13.1 mg) and **8** (16.3 mg). Fraction 11, eluted with *n*-hexane−EtOAc (1:3), was rechromatographed on a silica gel column using *n*-hexane−acetone (1:1) followed by purification by normal-phase HPLC, using *n*-hexane−acetone (3:2), to afford **2** (10.2 mg) and **7** (15.3 mg). Fraction 12, eluted with pure EtOAc, was purified on a silica gel column using *n*-hexane−acetone (1:1) followed by normal-phase HPLC, using *n*-hexane−acetone (1:1), to afford **4** (8.2 mg).

The sliced tissues of the soft coral *S. granosa* (0.8 kg, wet wt) were exhaustively extracted with EtOH (1 L × 5). The combined EtOH extract was filtered and concentrated under reduced pressure. The residue was partitioned between EtOAc and H_2O. The EtOAc extract (8.7 g) was chromatographed over silica gel by column chromatography and eluted with EtOAc in *n*-hexane (0−100%, stepwise) then with MeOH in EtOAc (5−50%, stepwise) to yield 26 fractions. Fraction 14, eluted with *n*-hexane−EtOAc (10:1), was further subjected to a Sephadex LH-20 column, using acetone as the mobile phase, to afford two subfractions. Subfraction 2 was further separated by normal-phase HPLC using *n*-hexane−-acetone (8:1) to yield **6** (3.5 mg). Fraction 17, eluted with *n*-hexane−EtOAc (1:1), was further separated by normal-phase HPLC using *n*-hexane−acetone (4:1) to yield **5** (3.0 mg). Fraction 19, eluted with *n*-hexane−EtOAc (1:2), was further purified by normal-phase HPLC using *n*-hexane−EtOAc (1:2) to afford **2** (3.0 mg).

Querciformolide A (1): white powder; mp 120−122 °C; $[\alpha]^{25}_D$ −2.6 (*c* 0.8, $CHCl_3$); UV (MeOH) λ_{max} 218 (log ε = 3.8); IR (KBr) ν_{max} 3471, 2924, 1732, 1715, 1456, 1376, and 1257 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 431 [M + Na]$^+$; HRESIMS *m/z* 431.2044 [M + Na]$^+$ (calcd for $C_{22}H_{32}O_7Na$, 431.2046).

Querciformolide B (2): colorless needless; mp 109−111 °C; $[\alpha]^{25}_D$ −28.0 (*c* 0.6, $CHCl_3$); UV (MeOH) λ_{max} 216 (log ε = 3.8); IR (KBr) ν_{max} 3445, 2926, 1738, 1718, 1373, and 1240 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 433 [M + Na]$^+$; HRESIMS *m/z* 433.2204 [M + Na]$^+$ (calcd for $C_{22}H_{34}O_7Na$, 433.2202).

Querciformolide C (3): white powder; mp 149−150 °C; $[\alpha]^{25}_D$ +0.96 (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} 217 (log ε = 3.7); IR (KBr) ν_{max} 3461, 2927, 1737, 1716, 1373, and 1238 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 475 [M + Na]$^+$; HRESIMS *m/z* 475.2310 [M + Na]$^+$ (calcd for $C_{24}H_{36}O_8Na$, 475.2308).

Querciformolide D (4): white powder; mp 111−113 °C; $[\alpha]^{25}_D$ −2.0 (*c* 0.4, $CHCl_3$); UV (MeOH) λ_{max} 216 (log ε = 3.7); IR (KBr) ν_{max} 3403, 2930, 1715, 1682, 1456, 1377, and 1256 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 391 [M + Na]$^+$; HRESIMS *m/z* 391.2097 [M + Na]$^+$ (calcd for $C_{20}H_{32}O_6Na$, 391.2096).

Granosolide A (5): white powder; mp 187−189 °C; $[\alpha]^{25}_D$ −8.1 (*c* 0.7, $CHCl_3$); UV (MeOH) λ_{max} 215 (log ε = 3.8); IR (KBr) ν_{max} 3460, 2932, 1739, 1712, 1454, 1372, and 1239 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 415 [M + Na]$^+$; HRESIMS *m/z* 415.2094 [M + Na]$^+$ (calcd for $C_{22}H_{32}O_6Na$, 415.2096).

Granosolide B (6): white powder; mp 175−177 °C; $[\alpha]^{25}_D$ −38.0 (*c* 0.4, $CHCl_3$); UV (MeOH) λ_{max} 215 (log ε = 3.8); IR (KBr) ν_{max} 3461, 2935, 1732, 1714, 1455, 1373, and 1237 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 457 [M + Na]$^+$; HRESIMS *m/z* 457.2205 [M + Na]$^+$ (calcd for $C_{24}H_{34}O_7Na$, 457.2202)

Preparation of (*S*)- and (*R*)-MTPA Esters of 1. To a solution of **1** (2.0 mg) in pyridine (100 μL) was added *R*-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (10 μL), and the solution was allowed to stand overnight at room temperature. The reaction mixture was added to 1.0 mL of H_2O, followed by extraction with EtOAc (1.0 mL × 3). The EtOAc-soluble layers were combined, dried over anhydrous $MgSO_4$, and evaporated. The residue was purified by a short silica gel column using acetone−*n*-hexane (1:6) to yield the (*S*)-MTPA ester **1a** (3.2 mg, 94%). The same procedure was applied to obtain the (*R*)-MTPA ester **1b** (3.1 mg, 91%) from the reaction of *S*-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride with **1** in pyridine. Selective ^{1}H NMR ($CDCl_3$, 500 MHz) data of **1a**: δ 6.340 (1H, s, H-17a), 5.398 (1H, s, H-17b), 4.703 (1H, dd, *J* = 12.0, 2.0 Hz, H-3), 4.691 (1H, dd, *J* = 11.5, 4.5 Hz, H-7), 3.097 (1H, ddd, *J* = 11.5, 11.5, 5.5 Hz, H-1), 2.406 (1H, dd, *J* = 14.0, 7.0 Hz, H-9a), 2.042 (3H, s, OAc), 1.905 (1H, m, H-2a), 1.468 (3H, s, H-20), 1.234 (3H, s, H-19); selective ^{1}H NMR ($CDCl_3$, 500 MHz) data of **1b**: δ 6.353 (1H, s, H-17a), 5.414 (1H, s, H-17b), 4.723 (1H, dd, *J* = 11.5, 2.0 Hz, H-3), 4.668 (1H, dd, *J* = 12.0, 4.5 Hz, H-7), 3.131 (1H, ddd, *J* = 11.5, 11.5, 5.5 Hz, H-1), 2.398 (1H, dd, *J* = 14.0, 7.0 Hz, H-9a), 2.033 (3H, s, OAc), 2.022 (1H, m, H-2a), 1.469 (3H, s, H-20), 1.231 (3H, s, H-19).

Preparation of (*S*)- and (*R*)-MTPA esters of 2. By using the same procedure as the preparation of **1a**, the reaction of **2** (2 mg) with *S*-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride afforded a crude product, which was subjected to column chromatography over silica gel using acetone−*n*-hexane (1:2) to yield the (*S*)-MTPA ester **2a** (3.1 mg, 91%). The same procedure was applied to obtain the (*R*)-MTPA ester **2b** (3.0 mg, 88%) from the reaction of *S*-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride with **2** in pyridine. Selective ^{1}H NMR ($CDCl_3$, 500 MHz) data of **2a**: δ 6.342 (1H, d, *J* = 13.5 Hz, H-11), 6.278 (1 H, s, H-17a), 5.384 (1H, s, H-17b), 5.136 (1H, dd, *J* = 14.0, 5.5 Hz, H-3), 4.049 (1H, dd, *J* = 8.0, 8.0 Hz, H-7), 3.324 (1H, m, H-1), 2.442 (1H, dddd, *J* = 16.0, 16.0, 8.0, 8.0 Hz, H-14a), 2.128 (3H, s, OAc), 1.437 (1H, m, H-5), 1.363 (3H, s, H_3-20), 1.176 (3H, s, H_3-19); selective ^{1}H NMR ($CDCl_3$, 500 MHz) data of **2b**: δ 6.347 (1H, d, *J* = 14.5 Hz, H-11), 6.295 (1 H, s, H-17a), 5.423 (1H, s, H-17b), 5.149 (1H, dd, *J* = 14.0, 6.0 Hz, H-3), 4.028 (1H, dd, *J* = 7.5, 7.5 Hz, H-7), 3.351 (1H, m, H-1), 2.452 (1H, dddd, *J* = 15.0, 15.0, 7.5, 7.5 Hz, H-14a), 2.131 (3H, s, OAc), 1.365 (3H, s, H_3-20), 1.300 (1H, m, H-5), 1.166 (3H, s, H_3-19).

Acetylation of 2. A solution of **2** (2.0 mg) in pyridine (0.2 mL) was mixed with Ac_2O (0.1 mL), and the mixture was stirred at room temperature for 24 h. After evaporation of excess reagent, the residue was subjected to column chromatography over Si gel using *n*-hexane−acetone (6:1) to yield the diacetyl derivative **3** (2.5 mg, 91%).

Acetylation of 4. A solution of **4** (2.0 mg) in pyridine (0.2 mL) was mixed with Ac_2O (0.1 mL), and the mixture was stirred at room temperature for 24 h. After evaporation of excess reagent, the residue was subjected to column chromatography over Si gel using *n*-hexane−acetone (6:1) to yield the diacetyl derivative **3** (2.1 mg, 85%).

Acetylation of 5. A solution of **5** (2.0 mg) in pyridine (0.2 mL) was mixed with Ac_2O (0.1 mL), and the mixture was stirred at room temperature for 24 h. After evaporation of excess reagent, the residue was subjected to column chromatography over Si gel using *n*-hexane−acetone (8:1) to give **6** (1.9 mg, 86%).

X-ray Diffraction Analysis of Querciformolide B (2). [18] A suitable colorless crystal (0.8 × 0.6 × 0.2 mm^3) of **2** was grown by slow evaporation of the EtOAc solution. Diffraction intensity data were acquired with a Rigaku AFC7S single-crystal X-ray diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). Crystal data for **2**: $C_{22}H_{34}O_7$ (formula weight 410.49), monoclinic, space group, $P2_1$ (# 4), *T* = 298(2) K, *a* = 9.2866(14) Å, α = 90°, *b* = 12.216(2) Å, β = 105.172 (11)°, *c* = 9.8166(12) Å, γ= 90°, *V* = 1074.8(3) Å^3, D_c = 1.268 Mg/m^3, *Z* = 2, *F*(000) = 444, $\mu_{(Mo\ K\alpha)}$ = 0.093 mm^{-1}. A total of 2354 reflections were collected in the range 2.15° < θ < 25.99°, with 2225 independent reflections [*R*(int) = 0.0103], completeness to θ_{max} was 100.0%; psi-scan absorption correction applied; full-matrix least-squares refinement on F^2, the number of data/restraints/parameters were 2225/1/272; goodness-of-fit on F^2 = 1.060; final *R* indices [*I* >

$2\sigma(I)$], $R_1 = 0.0364$, $wR_2 = 0.0932$; R indices (all data), $R_1 = 0.0465$, $wR_2 = 0.0995$, largest difference peak and hole, 0.156 and −0.224 e/Å^3.

***In Vitro* Anti-inflammatory Assay.** The anti-inflammatory assay was modified from Ho et al.[19] and Park et al.[20] Murine RAW 264.7 macrophages were obtained from the American Type Culture Collection (ATCC, No. TIB-71) and cultured in Dulbecco's modified essential medium (DMEM) containing 10% heat-inactivated fetal bovine serum, at 37 °C in a humidified 5% CO_2−95% air incubator under standard conditions. The macrophage cells were plated in 60 mm culture dishes (3×10^6 cells per dish). Inflammation in macrophages was induced by incubating them for 16 h in a medium containing LPS (0.01 μg/mL; Sigma) without the presence of test compounds. For the anti-inflammatory activity assay, compounds **1**−**8** were added to the cells 5 min before LPS challenge, respectively. Then, cells were washed with ice-cold PBS, lysed in ice-cold lysis buffer, and then centrifuged at 20000*g* for 30 min at 4 °C. The supernatant was decanted from the pellet and retained for Western blot analysis. Protein concentrations were determined by the DC protein assay kit (Bio-Rad) modified by the method of Lowry et al.[21] Samples containing equal quantities of protein were subjected to SDS-polyacrylamide gel electrophoresis, and the separated proteins were electrophoretically transferred to polyvinylidene difluoride membranes (PVDF; Immobilon-P, Millipore, 0.45 μm pore size). The resultant PVDF membranes were incubated with blocking solution first and then incubated for 180 min at room temperature with antibodies against inducible nitric oxide synthase (iNOS; 1:1000 dilution; Transduction Laboratories) and cyclooxygenase-2 (COX-2; 1:1000 dilution; Cayman Chemical) proteins. The blots were detected using ECL detection reagents (Perkin-Elmer, Western Blot Chemiluminescence Reagent Plus) according to the manufacturer's instructions and finally exposed to X-ray film (Kodak X-OMAT LS, Kodak, U.S.A.). The membranes were reprobed with a monoclonal mouse anti-β-actin antibody (1:2500, Sigma) as the loading control. For the immunoreactivity data, the intensity of each drug-treated band is expressed as the integrated optical density (IOD), calculated with respect to the average optical density of the corresponding control (treated with LPS only) band. For statistical analysis, all of the data were analyzed by a one-way analysis of variance (ANOVA), followed by the Student−Newman−Keuls post hoc test for multiple comparisons. A significant difference was defined as a P value of <0.05.

Cytotoxicity Testing. Cell lines were purchased from the American Type Culture Collection (ATCC). Cytotoxicity assays of the test compounds **1**−**8** were performed using the MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method. [22,23]

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of **1**−**6** and description of X-ray crystal structure data of compound **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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