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Cytotoxic and anti-inflammatory cembranoids from the Dongsha Atoll soft coral *Sarcophyton crassocaule*

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ABSTRACT

Five new cembranoids, sarcocrassocolides A–E (**1**–**5**), along with three known cembranoids **6**–**8**, have been isolated from a Formosan soft coral *Sarcophyton crassocaule*. The structures of the new metabolites were elucidated by extensive spectroscopic analysis and the absolute configuration of **1** was determined by a modified Mosher's method. Compounds **1**–**4** exhibited significantly cytotoxic activity against a limited panel of cancer cell lines. Compounds **1**–**4**, **6** and **8** were shown to exert significant in vitro anti-inflammatory activity in LPS-stimulated RAW264.7 macrophage cells. Compound **6** also significantly inhibited the accumulation of pro-inflammatory COX-2 protein.

1. Introduction

Many cembrane-type compounds have been shown to exhibit cytotoxicity[1–6] and anti-inflammatory activity.[7–11] During the course of our search for bioactive metabolites from marine invertebrates, many bioactive cembrane-derived compounds have been discovered from soft corals of the genera *Sinularia*,[1–5,7–9] *Lobophytum*,[10,11] and *Sarcophyton*.[12,13] Our investigation on the chemical constituents of the Dongsha Atoll soft coral *Sarcophyton crassocaule* yielded five new cembranoids, sarcocrassocolides A–E (**1**–**5**), along with three known cembranoids, sarcocrassolide (**6**),[14] sinularolide (**7**)[15] and 13-acetoxysarcocrassolide (**8**).[14] The structures of **1**–**5** were established by detailed spectroscopic analysis, including extensive examination of 2D NMR (^{1}H–^{1}H COSY, HMQC, HMBC and NOESY) correlations. The absolute configuration of **1** was further determined using a modified Mosher's method. The cytotoxicity of compounds **1**–**4** against human breast carcinoma (MCF-7), human colon carcinoma (WiDr), human laryngeal carcinoma (HEp-2) and human medulloblastoma (Daoy) cells was studied, and the ability of **1**–**4** and **6**–**8** to inhibit the up-regulation of pro-inflammatory iNOS (inducible nitric oxide synthase) and COX-2 (cyclooxygenase-2) proteins in LPS (lipopolysaccharide)-stimulated RAW264.7 macrophage cells was also evaluated. The results revealed that compounds **1**–**4** are all cytotoxic towards the above cancer cells, **3** being the most cytotoxic. Compounds **1**–**4**, **6** and **8** were found to effectively inhibit the expression of iNOS protein, while **6** was the only one seen also to significantly reduce the level of COX-2 protein in the above assay.

2. Results and discussion

The HRESIMS (*m/z* 413.1938 $[M+Na]^+$) of **1** established the molecular formula $C_{22}H_{30}O_6$, appropriate for eight degrees of unsaturation, and the IR spectrum revealed the presence of hydroxy (3487 cm^{-1}) and lactonic carbonyl (1769 cm^{-1}) groups. The ^{13}C NMR (Table 1) and DEPT spectroscopic data showed signals of three methyls (including one acetate methyl), five sp^3 methylenes, two sp^2 methylenes, five sp^3 methines (including four oxymethines), one sp^2 methine, one sp^3 and five sp^2 quaternary carbons (including two ester carbonyls). The NMR signals (Tables 1 and 2) observed at δ_C 170.1 (qC), 140.8 (qC), 120.5 (CH_2), 81.0 (CH), and 36.3 (CH), and δ_H 6.20, 5.65 (each, 1H, d, J = 2.0 Hz), 4.50 (1H, t, J = 2.8 Hz), and 3.64 (1H, dd, J = 6.4, 3.2 Hz) revealed the presence of an α-methylene-γ-lactonic group by comparing the very similar NMR data of the five-membered lactone ring in

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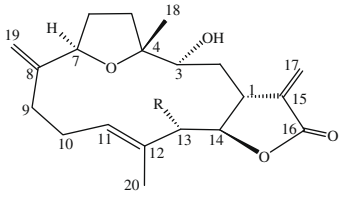

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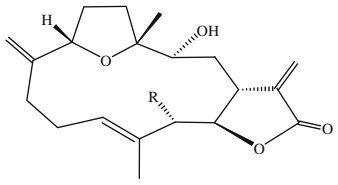
3: R = H



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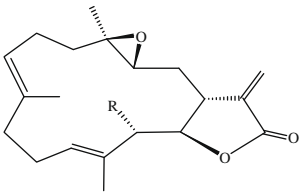


5: R = OCOEt
6: R = H
7: R = OH
8: R = OAc

Table 1
^{13}C NMR data for compounds **1**–**5**

C#	**1**[a]	**2**[b]	**3**[a]	**4**[a]	**5**[b]
1	36.3, CH [c]	35.4, CH	41.8, CH	41.4, CH	36.9, CH
2	39.4, CH_2	41.4, CH_2	38.2, CH_2	40.9, CH_2	34.7, CH_2
3	73.5, CH	74.9, CH	73.3, CH	74.9, CH	60.1, CH
4	84.4, qC	86.0, qC	84.3, qC	85.7, qC	60.5, qC
5	37.9, CH_2	37.8, CH_2	38.4, CH_2	38.4, CH_2	37.4, CH_2
6	30.9, CH_2	28.2, CH_2	31.9, CH	29.1, CH_2	23.4, CH_2
7	79.4, CH	82.6, CH	79.6, CH	82.4, CH	123.0, CH
8	149.8, qC	148.3, qC	149.1, qC	147.2, qC	135.1, qC
9	33.4, CH_2	28.0, CH_2	34.1, CH_2	29.3, CH_2	37.9, CH_2
10	26.9, CH_2	26.9, CH_2	28.5, CH_2	28.1, CH_2	24.8, CH_2
11	129.1, CH	126.4, CH	129.8, CH	127.8, CH	129.0, CH
12	127.4, qC	128.5, qC	127.6, qC	127.7, qC	128.7, qC
13	77.1, CH	77.0, CH	46.0, CH	46.2, CH_2	77.2, CH
14	81.0, CH	81.8, CH	80.7, CH	81.4, CH	80.9, CH
15	140.8, qC	140.9, qC	139.1, qC	138.9, qC	139.7, qC
16	170.1, qC	170.1, qC	168.9, qC	168.6, qC	169.4, qC
17	120.5, CH_2	120.9, CH_2	121.6, CH_2	122.0, CH_2	121.0, CH_2
18	21.4, CH_3	18.9, CH_3	22.3, CH_3	20.4, CH_3	17.4, CH_3
19	111.0, CH_2	112.3, CH_2	110.2, CH_2	111.3, CH_2	16.8, CH_3
20	14.5, CH_3	14.8, CH_3	17.7, CH_3	17.9, CH_3	14.7, CH_3
13-Acetate	20.7, CH_3	20.7, CH_3			
	169.4, qC	169.3, qC			
13-Propionate					8.9, CH_3
					27.5, CH_2
					172.5, qC

[a] Spectra recorded at 100 MHz in $CDCl_3$.
[b] Spectra recorded at 125 MHz in $CDCl_3$.
[c] Attached protons were deduced by DEPT experiments.

the known compounds **6** and **8**.[14] Signals appearing at δ_C 111.0 (CH_2), 149.8 (qC), δ_H 4.90 (1H, s) and 4.92 (1H, s) revealed the presence of the other 1,1-disubstituted carbon–carbon double bond. One trisubstituted double bond was also identified from the NMR signals at δ_C 127.4 (qC) and 129.1 (CH) and at δ_H 5.48 (1H, dd, J = 10.0, 5.6 Hz). In the ^{1}H–^{1}H COSY spectrum, it was possible to identify three different structural units, which were assembled with the assistance of an HMBC experiment (Fig. 1). Key HMBC correlations of H_3-18 to C-3, C-4 and C-5; H_2-19 to C-7, C-8 and C-9; H_3-20 to C-11, C-12 and C-13; H_2-17 to C-1, C-15 and C-16; and H_2-5 and H_2-6 to C-4 permitted connection of the carbon skeleton. Furthermore, the acetoxy group positioned at C-13 was confirmed from the HMBC correlations of H-13 (δ 5.36) and protons of an acetate methyl (δ 2.01) to the ester carbonyl carbon at δ 169.4 (C). 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 configuration (later discussed), 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 has to be placed between C-4 and C-7. On the basis of the above analysis, the planar structure of **1** was established unambiguously.

The relative configuration of **1** was elucidated by the analysis of NOE correlations, as shown in Figure 2. It was found that H-1 (δ 3.64, dd, J = 6.4, 3.0 Hz) showed NOE interaction with H_3-18 (δ 1.21, s); therefore, assuming a β-orientation of H-1, H_3-18 should also be positioned on the β-face. One of the methylene protons at C-2 (δ 1.94, m) exhibited NOE correlations with both H-1 and H_3-18 and was characterized as H-2β, while the other proton was assigned as H-2α (δ 1.75, m). NOE correlations observed between H-2α and H-14, and H-14 and H-3, reflected the α-orientation of H-14, and hence the S^* configuration of C-3. Furthermore, H-14 exhibited NOE correlation with H-13 and H-3 exhibited NOE interaction with H-7, revealing the α-orientations of H-7 and H-13, and hence the S^* configuration of C-13. The *E* geometry of the trisubstituted C-11/C-12 double bond was also assigned from the NOE correlation of H_3-20 (δ 1.71, s) with H-10 (δ 2.15, m), but not with olefinic proton H-11, and also the upper field chemical shift of C-20 (δ 14.5). On the basis of the above findings and detailed examination of other NOE correlations (Fig. 2), the relative configuration of compound **1** was determined. Furthermore, the absolute configuration of **1** was finally determined using a modified Mosher's esterification method.[16] The (*S*)- and (*R*)-MTPA esters of **1** (**1a** and **1b**, respectively) were prepared using the corresponding (*R*)-

Table 2
^{1}H NMR data for compounds **1**–**5**

H#	**1**[a]	**2**[b]	**3**[a]	**4**[a]	**5**[b]
1	3.64, dd (6.4, 3.2)	3.66, br d (10.0)	3.43, ddd (5.6, 3.2, 1.6)	3.40, d (8.4)	3.06, d (12.0)
2	1.94, m	1.88, m	1.94, m	1.83, m	1.83, ddd (15.0, 12.5, 3.5)
	1.75, m	1.69, m	1.84, m	1.75, m	1.69, ddd (15.0, 7.0, 3.5)
3	3.49, dd (9.2, 3.2) [c]	3.37, dd (9.5, 3.0)	3.53, dd (8.0, 4.0)	3.43, t (4.0)	2.64, dd (7.5, 3.5)[c]
5	1.98, m; 1.90, m	1.90, m; 1.75, m	1.89, m; 1.78, m	1.86, m; 1.77, m	1.34, m
6	2.05, m; 1.78, m	2.15, m; 1.76, m	2.00, m; 1.77, m	2.14, m; 1.79, m	2.14, m; 2.09, m
7	4.17, dd (10.4, 4.4)	4.52, dd (9.5, 4.5)	4.14, dd (11.2, 4.0)	4.49, dd (8.8, 4.0)	5.02, t (6.5)
9	2.37, m	2.33, m	2.29, m	2.32, d (14.0)	2.22, dd (14.0, 6.5)
		1.82, m		1.83, m	2.04, m
10	2.28, m	2.45, dddd (14.0, 10.5, 7.0, 3.5)	2.25, m	2.40, m	2.39, ddd (15.0, 9.5, 9.5)
	2.15, m	2.14, m	2.13, m	2.12, m	2.15, m
11	5.48, dd (10.0, 5.6)	5.49, t (7.5)	5.29, dd (10.4, 5.2)	5.34, t (7.2)	5.30, br t (7.0)
13	5.36, s	5.27, s	2.55, d (14.0)	2.48, d (12.8)	5.39, s
			2.12, m	2.02, t (12.4)	
14	4.50, t (2.8)	4.39, br s	4.40, dt (10.4, 3.2)	4.33, d (11.6)	4.60, t (2.5)
17	6.20, d (2.0)	6.21, d (2.0)	6.25, d (2.0)	6.25, s	6.25, d (2.0)
	5.65, d (2.0)	5.67, d (2.0)	5.69, d (2.0)	5.70, s	5.62, d (1.5)
18	1.21, s	1.20, s	1.22, s	1.20, s	1.29, s
19	4.92, s; 4.90, s	5.10, s; 4.99, s	4.93, s; 4.89, s	5.08, s; 5.00, s	1.63, s
20	1.71, s	1.73, s	1.67, s	1.69, s	1.72, s
13-Acetate	2.01, s	1.97, s			
13-Propionate					2.26, q (7.5)
					1.09, t (7.5)

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

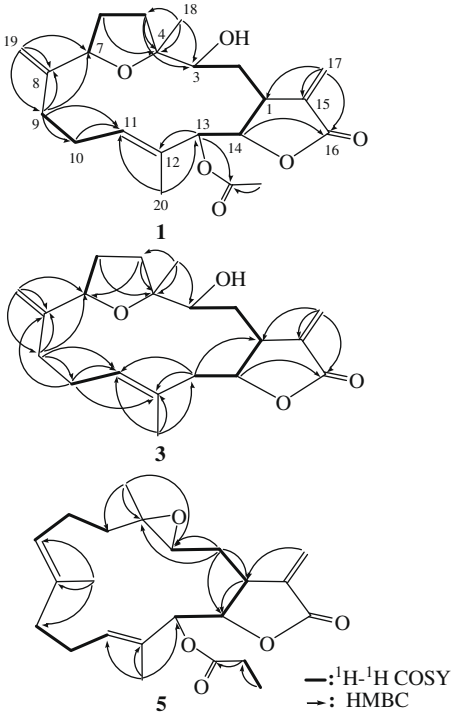


Figure 1. ^{1}H–^{1}H COSY and HMBC correlations for **1**, **3** and **5**.

(−) and (*S*)-(+)-MTPA chloride, respectively. The determination of $\Delta\delta$ values (δ_S–δ_R) for protons neighboring C-3 led to the assignment of the *S* configuration at C-3 in **1** (Fig. 3).

Compound **2** possessed the same molecular formula ($C_{22}H_{30}O_6$) as that of **1**, as revealed from HRESIMS. Furthermore, it was found that the NMR spectroscopic data of **2** were very similar to those of **1**. By analysis of the 2D NMR (^{1}H–^{1}H COSY, HMQC, and HMBC) correlations, compound **2** was shown to possess the same planar structure as that of **1**. From the NOESY spectrum (Fig. 2), it was found that H-7 (δ 4.52, dd, J = 9.5, 4.5 Hz) showed an NOE interaction with H_3-18 (δ 1.20, s), but not with H-3 and H_3-20, revealing the β-orientation of H-7. Further analysis of other NOE interactions revealed that **2** possessed the same relative configurations at C-1, C-3, C-4, C-13 and C-14 as those of **1** (Fig. 2). Therefore, **2** was found to be the C-7 epimer of **1**.

Compound **3** was shown by HRESIMS to possess the molecular formula $C_{20}H_{28}O_4$ (*m/z* 355.1883 [M+Na]$^+$). The IR spectrum of **3** revealed the presence of hydroxy (3472 cm^{-1}) and lactonic carbonyl (1760 cm^{-1}) groups. Comparison of the ^{1}H and ^{13}C NMR data (Tables 1 and 2) of compounds **1** and **3** showed that the structure of **3** should be very close to that of **1,** with the exception of signals assigned to C-13, where an acetoxymethine (δ_H 5.36, 1H, s; δ_C 77.1) in **1** is replaced by a methylene (δ_H 2.55, 1H, d, J = 14.0 Hz, δ_H 2.12, 1H, m; δ_C 26.6) in **3**. The overall planar structure of **3** was fully established by analyzing the ^{1}H–^{1}H COSY and HMBC correlations (Fig. 1). The relative stereochemistry of **3** was confirmed to be $1R^*$, $3S^*$, $4R^*$, $7R^*$ and $14S^*$ from the following NOESY correlations (Fig. 4): both H-7 (δ 4.14) and H-14 (δ 4.40) with H-3 (δ 3.53), and H_3-18 (δ 1.22) with H-1 (δ 3.43). These results, together with other detailed NOE correlations of **3** (Fig. 4), unambiguously established the structure of sarcocrassocolide C, as shown in formula **3**. Thus, **3** is the 13-deacetoxy derivative of **1**.

Compound **4** was found to have the molecular formula $C_{22}H_{30}O_6$, as indicated by HRESIMS, suggesting **4** to be an isomer of **3**. By 2D NMR spectroscopic data, including ^{1}H–^{1}H COSY, HMQC and HMBC, compound **4** was shown to possess the same planar structure as that of **3**. By comparison of the related ^{1}H and ^{13}C NMR spectroscopic data of **4** (Tables 1 and 2) with those of **3**, **4** was found to be the C-7 epimer of **3**; this was further confirmed by analysis of the NOE correlations (Fig. 4) of **4**, which revealed that H-7 was correlated with H_3-18.

The HRESIMS spectrum of **5** exhibited a molecular ion peak at *m/z* 411.2150 [M+Na]$^+$, consistent with the molecular formula $C_{23}H_{32}O_5$ and implying eight degrees of unsaturation. Comparison of the ^{1}H and ^{13}C NMR data of **5** (Tables 1 and 2) with those of **8** showed that the structure of both compounds are very similar. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) revealed that **5** is the 13-*O*-deacetyl-13-*O*-propionyl derivative of **8**.

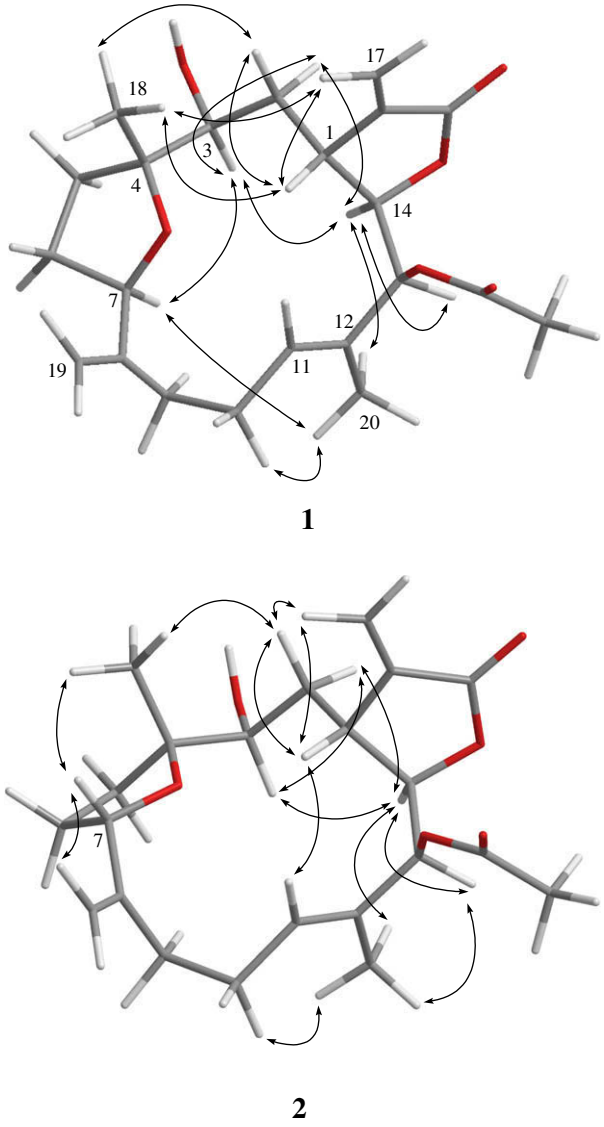


Figure 2. Key NOESY correlations for **1** and **2**.

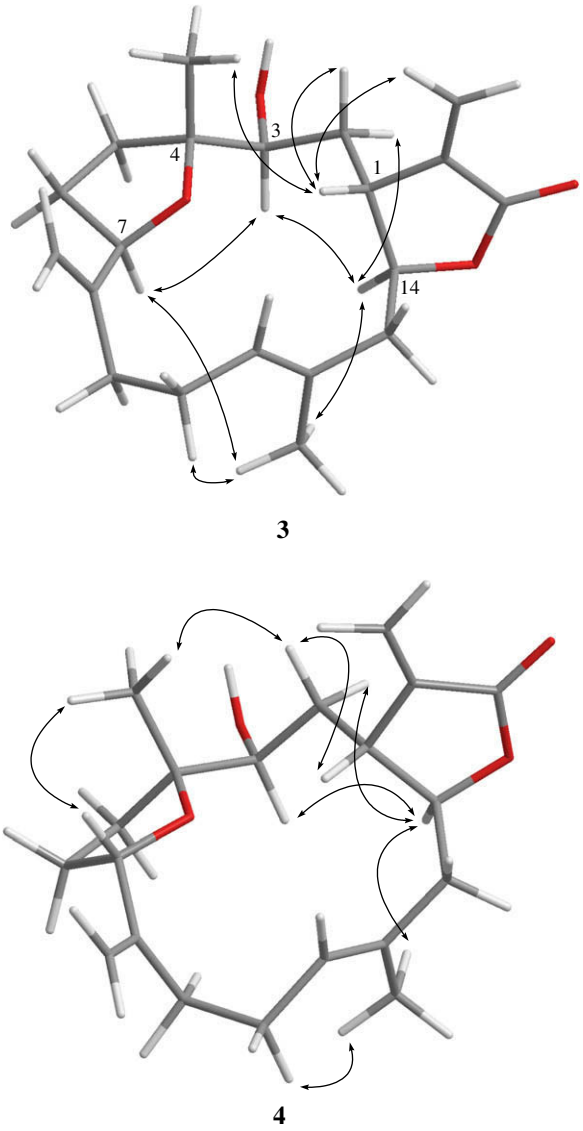
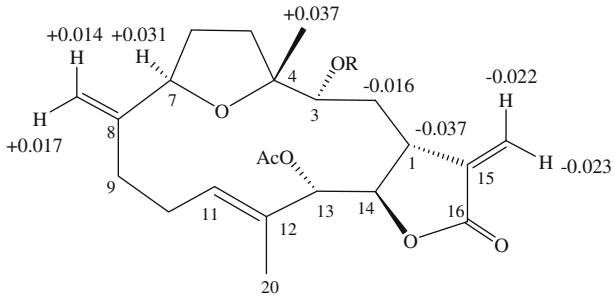


Figure 4. Key NOESY correlations for **3** and **4**.



1a: R= (*S*)-MTPA
1b: R= (*R*)-MTPA

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

It is noteworthy to mention that metabolites **1**–**4** are cembranoids possessing a tetrahydrofuran moiety with a rarely found 4,7-ether linkage, which has been discovered previously only in the soft coral *Eunicea mammosa*.[17] As the known compounds **6**–**8** have been found to exhibit cytotoxicity toward several cancer cell lines,[14,15] the cytotoxicity of compounds **1**–**4** against the proliferation of a limited panel of cancer cell lines, including MCF-7, WiDr, HEp-2 and Daoy carcinoma cells was then evaluated. The results showed that compound **3**, the most potent of compounds **1**–**4,** exhibited cytotoxicity against MCF-7, WiDr, HEp-2 and Daoy cancer cell lines with ED_{50}s of 2.0, 1.2, 2.6 and 3.2 μg/mL, respectively. Furthermore, metabolites **1**, **2** and **4** also were found to exhibit significant cytotoxicity towards some of the above four cancer cells (Table 3). The in vitro anti-inflammatory effects of compounds **1**–**4** and **6**–**8** were also tested. The inhibition of LPS-induced up-regulation of pro-inflammatory proteins, iNOS and COX-2 in macrophage cells was assayed by immunoblot analysis. At a concentration of 10 μM, compounds **1**–**4**, **6** and **8** were found to

Table 3
Cytotoxicity (ED_{50} μg/mL) of compounds **1**–**4**

Compound	MCF-7	WiDr	HEp 2	Daoy
1	4.2	4.2	6.2	8.8
2	3.2	3.2	4.5	5.6
3	2.0	1.2	2.6	3.2
4	4.1	1.8	4.0	5.4
Mitomycin C	0.14	0.15	0.07	0.14

significantly reduce the levels of iNOS protein to 13.7 ± 5.2%, 13.3 ± 5.0%, 4.6 ± 1.3%, 7.0 ± 3.1%, 1.1 ± 0.9% and 6.2 ± 0.5%, respectively, relative to control cells stimulated with LPS only. Furthermore, at the same concentration, metabolite **6** could strongly reduce COX-2 expression (3.9 ± 2.3%) with LPS treatment. Thus, compounds **1**–**4**, **6** and **8** might be useful anti-inflammatory agents, while **6** is the most promising as it showed potent inhibitory activity against expression of both iNOS and COX-2 proteins (Fig. 5). On the basis of the above results, we suggest that further investigation of **6** in terms of its anti-inflammatory activities would be worthwhile for future drug development.

3. Experimental

3.1. 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 INOVA500 FT NMR) instrument at 400 MHz (or 500 MHz) for ^{1}H and 100 MHz (or 125 MHz) for ^{13}C in $CDCl_3$. 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) and on a Hitachi L-2455 HPLC apparatus with a Inertsil ODS-3 column (250 × 20 mm, 5 μm).

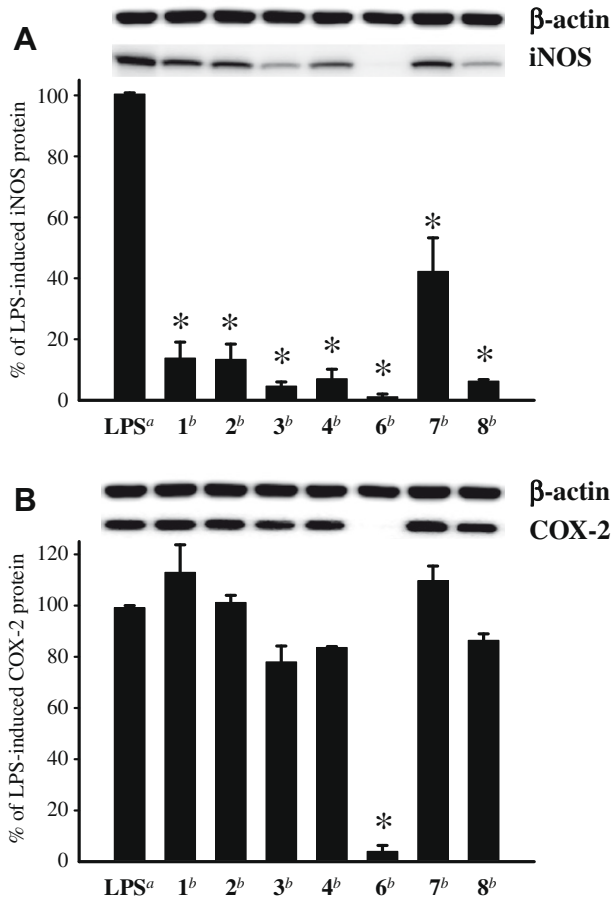


Figure 5. Effect of compounds **1**–**4** and **6**–**8** on iNOS and COX-2 proteins expression of RAW264.7 macrophage cells by immunoblot analysis. (A) Immunoblots of iNOS and β-actin; (B) immunoblots of COX-2 and β-actin. The values are mean ± SEM (n = 6). Relative intensity of the LPS alone stimulated group was taken as 100%. Under the same experimental condition CAPE (caffeic acid phenylethyl ester, 10 μM) reduced the levels of the 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**–**4** and **6**–**8** (10 μM).

3.2. Organism

S. crassocaule (specimen no. 20070402) was collected by hand using scuba off the coast of Dongsha, Taiwan, in April 2007, at a depth of 5–10 m, and stored in a freezer until extraction. A voucher sample was deposited at the Department of Marine Biotechnology and Resources, National Sun Yat-sen University.

3.3. Extraction and separation

The frozen bodies of *S. crassocaule* (0.5 kg, wet wt) were minced and exhaustively extracted with EtOAc (1 L × 5). The EtOAc extract (7.3 g) was chromatographed over silica gel by column chromatography and eluted with EtOAc in *n*-hexane (0–100%, stepwise) then with acetone in EtOAc (50–100%, stepwise) to yield 28 fractions. Fraction 10 eluted with *n*-hexane–EtOAc (6:1) and was further purified over silica gel using *n*-hexane–EtOAc (7:1) to afford six subfractions (A1–A6). Subfraction A2 was separated by silica gel using *n*-hexane–CH_2Cl_2 (8:1) to afford **6** (20.0 mg). Fraction 13 eluted with *n*-hexane–EtOAc (3:1) and was further purified over silica gel using *n*-hexane–acetone (5:1) to afford six subfractions (B1–B6) and **8** (60.0 mg). Subfraction B6 was separated by normal-phase HPLC using *n*-hexane–acetone (4:1) and further purified by reverse-phase HPLC using MeOH–H_2O (4:1) to afford **5** (1.0 mg). Fraction 15 eluted with *n*-hexane–EtOAc (2:1) and was further purified over silica gel using *n*-hexane–acetone (3:1) to afford seven subfractions (C1–C7). Subfraction C3 was separated by normal-phase HPLC using *n*-hexane–acetone (7:2) and further purified by reverse-phase HPLC using MeOH–H_2O (2.3:1) to afford **3** (6.2 mg), **4** (5.7 mg) and **7** (1.3 mg). Fraction 18 eluted with *n*-hexane–EtOAc (1:1) and was further purified over silica gel using *n*-hexane–acetone (3:1) to afford seven subfractions (D1–D7). Subfraction D3 was separated by reverse-phase HPLC using MeOH–H_2O (1.5:1) to afford **1** (10.2 mg) and **2** (3.0 mg).

3.3.1. Sarcocrassocolide A (1)

White solid; $[\alpha]_D^{25}$ +17.7 (c 0.4, $CHCl_3$); UV (MeOH) λ_{max} 204 (log ε = 3.5); IR (neat) ν_{max} 3487, 2971, 2940, 1769, 1748, 1436, 1374, 1358,1275,1228 cm^{-1}; ^{1}H NMR and ^{13}C NMR data see Tables 1 and 2; ESIMS m/z 413 [M+Na]$^+$; HRESIMS m/z 413.1938 [M+Na]$^+$ (calcd for $C_{20}H_{30}O_6Na$, 413.1940).

3.3.2. Sarcocrassocolide B (2)

White solid; $[\alpha]_D^{25}$ −71.7 (c 0.3, $CHCl_3$); UV (MeOH) λ_{max} 204 (log ε = 3.5); IR (neat) ν_{max} 3481, 2965, 2951, 1755, 1456, 1444, 1386, 1370, 1346, 1270, 1227 cm^{-1}; ^{1}H NMR and ^{13}C NMR data see Tables 1 and 2; ESIMS m/z 413 [M+Na]$^+$; HRESIMS m/z 413.1943 [M+Na]$^+$ (calcd for $C_{20}H_{30}O_6Na$, 413.1940).

3.3.3. Sarcocrassocolide C (3)

White solid; $[\alpha]_D^{25}$ +89.4 (c 0.6, $CHCl_3$); UV (MeOH) λ_{max} 207 (log ε = 3.6); IR (neat) ν_{max} 3472, 2969, 2930, 1760, 1442, 1405, 1269, 1213 cm^{-1}; ^{1}H NMR and ^{13}C NMR data see Tables 1 and 2; ESIMS m/z 355 [M+Na]$^+$; HRESIMS m/z 355.1883 [M+Na]$^+$ (calcd for $C_{20}H_{28}O_4Na$, 355.1885).

3.3.4. Sarcocrassocolide D (4)

White solid; $[\alpha]_D^{25}$ +17.2 (c 0.6, $CHCl_3$); UV (MeOH) λ_{max} 215 (log ε = 3.8); IR (neat) ν_{max} 3462, 2968, 2949, 1748, 1456, 1268,

1215 cm^{-1}; ^1H NMR and ^{13}C NMR data see Tables 1 and 2; ESIMS *m/z* 355 $[M+Na]^+$; HRESIMS *m/z* 355.1883 $[M+Na]^+$ (calcd for $C_{20}H_{28}O_4Na$, 355.1885).

3.3.5. Sarcocrassocolide E (5)

Colorless oil; $[\alpha]_D^{25}$ −47.5 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} 203 (log ε = 3.5); IR (neat) ν_{max} 2923, 2853, 1770, 1749, 1385, 1271, 1223 cm^{-1}; ^1H NMR and ^{13}C NMR data see Tables 1 and 2; ESIMS *m/z* 411 $[M+Na]^+$; HRESIMS *m/z* 411.2150 $[M+Na]^+$ (calcd for $C_{20}H_{30}O_6Na$, 411.2147).

3.3.6. 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 then 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 (1:2) to yield the (*S*)-MTPA ester **1a** (1.8 mg, 60%). The same procedure was applied to obtain the (*R*)-MTPA ester **1b** (1.6 mg, 52%) from the reaction of (*S*)-(+)-α-methoxy-α-(trifluoromethyl) phenylacetyl chloride with **1** in pyridine. Selective ^{1}HNMR ($CDCl_3$, 400 MHz) data of **1a**: δ 3.544 (1H, d, *J* = 12.4 Hz, H-1), 1.624 (1H, m, H-2b), 4.125 (1H, dd, *J* = 10.4, 4 Hz, H-7), 6.156 (1H, d, *J* = 1.6 Hz, H-17a), 5.569 (1H, d, *J* = 1.6 Hz, H-17b), 1.255 (3H, s, H_3-18), 5.043 (1H, s, H-19a), 4.934 (1H, s, H-19b); selective ^1H NMR ($CDCl_3$, 400 MHz) data of **1b**: δ 3.581 (1H, d, *J* = 12.4 Hz, H-1), 1.640 (1H, m, H-2b), 4.099 (1H, br s, H-7), 6.178 (1H, s, H-17a), 5.592 (1H, s, H-17b), 1.218 (3H, s, H_3-18), 5.026 (1H, s, H-19a), 4.920 (1H, s, H-19b).

3.4. Cytotoxicity testing

Cell lines were purchased from the American Type Culture Collection (ATCC). Cytotoxicity assays of compounds **1**–**4** were performed using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method.[18,19]

3.5. In vitro anti-inflammatory assay

Macrophage (RAW264.7) cell line was purchased from ATCC. In vitro anti-inflammatory activities of compounds **1**–**4** and **6**–**8** were measured by examining the inhibition of lipopolysaccharide (LPS) induced up-regulation of iNOS (inducible nitric oxide synthetase) and COX-2 (cyclooxygenase-2) proteins in macrophages cells using western blotting analysis.[20,21]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.01.036.

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