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Cytotoxic and anti-inflammatory diterpenoids from the Dongsha Atoll soft coral *Sinularia flexibilis*

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ABSTRACT

Seven new diterpenoids, namely, flexibilisolides C–G (**1**–**5**), flexibilisin C (**6**), and a novel 11,12-secoflexibillin (**7**), along with seven known compounds, **8**–**14**, were isolated from the Dongsha Atoll soft coral *Sinularia flexibilis*. The structures of the new metabolites were elucidated by extensive spectroscopic analysis and comparison of the NMR data with those of known analogues. Compounds **1**, **8**, and **11** were shown to exhibit moderate cytotoxic activity against HeLa and B16 cancer cell lines, and compound **10** was found to exhibit more potent cytotoxic activity against SK-Hep1 and B16 cancer cell lines. Moreover, compounds **1**, **2**, **8**, **9**, and **11**–**14** could significantly inhibit the accumulation of the pro-inflammatory iNOS protein and **1**, **8**, **11**, and **14** could reduce the accumulation of COX-2 protein in LPS-stimulated RAW264.7 macrophage cells.

1. Introduction

Soft corals of the genus *Sinularia* have been known to produce 14-membered ring cembrane diterpenes.[1–7] Formosan soft corals of the *Sinularia* have been proven to be rich sources of terpenoids.[8–20] Our previous chemical investigations on *Sinularia flexibilis* afforded a sulfur-containing biscembranoid[21] and several cembranoids, including flexibilisolides A and B, and flexibilisins A and B.[22] The first investigation on the chemical constituents of the Dongsha Atoll, located in South China Sea soft coral *S. flexibilis* by our group again yielded seven new metabolites, flexibilisolides C–G (**1**–**5**), flexibilisin C (**6**), and 11,12-secoflexibillin (**7**), along with seven known cembranoids, 11-dehydrosinulariolide (**8**),[23] flexilarin D (**9**),[24] 14-deoxycrassin (**10**),[25] 11-*epi*-sinulariolide acetate (**11**),[26] 3,4:8,11-bisepoxy-7-acetoxycembra-15(17)-en-1,12-olide (**12**),[27] sinulariolide (**13**),[26] and 11-*epi*-sinulariolide (**14**).[28] The structures of these compounds were determined on the basic of extensive spectroscopic analysis, including 1D and 2D NMR (^{1}H and ^{13}C NMR, ^{1}H–^{1}H COSY, HMQC, HMBC, and NOESY) spectroscopy. Cytotoxicity of compounds **1**–**14** against a limited panel of human tumor cell lines, including human cervical epitheloid carcinoma (HeLa), human liver carcinoma (SK-Hep1), and human melanin carcinoma (B16) cells was studied, and the ability of **1**–**14** to inhibit up-regulation of the pro-inflammatory iNOS (inducible nitric oxide synthase) and COX-2 (cyclooxygenase-2) proteins in LPS (lipopolysaccharide)-stimulated RAW264.7 macrophage cells was also evaluated.

2. Results and discussion

HRESIMS of flexibilisolide C (**1**) exhibited a pseudo-molecular ion peak at m/z 347.1863 $[M+H]^+$, consistent with the molecular formula $C_{20}H_{26}O_5$, and 8° of unsaturation. The IR spectrum of **1** revealed the presence of carbonyl groups (ν_{max} 1723 and 1694 cm^{-1}). The ^{13}C NMR spectrum of **1** (Table 1) displayed 20 carbon signals, and a DEPT experiment confirmed the presence of three methyls, six methylenes, five methines, and six quaternary carbons. From signals (Tables 1 and 2) appearing at δ_C 168.1 (C), 144.0 (C), 125.6 (CH_2), 91.1 (C), 35.4 (CH_2), 34.2 (CH), and 31.3 (CH_2) and 6.38 (1H, s), 5.51 (1H, s), and 2.62 (1H, m), and a tertiary oxygenated carbon signal resonating at δ 91.1 suggested the presence of an α-exomethylene-ε-lactone moiety,[23] which was further supported by ^{1}H–^{1}H COSY and HMBC experiment (Fig. 1). From the ^{1}H–^{1}H COSY spectrum of **1**, it was also possible to identify three different structural units, which were assembled with the assistance of an HMBC experiment. Key HMBC correlations of H-10 to C-11; 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

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Table 1
^{13}C NMR spectroscopic data for compounds **1**–**6**

Position	1[a]	2[b]	3[b]	4[a]	5[b]	6[b]
1	34.2 (CH)[c]	36.4 (CH)	35.4 (CH)	32.9 (CH)	35.0 (CH)	38.9 (CH)
2	33.0 (CH_2)	30.3 (CH_2)	33.4 (CH_2)	38.7 (CH_2)	31.8 (CH_2)	33.4 (CH_2)
3	62.3 (CH)	59.5 (CH)	61.1 (CH)	73.9 (CH)	61.1 (CH)	61.9 (CH)
4	60.0 (C)	60.3 (C)	60.1 (C)	86.2 (C)	60.4 (C)	60.0 (C)
5	35.5 (CH_2)	41.2 (CH_2)	34.0 (CH_2)	37.5 (CH_2)	43.4 (CH_2)	38.4 (CH_2)
6	23.8 (CH_2)	125.0 (CH)	26.9 (CH_2)	29.8 (CH_2)	126.6 (CH)	24.2 (CH_2)
7	68.9 (CH)	138.3 (CH)	75.1 (CH)	81.7 (CH)	135.0 (CH)	122.9 (CH)
8	58.0 (C)	72.4 (C)	88.1 (C)	146.3 (C)	85.5 (C)	135.1 (C)
9	148.0 (CH)	34.3 (CH_2)	40.3 (CH_2)	27.0 (CH_2)	32.0 (CH_2)	33.9 (CH_2)
10	125.2 (CH)	35.4 (CH_2)	93.4 (CH)	37.1 (CH_2)	23.3 (CH_2)	30.5 (CH_2)
11	212.0 (C)	214.5 (C)	157.2 (C)	212.4 (C)	75.7 (CH)	66.9 (CH)
12	91.1 (C)	91.4 (C)	83.1 (C)	91.5 (C)	87.0 (C)	138.1 (C)
13	35.4 (CH_2)	34.2 (CH_2)	38.5 (CH_2)	34.6 (CH_2)	32.4 (CH_2)	126.5 (CH)
14	31.3 (CH_2)	33.3 (CH_2)	32.4 (CH_2)	30.7 (CH_2)	29.6 (CH_2)	29.5 (CH_2)
15	144.0 (C)	143.4 (C)	145.1 (C)	144.9 (C)	143.1 (C)	143.1 (C)
16	168.1 (C)	167.8 (C)	168.4 (C)	168.6 (C)	168.4 (C)	167.4 (C)
17	125.6 (CH_2)	125.8 (CH_2)	123.8 (CH_2)	124.6 (CH_2)	124.9 (CH_2)	124.2 (CH_2)
18	15.8 (CH_3)	16.2 (CH_3)	16.7 (CH_3)	18.5 (CH_3)	16.4 (CH_3)	16.4 (CH_3)
19	16.2 (CH_3)	29.9 (CH_3)	20.7 (CH_3)	114.3 (CH_2)	26.5 (CH_3)	16.8 (CH_3)
20	27.4 (CH_3)	28.3 (CH_3)	32.5 (CH_3)	29.0 (CH_3)	24.3 (CH_3)	17.3 (CH_3)
7-OAc			21.0 (CH_3) 171.1 (C)			
11-OAc					21.1 (CH_3) 171.5 (C)	
16-OMe						51.9 (CH_3)

[a] Recorded at 125 MHz in $CDCl_3$ at 25 °C.
[b] Recorded at 100 MHz in $CDCl_3$ at 25 °C.
[c] Multiplicities deduced by DEPT.

Table 2
^{1}H NMR spectroscopic data for compounds **1**–**6**

Position	1[a]	2[b]	3[b]	4[a]	5[b]	6[b]
1	2.62 m	2.42 m	2.95 m	3.03 m	2.83 m	2.56 m
2	1.69 m 1.90 m	1.37 m 2.05 m	1.48 m 2.03 m	1.57 m 2.03 m	1.43 m 2.13 m	1.52 ddd (14.0, 8.4, 2.8) 1.77 ddd (14.0, 9.2, 4.8)
3	2.59 dd (10.5,1.5)	2.95 dd (10.0, 4.4)[c]	3.33 dd (7.6, 4.4)	3.51 m	2.93 dd (9.6, 4.4)	2.84 dd (8.4, 4.8)
5	1.08 td (14.0, 4.0) 2.27 dt (13.5, 4.0)	1.88 dd (12.8, 7.6) 2.58 dd (12.8, 8.8)	1.02 m 2.03 m	1.81 m 2.03 m	1.77 dd (13.2, 9.6) 2.76 dd (13.2, 4.4)	1.22 m 2.11 m
6	1.52 m 2.07 m	5.71 ddd (15.6, 8.8, 7.6)	1.81 m	1.80 m 1.94 m	5.80 ddd (16.0, 9.6, 4.4)	2.21 m
7	2.85 dd (11.0, 2.5)	5.46 d (15.6)	5.12 m	4.36 dd (9.0, 6.0)	5.73 d (16.0)	5.18 dd (7.6, 7.6)
9	6.94 d (15.5)	1.73 m 2.18 m	2.39 m 2.50 dd (15.6, 2.4)	2.23 m 2.55 m	1.55 m 2.01 m	1.91 m 2.20 m
10	7.04 d (15.5)	2.75 ddd (20.8, 6.8, 3.6) 3.17 ddd (20.8, 9.2, 3.2)	4.51 t (2.4)	2.60 m 3.54 m	1.64 m 1.68 m	1.66 m 1.85 m
11					5.57 dd (12.0, 3.2)	4.59 dd (6.4, 6.4)
13	1.98 m 2.11 m	1.97 m 2.33 m	1.91 ddd (16.8, 9.6, 1.6) 2.16 m	1.86 m 2.33 dd (15.0, 5.0)	1.93 m	5.33 dd (9.2, 4.0)
14	1.30 m 1.97 m	1.43 m 2.10 m	1.37 m 2.38 m	1.08 m 2.05 m	1.28 m 2.21 m	2.04 m 2.78 m
17	5.51 s 6.38 s	5.54 s 6.37 s	5.43 s 6.24 s	5.49 s 6.31 s	5.49 s 6.30 s	5.61 s 6.28 s
18	1.33 s	1.25 s	1.21 s	1.15 s	1.50 s	1.28 s
19	1.50 s	1.34 s	1.38 s	5.04 s 5.05 s	1.36 s	1.67 s
20	1.56 s	1.48 s	1.61 s	1.46 s	1.38 s	1.69 s
7-OAc			2.09 s			
11-OAc					2.15 s	
16-OMe						3.75 s

[a] Recorded at 500 MHz in $CDCl_3$ at 25 °C.
[b] Recorded at 400 MHz in $CDCl_3$ at 25 °C.
[c] *J* values (in Hz) in parentheses.

connection of the carbon skeleton. Thus, **1** was revealed as a cembranoid possessing a ε-lactone ring, on the basis of the above analysis.

The relative structure of **1** was elucidated by the analysis of NOE correlations, as shown in Fig. 2. It was found that H-1 (δ 2.62, m) showed NOE interaction with H_3-18 (δ 1.33, 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-2 (δ 1.69, m) exhibited NOE correlation with H-1 and was characterized as H-2α, while the other (δ 1.90, m) was assigned as H-2β. NOE correlations observed between H-2α and H_3-18, H_3-18 and H-5α (δ 2.27, dt, J=13.5, 4.0 Hz), H-5α and H-6α (δ2.07, m), H-6α and H-7 (δ 2.85, dd, J=11.0, 2.5 Hz), H-1 and H-14α (δ 1.30, m), and H-14α and H-13α (δ 1.98, m), reflected the α-orientation of the H-7. Also, the NOE correlations observed for H-2β with H-3 (δ 2.59, dd, J=10.5, 1.5 Hz), H-6β (δ 1.52, m) with H_3-19 (δ 1.50, s), and H-13β (δ 2.11, m) with H_3-20 (δ 1.56, s), reflected the β-orientations of the H-3, H_3-19, and H_3-20.