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Eunicellin-based diterpenoids from the cultured soft coral *Klyxum simplex*

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ABSTRACT

Eight new eunicellin-base diterpenoids, klysimplexins A–H (**1**–**8**), were isolated from a cultured soft coral *Klyxum simplex*. Their structures were elucidated by spectroscopic methods, particularly in 1D and 2D NMR experiments. The structure of **1** was further confirmed by a single-crystal X-ray diffraction analysis and the application of modified Mosher's method. Metabolites **2** and **8** were found to be cytotoxic toward a limited panel of cancer cell lines.

1. Introduction

Worldwide marine secondary metabolites are of considerable interest due to the highly diversified structures and wide range of biological activites.[1] The eunicellin-based diterpenoids are secondary metabolites which possess a cladiellane skeleton with a C-2, C-9 ether bridge. Previously reported eunicellin-based diterpenoids have been mostly isolated from octocorals (Alcyonaceae) belonging to the genera *Acalycigorgia*,[2] *Alcyonium*,[3] *Astrogorgia*,[4] *Briareum*,[5] *Cladiella*,[6] *Eleutherobia*,[7] *Eunicella*,[8] *Klyxum*,[9] *Litophyton*,[10] *Muricella*,[11] *Pachyclavularia*,[12,13] *Sclerophytum*,[14] *Sinularia*[15] and *Solenopodium*.[16] Some of these metabolites have been shown to exhibit cytotoxic activity against the growth of various cancer cell lines.[5–7,12–14] In continuation of our investigation on the bioactive substances from marine invertebrates,[17–25] the chemical content of a cultured soft coral *Klyxum simplex* has been studied. In this paper, we report the isolation, structure determination and biological activity of eight new eunicellin-based metabolites, klysimplexins A–H (**1**–**8**), from this soft coral. The structures of **1**–**8** were established by extensive spectroscopic analysis, including 2D NMR (^{1}H–^{1}H COSY, HSQC, HMBC, and NOESY) spectroscopy. Cytotoxicity of metabolites **1**–**8** against a limited panel of human tumor cell lines including human liver carcinoma (Hep G2 and Hep G3B), human breast carcinoma (MDA-MB-231 and MCF-7), human lung carcinoma (A-549), and human oral cancer cells (Ca9-22) was also evaluated. Klysimplexin B (**2**) and H (**8**) have been shown to exhibit moderate cytotoxicity against the growth of the above six cancer cell lines.

2. Results and discussion

The octocoral (1.5 kg fresh wt) was collected and freeze-dried. The freeze-dried material was minced and extracted exhaustively with EtOH (3×10 L). The organic extract was concentrated to an aqueous suspension and was further partitioned between CH_2Cl_2 and water. The combined CH_2Cl_2-soluble fraction was concentrated under reduced pressure and the residue was repeatedly purified by chromatography to yield metabolites **1**–**8**.

Klysimplexin A (**1**) was isolated as colorless crystals following recrystallization from acetone. The HRESIMS of **1** exhibited a $[M+Na]^+$ peak at *m*/*z* 489.2832 and established a molecular formula $C_{26}H_{42}O_7$, implying six degrees of unsaturation. The IR spectrum of **1** revealed the presence of hydroxy and carbonyl functionalities from absorptions of 3442 and 1728 and 1712 cm^{-1}. The ^{13}C NMR spectroscopic data of **1** exhibited 26 carbon signals (Table 1), which were assigned by the assistance of DEPT spectrum

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Table 1
^{13}C NMR data for compounds **1**–**8**

Position	**1**[a]	**2**[b]	**3**[c]	**4**[c]	**5**[d]	**6**[c]	**7**[e]	**8**[d]
1	45.5 (CH)[f]	45.0 (CH)	43.0 (CH)	43.1 (CH)	43.0 (CH)	44.2 (CH)	42.3 (CH)	42.5 (CH)
2	93.0 (CH)	91.5 (CH)	91.6 (CH)	91.5 (CH)	91.5 (CH)	93.6 (CH)	92.0 (CH)	93.7 (CH)
3	84.9 (qC)	83.9 (qC)	84.5 (qC)	84.4 (qC)	84.8 (qC)	85.9 (qC)	86.2 (qC)	84.3 (qC)
4	34.2 (CH_2)	34.2 (CH_2)	29.9 (CH_2)	29.9 (CH_2)	29.7 (CH_2)	36.6 (CH_2)	36.2 (CH_2)	29.6 (CH_2)
5	35.5 (CH_2)	36.1 (CH_2)	35.4 (CH_2)	29.7 (CH_2)	29.7 (CH_2)	30.5 (CH_2)	30.5 (CH_2)	30.8 (CH_2)
6	211.4 (qC)	200.3 (qC)	73.5 (CH)	87.2 (CH)	73.7 (CH)	80.6 (CH)	80.6 (CH)	71.0 (CH)
7	45.0 (CH)	147.5 (qC)	150.0 (qC)	145.5 (qC)	150.1 (qC)	77.2 (qC)	77.2 (qC)	136.0 (qC)
8	42.2 (CH_2)	42.1 (CH_2)	41.2 (CH_2)	41.9 (CH_2)	41.3 (CH_2)	47.5 (CH_2)	47.6 (CH_2)	124.9 (CH)
9	76.8 (CH)	78.6 (CH)	79.2 (CH)	79.0 (CH)	79.2 (CH)	76.1 (CH)	75.8 (CH)	79.4 (CH)
10	52.0 (CH)	49.7 (CH)	45.5 (CH)	45.3 (CH)	45.3 (CH)	52.5 (CH)	52.4 (CH)	56.9 (CH)
11	84.1 (qC)	82.6 (qC)	83.5 (qC)	83.4 (qC)	83.4 (qC)	83.7 (qC)	82.2 (qC)	73.0 (qC)
12	43.3 (CH_2)	43.3 (CH_2)	42.2 (CH_2)	42.4 (CH_2)	42.5 (CH_2)	41.9 (CH_2)	32.4 (CH_2)	76.4 (CH)
13	66.1 (CH)	66.5 (CH)	66.8 (CH)	66.8 (CH)	66.8 (CH)	66.4 (CH)	17.6 (CH_2)	70.4 (CH)
14	50.2 (CH)	49.7 (CH)	50.3 (CH)	49.9 (CH)	50.2 (CH)	50.4 (CH)	42.5 (CH)	48.0 (CH)
15	22.8 (CH_3)	23.6 (CH_3)	22.7 (CH_3)	22.9 (CH_3)	22.7 (CH_3)	23.5 (CH_3)	23.1 (CH_3)	24.5 (CH_3)
16	14.4 (CH_3)	117.9 (CH_2)	117.0 (CH_2)	118.2 (CH_2)	116.9 (CH_2)	22.8 (CH_3)	22.8 (CH_3)	19.6 (CH_3)
17	25.5 (CH_3)	25.9 (CH_3)	25.2 (CH_3)	25.3 (CH_3)	25.2 (CH_3)	24.6 (CH_3)	24.7 (CH_3)	26.6 (CH_3)
18	31.1 (CH)	30.7 (CH)	28.4 (CH)	28.5 (CH)	28.4 (CH)	30.5 (CH)	29.0 (CH)	30.7 (CH)
19	25.5 (CH_3)	25.8 (CH_3)	24.7 (CH_3)	24.8 (CH_3)	24.7 (CH_3)	24.7 (CH_3)	21.8 (CH_3)	24.3 (CH_3)
20	16.6 (CH_3)	16.8 (CH_3)	15.8 (CH_3)	15.7 (CH_3)	15.8 (CH_3)	16.3 (CH_3)	15.4 (CH_3)	17.4 (CH_3)
3-*n*-Butyrate	14.3 (CH_3)	14.8 (CH_3)	13.6 (CH_3)	13.6 (CH_3)		13.7 (CH_3)		14.8 (CH_3)
	19.6 (CH_2)	20.0 (CH_2)	18.6 (CH_2)	18.5 (CH_2)		18.7 (CH_2)		19.2 (CH_2)
	37.9 (CH_2)	37.9 (CH_2)	37.4 (CH_2)	37.3 (CH_2)		37.3 (CH_2)		37.4 (CH_2)
	173.4 (qC)	170.4 (qC)	172.6 (qC)	172.6 (qC)		172.6 (qC)		170.8 (qC)
3-OAc					22.4 (CH_3)		22.3 (CH_3)	
					169.8 (qC)		169.8 (qC)	
11-OAc	22.8 (CH_3)	22.8 (CH_3)	22.4 (CH_3)	22.4 (CH_3)	22.4 (CH_3)	22.5 (CH_3)	22.6 (CH_3)	
	170.6 (qC)	167.6 (qC)	170.0 (qC)	169.9 (qC)	169.9 (qC)	170.1 (qC)	170.3 (qC)	
12-OAc								21.8 (CH_3)
								168.4 (qC)
13-*n*-Butyrate								15.0 (CH_3)
								19.3 (CH_2)
								37.6 (CH_2)
								170.8 (qC)

[a] Spectra recorded at 125 MHz in C_5D_5N at 25 °C.
[b] Spectra recorded at 100 MHz in C_6D_6 at 25 °C.
[c] Spectra recorded at 75 MHz in $CDCl_3$ at 25 °C.
[d] Spectra recorded at 100 MHz in $CDCl_3$ at 25 °C.
[e] Spectra recorded at 125 MHz in $CDCl_3$ at 25 °C.
[f] Multiplicities deduced by DEPT.

to seven methyls, six sp^3 methylenes, eight sp^3 methines (including three oxymethines), three sp^2 carbonyls and two sp^3 oxygenated quaternary carbons. The ^{13}C NMR spectrum of **1** showed the presence of a ketone (δ_C 211.4). Two ester carbonyls (δ_C 170.6 and 173.4) were also assigned from the ^{13}C NMR spectrum and were HMBC correlated with an acetate methyl (δ_H 2.06 s) and methylenes (δ_H 1.78 m and 2.58 dd, J=14.5 and 7.0 Hz, each 2H) of an *n*-butyrate, respectively. Therefore, the remaining three degrees of unsaturation identified compound **1** as a tricyclic compound. In the ^{1}H NMR of **1** (Table 2), a doublet at δ_H 1.31 (3H, d, J=7.0 Hz) was attributed to H_3-16 and two doublet at δ_H 1.21 and 1.49 (each 3H, d, J=7.0 Hz) were arised from two methyls of an isopropyl group. Furthermore, two singlets of the tertiary methyls bonded to oxygenated carbons at δ_H 1.53 and 1.62 (each, 3H, s) were due to the resonances of H_3-15 and H_3-17. Signals resonating at δ_H 2.51 (1H, dd, J=12.0, 7.0 Hz), 3.54 (1H, br t, J=7.5 Hz), 3.78 s and 4.20 (1H, ddd, 13.0, 7.0, 4.0 Hz), and at δ_C 45.5, 52.0, 93.0 and 76.8, indicated the presence of a tetrahydrofuran structural unit.[6] The gross structure of metabolite **1** was elucidated by analysis of ^{1}H–^{1}H COSY and HMBC correlations (Fig. 2). From the ^{1}H–^{1}H COSY spectrum of **1**, it was possible to identify three structural units, which were further assembled by HMBC correlations (Fig. 1). Key HMBC correlations from H-2 to C-1, C-9 and C-10; H_2-4 to C-5 and C-6; H_3-15 to C-2, C-3 and C-4; H_3-16 to C-6, C-7 and C-8; H_3-17 to C-10, C-11 and C-12; and both H_3-19 and H_3-20 to C-14 and C-18 permitted the connection of the carbon skeleton. Furthermore, the detailed structure and relative configuration of **1** were established unambiguously from a single-crystal X-ray diffraction analysis (Fig. 3). Finally, in order to resolve the absolute structure of **1**, we determined the configuration at C-13 using a modified Mosher's method.[26,27] The *S*- and *R*-α-methoxy-α-trifluoromethylphenylacetic (MPTA) esters of **1** (**1a** and **1b**, respectively) were prepared by using the corresponding *R*-(−)- and *S*-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chlorides, respectively. The values of $\Delta\delta$ [δ(*S*-MPTA ester)−δ(*R*-MPTA ester)] for H-9, H-10, H-12 and H_3-17 were positive, while the values of $\Delta\delta$ for H-18, H_3-19, H_3-20 were negative, revealing the *R*-configuration at C-13 (Fig. 4).

Klysimplexin B (**2**) was isolated as a colorless oil. Its molecular formula $C_{26}H_{40}O_7$ was established by HRESIMS (*m*/*z* 487.2669, $[M+Na]^+$). Thus, **2** has seven degrees of unsaturation. Its IR spectrum exhibited strong absorptions at 3442, 1738 and 1696 cm^{-1}, indicative of hydroxy, ester carbonyl and conjugated carbonyl groups. The ^{13}C NMR signals of **2** were found to be very closely related to those of compound **1**, suggesting the very similar eunicellin-based skeleton for both compounds, except that the single bond between C-7 and C-16 in **1** was oxidized to a double bond in **2**, as evidenced by two exocyclic methylene protons resonating at δ 4.96 (s) and 5.61 (s). The structure of **2** was unambiguously determined by the extensive analysis of ^{1}H–^{1}H COSY and HMBC (Fig. 2), and NOESY correlations (Fig. 5).

Klysimplexin C (**3**) was obtained as a colorless oil that gave a pseudomolecular ion peak at *m*/*z* 489.2825 $[M+Na]^+$ in the HRESIMS, consistent with the molecular formula $C_{26}H_{42}O_7$ and implying six degrees of unsaturation. The IR absorptions at ν_{max} 3431 (br) and 1733 cm^{-1} revealed the presence of hydroxy and ester carbonyl functionalities. The assignments of ^{1}H and ^{13}C NMR spectroscopic