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Monanchoramides A–D, ceramides from the marine sponge *Monanchora clathrata* with cytotoxic activity

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ABSTRACT

Four new ceramides (**1–4**) were isolated from the sponge *Monanchora clathrata*, along with four epidioxysterols (**5–8**), two sterols (**9**, **10**), uracil (**11**), and three triglycerides (**12–14**). All compounds were isolated for the first time from the genus *Monanchora*, also this is the first time for isolation and identification of compounds **5–8**, and the ceramide moieties from the family Crambeidae. Acetylation of compounds (**5–8**) yielded two new chemically modified compounds (**15**, **16**), in addition to the known **17**, **18**. Their chemical structures were elucidated using a combination of spectroscopic methods, including extensive 1D and 2D NMR, IR, HRESIMS, and GC/MS. The configuration of compounds **1–4** were assigned as 2*S*,3*S*,4*R*,2′*R* based on the modified Mosher's reaction, optical rotation measurements and spectroscopic data comparison. The compounds were evaluated for their, cytotoxic, antiprotozoal, antimicrobial, and antimalarial potentials. Compound **1** showed remarkable cytotoxicity against MES-SA, MCF-7, and HK-2 cell lines with IC_{50} values of 3.29, 17.95 and 4.45 μM, respectively.

1. Introduction

The term ceramide describe specifically a long-chain base and an amide-linked fatty acid (the *N*-acyl chain) (Bikman and Summers, 2011). In most mammalian cells, the predominant long chain base is sphingosine, and the majority of the *N*-acyl chain is 14 to 26 carbons in length saturated or monounsaturated and the *N*-acyl chain can be hydroxylated to various degrees depending on cell types (Kota et al., 2013; Hama, 2010). Ceramides are an essential component of sphingolipid mediated cell signaling pathways that regulate various cellular processes including cell growth and apoptosis, differentiation, and motility (Kota et al., 2013; Hannun and Obeid, 2008; Zheng et al., 2006). There is increasing evidence that 2′-hydroxy ceramide has distinct cell signaling function. Four purified ceramide fractions isolated from equine kidney induced apoptosis in cancer cell lines (Kyogashima et al., 2008). Interestingly, the pro-apoptotic activity of the ceramide was stronger when ceramide contained phytoceramide (C4-hydroxyl group in the long-chain base) or 2′-hydroxyl group in the *N*-acyl chain. More recent studies showed that synthetic (*R*) 2′-hydroxy-C6-ceramide inhibited growth of MCF-7 cells at lower concentrations compared to the (*S*)-2′ isomer or non-hydroxy-C6-ceramide (Szulc et al., 2010), though the mechanism of the growth inhibition was not determined. These findings strongly suggest that 2′-hydroxyceramide regulates cell growth and apoptosis. Marine ceramides exhibit variable and promising biological activities, such as antihepatotoxic, antitumor, immunostimulant, and antifouling, in addition to anticholinesterase activity (Abdelhameed et al., 2016; Abboushi et al., 2004; Hattori et al., 1998; Kim et al., 1997; Natori et al., 1994). In the course of an ongoing program toward the isolation of biologically active metabolites from marine organisms (Eltamany et al., 2015; Radwan et al., 2015), chemical investigation of the extract of the marine sponge *Monanchora clathrata* (class Demospongiae, order Poecilosclerida, family Crambeidae) collected from the Philippine islands, afforded four new ceramides, monanchoramides A-D (**1–4**), four known epidioxysterols

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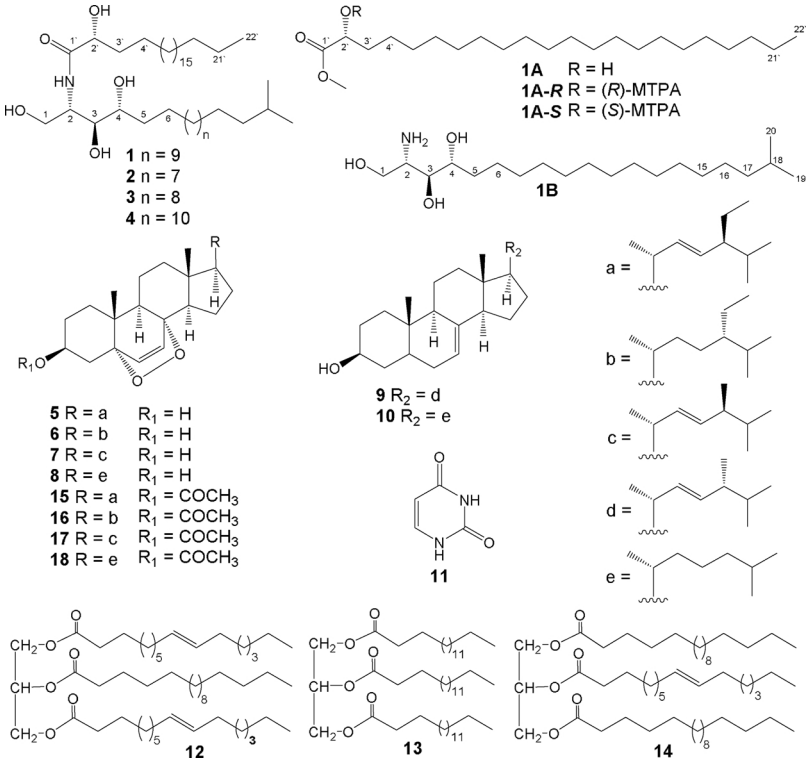

Fig. 1. Structures of compounds **1–18**.

(**5–8**), two known sterols (**9**, **10**), the known nucleobase uracil (**11**), together with three known triglycerides (**12–14**) (Fig. 1).

2. Results and discussion

Compound **1** was isolated as white amorphous powders and exhibited a cholorine adduct ion $[M + Cl]^-$ at *m/z* 718.6165, corresponding to a molecular formula of $C_{42}H_{85}NO_5Cl$ in the negative HRESIMS. It showed characteristic IR absorption bands at 3210 cm^{-1} (hydroxy), 3334 cm^{-1} (secondary amino), 1620 cm^{-1} (amide carbonyl) and 2917, 2850, 721 cm^{-1} (aliphatic) suggesting the presence of fatty-acid amide. The ^1H, ^{13}C and HSQC NMR spectroscopic data (Table 1) revealed the presence of an amide moiety [δ_H 8.61 (NH, d, J = 8.8 Hz), δ_C 175.8 (C-1′)]. In addition, hydroxymethylene and three oxymethine characteristic signals at δ_H 4.46 (H-1a), 4.55 (H-1b)/δ_C 62.5 (C-1), δ_H 4.32 (H-4)/δ_C 73.5 (C-4), δ_H 4.39 (H-3)/δ_C 77.2 (C-3) and δ_H 4.65 (H-2′)/δ_C 73.0 (C-2′). Also, it showed signals for an isopropyl moiety at δ_H 1.33 (H-18)/δ_C 28.3 (C-18) and δ_H 0.90 (H-19 and 20)/δ_C 23.3 (C-19 and 20) and a methyl group δ_H 0.89 (H-22)/δ_C 14.8 (C-22) along with a cluster of methylene groups at δ_H 1.27-2.29/δ_C 23.6-39.8. All the previous spectroscopic data, in addition to a characteristic nitrogenated methine signal at δ_H 5.15 (H-2)/δ_C 53.5 (C-2) suggested the

Table 1
^1H and ^{13}C NMR Spectroscopic Data (^1H 500 MHz, ^{13}C 125 MHz, C_5D_5N) for Monanchoramides A–D (**1–4**).

Position	Monanchoramide A (1)		Monanchoramide B (2)		Monanchoramide C (3)		Monanchoramide D (4)	
	δ_C, type	δ_H, mult (J in Hz)	δ_C, type	δ_H, mult (J in Hz)	δ_C, type	δ_H, mult (J in Hz)	δ_C, type	δ_H, mult (J in Hz)
1	62.5, CH_2	4.55, dd (3.6, 8.6) 4.46, dd (3.6, 8.6)	62.5, CH_2	4.47, dd (3.6, 8.6) 4.38, dd (3.6, 8.6)	62.5, CH_2	4.54, dd (3.7, 8.7) 4.47, dd (3.7, 8.7)	62.5, CH_2	4.51, dd (4.8, 10.8) 4.43, dd (4.8, 10.8)
2	53.5, CH	5.15, m	53.5, CH	5.05, m	53.5, CH	5.12, m	53.0, CH	5.12, m
3	77.2, CH	4.39, m	77.2, CH	4.32, m	77.2, CH	4.40, m	76.9, CH	4.36, m
4	73.5, CH	4.32, m	73.6, CH	4.24, m	73.5, CH	4.31, m	73.2, CH	4.28, m
5	34.7, CH_2	2.29, m, 1.95, m	34.7, CH_2	2.23, m, 1.92, m	34.7, CH_2	2.29, m, 1.96, m	34.3, CH_2	2.25, m, 1.92, m
6–14	26.3–32.7, CH_2	1.27–1.83	26.4–32.7, CH_2	1.25–1.76	26.4–28.7, CH_2	1.27–1.81	26.0–30.5, CH_2	1.23–1.75
15	26.3–32.7, CH_2	1.27–1.83	39.9, CH_2	1.16, m	26.4–28.7, CH_2	1.27–1.81	26.0–30.5, CH_2	1.23–1.75
16	26.3–32.7, CH_2	1.27–1.83	28.4, CH_2	1.45, m	39.8, CH_2	1.17, m	26.0–30.5, CH_2	1.23–1.75
17	39.8, CH_2	1.18, m	23.4, CH_3	0.89, d (5.2)	28.7, CH_2	1.50, m	26.0–30.5, CH_2	1.23–1.75
18	28.3, CH_2	1.33, m	23.4, CH_3	0.89, d (5.2)	23.3, CH_3	0.90, d (5.2)	39.4, CH_2	1.13, m
19	23.3, CH_3	0.90, d (5.2)	–	–	23.3, CH_3	0.90, d (5.2)	28.4, CH_2	1.43, m
20	23.3, CH_3	0.90, d (5.2)	–	–	–	–	22.9, CH_3	0.88, d (5.2)
21	–	–	–	–	–	–	22.9, CH_3	0.90, d (5.2)
1′	175.8, C	–	176.1, C	–	175.9, CH	–	175.4, C	–
2′	73.0, CH	4.65, dd (3.8, 7.8)	73.1, CH	4.60, dd (3.0, 6.2)	73.0, CH	4.66, dd (3.0, 6.2)	72.6, CH	4.65, dd (4.0, 7.6)
3′	36.2, CH_2	2.26, m, 2.05, m	36.0, CH_2	2.21, m, 2.02, m	36.2, CH_2	2.26, m, 2.07, m	35.9, CH_2	2.26, m, 2.06, m
4′–19′	26.3–32.7, CH_2	1.27–1.83	26.4–32.7, CH_2	1.25–1.76	26.3–28.7, CH_2	1.27–1.81	26.0–30.5, CH_2	1.23–1.75
20′	32.7, CH_2	1.32, m	32.8, CH_2	1.31, m	32.7, CH_2	1.40, m	32.7, CH_2	1.43, m
21′	23.5, CH_2	1.26, m	23.6, CH_2	1.27, m	23.5, CH_2	1.32, m	23.1, CH_2	1.28, m
22′	14.8, CH_3	0.89, t (5.4)	14.9, CH_3	0.88, t (5.6)	14.9, CH_3	0.88, t (5.6)	14.5, CH_3	0.85, t (5.6)
NH	–	8.61, d (8.8)	–	8.53, d (7.2)	–	8.61, d (7.2)	–	8.58, d (8.8)