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New compounds from the Red Sea marine sponge *Echinoclathria gibbosa*

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ABSTRACT

Three new compounds; β-sitosterol-3-*O*-(3*Z*)-pentacosenoate (**1**), 5α-pregna-3β-acetoxy-12β,16β-diol-20-one (**2**), and echinoclathriamide ((*R*)-2′-hydroxy-N-((2*S*,3*S*,4*R*)-1,3,4-trihydroxy-19-methylicosan-2-yl)heptadecanamide) (**3**), together with two known compounds; thymine (**4**) and uracil (**5**) were isolated from the EtOAc fraction of the Red Sea sponge *Echinoclathria gibbosa*. Their structures were unambiguously established on the basis of 1D and 2D NMR spectroscopy, in addition to mass spectrometry. The total MeOH extract (TME) and its fractions were evaluated for their antimicrobial, anti-inflammatory, antipyretic, and hepato-protective activities. The *in vitro* growth inhibitory activity of the isolated compounds was evaluated against three human cancer cell lines including the A549 non-small cell lung cancer (NSCLC), U373 glioblastoma (GBM), and PC-3 prostate cancer cell lines. Compound **1** showed weak activity against the three cancer cell lines.

1. Introduction

Marine sponges are a rich source of interesting class of compound metabolites with unique chemical features and pronounced biological activities (Blunt et al., 2013). The genus *Echinoclathria* has been subjected to extensive research leading to the isolation and identification of a wide variety of novel structures (Sarma et al., 2005). Previous phytochemical studies of genus *Echinoclathria* resulted in the isolation of steroids (Li et al., 1993) and pyridine alkaloids (Kitamura et al., 1999; Dembitsky et al., 2005; Ueoka et al., 2009). In this paper, we describe the isolation and structural elucidation of three new compounds from the EtOAc fraction of the Red Sea sponge *E. gibbosa*; β-sitosterol-3-*O*-(3*Z*)-pentacosenoate (**1**), 5α-pregna-3β-acetoxy-12β,16β-diol-20-one (**2**), and echinoclathriamide ((*R*)-2′-hydroxy-N-((2*S*,3*S*,4*R*)-1,3,4-trihydroxy-19-methylicosan-2-yl)heptadecanamide) (**3**), together with two known compounds; thymine (**4**) and uracil (**5**) (Fig. 1). In addition, the antimicrobial, anti-inflammatory, antipyretic, and hepato-protective activities of the total MeOH extract (TME) and its fractions were evaluated. The *in vitro* growth inhibitory activity of the isolated compounds was evaluated against three human cancer cell lines; NSCLC, GBM, and PC-3. Compound **1** showed weak activity against the tested cancer cell lines, while **2**–**5** were inactive.

2. Results and discussion

Compound **1** was isolated as a white greasy powder. It gave positive Liebermann–Burchard's test (Schmidt, 1964), suggesting its steroidal or triterpenoidal nature. The HRFABMS spectrum of **1** showed pseudo-molecular ion peak at *m*/*z* 777.7415 $[M+H]^+$, that is consistent with a formula of $C_{54}H_{96}O_2$. The IR spectrum of **1** showed characteristic absorption bands indicating the presence of an ester group (1720 cm^{-1}), unsaturation (1632 cm^{-1}), and long aliphatic chain (724 cm^{-1}). The ^1H NMR spectrum (Table 1) displayed signals for sterol and fatty acid moieties (Qi et al., 2003; Sayed et al., 2007). The appearance of six methyl groups at δ_H 0.68–1.02 including two tertiary methyl groups at δ_H 0.68 (3H, s, H-18) and δ_H 1.02 (3H, s, H-19), a methine proton at δ_H 4.59 (1H, m, H-3), and an olefinic proton at δ_H 5.34 (1H, m, H-6) suggesting the presence of sitosteryl moiety. Furthermore, **1** displayed signals for a primary methyl group at δ_H 0.89 (3H, t, J = 6.1 Hz, H-25′), two

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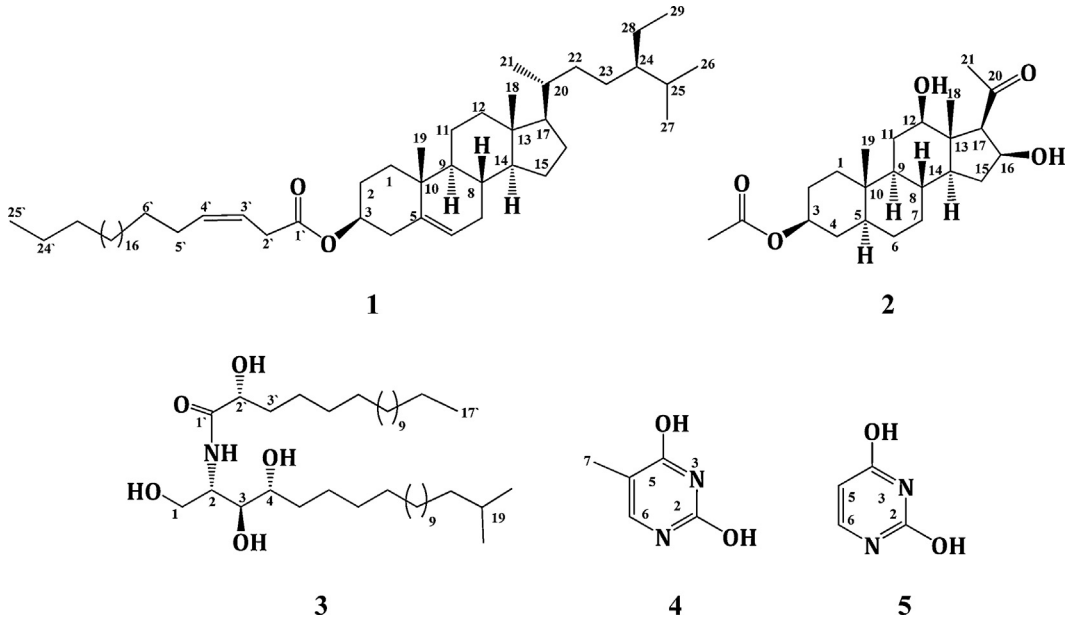

Fig. 1. Structures of the isolated compounds.

olefinic protons at δ_H 5.38 (2H, each dt, J = 7.5, 6.0 Hz, H-3′, 4′), and a large number of methylene protons at δ_H 1.22–1.38 suggesting the presence of an aliphatic fatty acid moiety. The ^{1}H–^{1}H COSY (Table 1) revealed different spin-coupling systems for sterol and fatty acid. The ^{13}C NMR and HSQC spectra displayed 54 signals, 29 of them were attributed to sitosteryl moiety with olefinic carbons at δ_C 139.7 (C-5) and 122.5 (C-6). A carbinol carbon at δ_C 73.6 (C-3), in addition to other carbon signals were in full agreement with those previously reported for β-sitosterol (Sayed et al., 2007; Bagri et al., 2009). They also showed an ester carbonyl group at δ_C 173.2, a terminal methyl group at δ_C 14.1, olefinic carbons at δ_C 122.5 and 129.9, and large number of methylene groups at δ_C 29.1–29.7 characteristic for the unsaturated fatty acid moiety. The geometry of the double bond was deduced to be *Z* from the coupling constant value (J = 6.0 Hz) (Vlahov, 2009). The attachment of the fatty acid to sterol moiety at C-3 was confirmed from HMBC cross-peak between H-3 and C-1′, as well as the downfield shift of H-3 (δ_H 4.59) (Fig. 2). Furthermore, the HMBC correlation correlations from H-2′ to C-4′ and H-5′ to C-3′ confirmed the position of the double bond between C-3′ and C-4′. This was further secured by the fragement ion peaks at *m*/*z* 99 (4%) and 86 (13%) in the GCMS of fatty acid methyl ester (FAME). Alkaline hydrolysis (Sayed et al., 2007) of **1** afforded β-sitosterol and FAME. β-Sitosterol was confirmed by co-TLC with authentic sample and the GCMS molecuar ion peak at *m*/*z* 414 (100%) $[M]^+$. The FAME was identified to be (3*Z*)-pentacosenoic acid methyl ester by the GCMS molecular ion peak at *m*/*z* 394 $[M]^+$ (100%). The fragment ion peaks at *m*/*z* 295 and 99 in the GCMS spectrum confirmed the position of double bond between C-3′ and C-4′ (Fig. 3). From the above mentioned data and by comparison with literature (Sayed et al., 2007; Bagri et al., 2009), **1** was deduced as β-sitosterol-3-*O*-(3*Z*)-pentacosenoate and considered a new natural product.

Compound **2** was isolated as white needles. It gave positive Salkowski's and Liebermann–Burchard's tests (Schmidt, 1964), suggesting its steroidal or triterpenoidal nature. Compound **2** possesses a molecular formula $C_{23}H_{36}O_5$ as confirmed from the HRESIMS molecular ion peak at *m*/*z* 392.2571. The ^{1}H NMR spectrum (Table 2) revealed characterisitic signals for two acetyls at δ_H 2.30 (3H, s, H-21) and 1.91 (3H, s, 3-OAc), two methyls at δ_H 0.99 (3H, s, H-19) and 0.84 (3H, s, H-18), downfield methines at δ_H 4.86 (1H, m, H-16), 4.31 (1H, m, H-17), 4.26 (1H, m, H-12), and 3.91 (1H, m, H-3). The ^{13}C NMR and HSQC spectra (Table 2) showed the presence of 23 carbon resonances including four methyls, three oxygenated methines at δ_C 73.1 (C-12), 72.8 (C-16), and 71.9 (C-3), five methines, seven methylenes, and four quaternary carbons two

Table 1
NMR data of compound **1** ($CDCl_3$, 400 and 100 MHz).

No.	δ_H [mult., J (Hz)]	δ_C[a] (mult.)	HMBC
1	2.10 m, 1.21 m	39.3 (CH_2)	–
2	1.90 m, 1.79 m	31.7 (CH_2)	–
3	4.59 m	73.6 (CH)	1′
4	2.30 dd (14.5, 7.2)	38.1 (CH_2)	3, 5, 6
5	–	139.7 (C)	–
6	5.34 m	122.5 (CH)	–
7	2.12 m, 2.16 m	31.9 (CH_2)	–
8	1.21 m	31.8 (CH)	–
9	1.11 m	50.0 (CH)	–
10	–	36.3 (C)	–
11	1.51 m	21.0 (CH_2)	–
12	2.00 m, 1.15 m	39.7 (CH_2)	–
13	–	42.3 (C)	–
14	1.20 m	56.7 (CH)	–
15	1.61 m	24.3 (CH_2)	–
16	1.62 m, 1.41 m	28.2 (CH_2)	–
17	1.09 m	56.0 (CH)	–
18	0.68 s	11.8 (CH_3)	13, 14
19	1.02 s	19.3 (CH_3)	5, 9, 10
20	1.42 m	37.0 (CH)	
21	0.95 d (6.2)	18.9 (CH_3)	–
22	1.11 m	36.6 (CH_2)	–
23	1.20 brs	26.3 (CH_2)	–
24	1.0 m	46.3 (CH)	–
25	1.02 m	29.5 (CH)	–
26	0.86 d (7.2)	19.3 (CH_3)	–
27	0.84 d (7.2)	19.6 (CH_3)	–
28	1.54 m, 1.68 m	25.0 (CH_2)	–
29	0.82 t (6.8)	12.3 (CH_3)	–
1′	–	173.2 (C)	–
2′	2.26 d (7.6)	34.7 (CH_2)	1′, 4′
3′	5.38 dt (7.6, 6.0)	129.9 (CH)	5′
4′	5.38 dt (7.5, 6.0)	122.5 (CH)	2′
5′	2.01 m	–	3′
16′–23′	1.22–1.38 m	29.1–29.7 (CH_2)	–
24′	1.49 brs	22.7 (CH_2)	–
25′	0.89 t (6.1)	14.1 (CH_3)	–

[a] Signals were assigned from multiplicity-edited HSQC.