SUPPLEMENTARY MATERIAL

New Cytotoxic Constituents from the Red Sea Soft Coral *Nephthea* sp.

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Abstract: *Nephthea* species are rich in sesquiterpenoids and steroids. The methylene chloride/methanol (1:1) extract of *Nephthea* sp. resulted in the isolation of a new steroid (**1**), as well as previously reported metabolites (**2-9**). Structures were elucidated by employing extensive NMR and HR-ESI-MS analyses. The total extract, fractions and isolated compounds showed differential cytotoxicity against breast cancer cells MCF-7 cell lines.

Keywords: *Nephthea* sp., Terpenes, Cytotoxicity, Breast cancer cells MCF-7

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Table S1. ^1H (600 MHz) and ^{13}C (150 MHz) NMR Data for **1** and **2**.

	1		2	
	^{13}C	^1H	^{13}C	^1H
1	38.0	1.30 m, 1.70 m	37.7	0.95 m, 1.75 m
2	30.1	1.45 m, 1.67 m	30.2	1.55 m, 1.80 m
3	76.0	2.95 m	76.0	3.02 m
4	38.3	1.28 m	38.3	1.65 m
5	51.9	0.74 m	51.9	0.96 m
6	20.4	1.44 m	18.8	1.47 m, 1.60 m
7	37.5	1.20 m, 1.80 m	39.4	1.70 m
8	71.5	----	72.7	---
9	56.1	0.87 m	56.7	0.80 m
10	36.2	----	36.2	---
11	18.5	1.56 m	18.3	1.55 m, 1.65 m
12	38.6	1.24 m, 1.80 m	41.4	2.0 m
13	46.5	----	43.2	---
14	61.0	1.38 m	59.9	1.42 m
15	20.4	0.86 m, 1.54 m	20.0	0.85 m, 1.58 m
16	28.5	1.40 m, 1.750 m	27.8	1.32 m, 1.90 m
17	57.0	1.12 m	57.1	0.98 m
18	62.1	3.54 d (11.7), 3.66 d (11.7)	13.0	0.96 s
19	12.6	0.98 s	12.9	0.98 s
20	40.0	1.92 m	33.0	2.0 m
21	19.9	1.00 d (6.2)	18.8	0.84 d (6.5)
22	135.4	5.08 dd (15.1, 8.3)	44.9	2.45 m
23	132.1	5.18 dd (15.1, 8.3)	203.3	---
24	42.9	1.80 m	155.9	---
25	33.0	1.45 m	27.8	2.90 m
26	18.8	0.80 d (6.2)	21.2	1.00 d (6.5)
27	19.1	0.81 d (6.8)	21.3	1.10 d (6.5)
28	16.8	0.90 d (6.8)	120.9	5.71 s, 5.98 s
29	14.4	0.93 d (6.2)	14.6	0.92 d (6.5)
CH₃-acetate	----	----	---	---
C=O acetate	----	-----	---	---

Table S2: Calculated IC₅₀ Values of Nephthea extract, fractions and pure compounds with human breast cancer cell lines MCF-7

Sample	IC ₅₀ * (µg/ml)
N1	142.4 (0.773)
N2	154.3 (0.997)
N3	155.9 (0.962)
N3-4A	146.9(0.772)
N3-8	339.2(0.982)
N4	37.0 (0.814)
N4-10-2	85.5 (0.898)
N5	155.8 (0.896)
N6	201.7 (0.986)
N6-12	113.6 (0.883)
N6-18-4	151.9 (0.823)
N6-19	124.3 (0.996)
N6-22	238.5 (0.925)
N6-26	56.6 (0. 895)
N6-30	84.3 (0.762)
N7	139.7 (0.941)

*IC₅₀ values shown with their corresponding goodness of the regression fit (r² value) given between parentheses.

Full assignment S1.

Assignment of known compounds (**2-9**) (^1H (600 MHz) and ^{13}C (150 MHz) NMR]

3.3.1 24-Methyl-cholesta-5,24(28)-diene-3 β ,7 β ,19-triol (3):

Amorphous powder, ^1H -NMR [CD_3OD] δ_{H} 5.54 (1H, s, H-6), 4.69, 4.63 (1H each, br s, H₂-28), 3.83, 3.57 (1H each, d, $J = 11.6$ Hz, H₂-19), 3.61 (1H, d, $J = 7.6$ Hz, H-7), 3.55 (1H, m, H-3), 1.02, 1.01 (6H, d, $J = 7.0$ Hz, Me-26, 27), 0.93 (3H, d, $J = 4.1$, Me-21), 0.74 (3H, s, Me-18),. ^{13}C -NMR [CD_3OD] δ_{C} 156.4 (C-24), 138.8 (C-5), 129.7 (CH, C-6), 105.9 (CH₂, C-28), 72.0 (CH, C-7), 70.7 (CH, C-3), 62.2 (CH₂, C-19), 57.2 (C-14), 55.6 (CH, C-17), 49.0 (CH, C-9), 43.0 (C-13), 41.5 (CH, C-8), 41.1 (C-10), 41.1 (CH₂, C-4), 40.1 (CH₂, C-12), 35.7 (CH₂, C-22), 34.8 (CH, C-20), 33.6 (CH, C-25), 33.1 (CH₂, C-1), 31.3 (CH, C-2), 30.9 (CH₂, C-23), 28.3 (CH₂, C-16) 25.9 (CH₂, C-15), 21.6 (CH₂, C-11), 21.2 (CH₃, C-27), 21.0 (CH₃, C-26), 18.1 (CH₃, C-21), 11.4 (CH₃, C-18) (Bortolotto et al. 1976).

3.3.2. 7 β -Acetoxy-24-methyl-cholesta-5,24(28)-diene-3 β ,19-diol (4):

Amorphous powder, ^1H NMR (CD_3OD): δ_{H} 5.39 (1H, br s, H-6), 4.91 (1H, d, $J = 8.2$ Hz, H-7), 4.70, 4.63 (1H each, br s, H₂-28), 3.83, 3.58 (1H each, d, $J = 11.6$ Hz, H₂-19), 3.45 (1H, m, H-3), 2.28, 2.17 (1H each, m, H-4), 1.96 (3H, s, OAc), 1.00 (3H, d, $J = 6.5$ Hz, CH₃-26), 1.00 (3H, d, $J = 6.5$ Hz, CH₃-27), 0.99 (3H, d, $J = 4.14$ Hz, CH₃-21), 0.75 (3H, s, CH₃-18). ^{13}C NMR(CD_3OD): δ_{C} 171.7 (C=O acetat), 156.4 (C-24), 141.6 (C-5), 124.4 (CH, C-6), 105.6 (CH₂, C-28), 75.5 (CH, C-7), 70.6 (CH, C-3), 62.0 (CH₂, C-19), 56.7 (CH, C-14), 55.5 (CH, C-17), 48.8 (CH, C-9), 43.0 (C-13), 41.4 (C-10), 41.2 (CH₂, C-4), 40.0 (CH₂, C-12), 37.6 (CH, C-8), 35.6 (CH₂, C-22), 34.7 (CH, C-20), 33.6 (CH, C-25), 33.1 (CH₂, C-1), 31.2 (CH₂, C-2), 30.8 (CH₂, C-23), 28.2 (CH₂, C-16), 24.9 (CH₂, C-15), 21.2 (CH₂, C-11), 21.1 (CH₃, C-27), 21.0 (CH₃, C-26), 20.3 (CH₃, CH₃-acetat), 18.0 (CH₃, C-21), 11.2 (CH₃, C-18) (Faheem et al. 2012).

3.3.3. 24-Methyl-cholesta-5,24(28)-diene-3 β -ol (5):

Amorphous powder, ^1H NMR (CDCl_3) δ_{H} 5.33 (1H, m, H-6), 4.69, 4.63 (1H each, s, H₂-28),

3.49 (1H, m, H-3), 1.01(3H, d, $J = 3.4$ Hz, H-27), 1.00 (3H, br s, H-19), 1.00 (3H, d, $J = 3.4$ Hz, H-26), 0.93 (3H, d, $J = 6.9$, H-21), 0.66 (3H, br s, H-18). ^{13}C NMR (CDCl_3) data: δ_{C} 156.9 (C-24), 140.8 (C-5), 121.7 (CH, C-6), 106.0 (CH_2 , C-28), 71.8 (CH, C-3), 56.8 (CH, C-14), 56.1 (CH, C-17), 50.2 (CH, C-9), 42.4 (C-13), 42.4 (CH_2 , C-4), 39.8 (CH_2 , C-12), 37.3 (CH_2 , C-1), 36.6 (C-10), 35.8 (CH_2 , C-22), 34.8 (CH, C-20), 33.9 (CH, C-25), 32.0 (CH, C-8), 31.9 (CH_2 , C-7), 31.7 (CH_2 , C-2), 31.0 (CH_2 , C-23), 28.3 (CH_2 , C-16), 24.3 (CH_2 , C-15), 22.0 (CH_3 , C-26), 21.9 (CH_3 , C-27), 21.1 (CH_2 , C-11), 19.4 (CH_3 , C-19), 18.8 (CH_3 , C-21), 11.9 (CH_3 , C-18) (Ahmed et al. 2006).

3.3.4. 4 α ,24R-Dimethyl-5 α -cholest-22-en-3 β -ol (6):

Amorphous powder, ^1H NMR (CDCl_3), δ_{H} 5.19 (1H, dd, $J = 15.1, 7.6$, H-23), 5.15 (1H, dd, $J = 15.1, 7.6$, H-22), 3.06 (1H, m, H-3), 0.98 (3H, d, $J = 3.4$ Hz, H-26), 0.93 (3H, d, $J = 6.2$, H-21), 0.91(3H, d, $J = 7.6$ Hz, H-27), 0.89 (3H, d, $J = 6.8$, H-28), 0.81 (3H, br s, H-19), 0.64 (3H, br s, H-18). ^{13}C NMR (CDCl_3) data: δ_{C} 135.9 (CH, C-22), 131.7 (CH, C-23), 76.7 (CH, C-3), 59.0 (CH, C-9), 57.0 (CH, C-17), 56.7 (CH, C-14), 51.0 (CH, C-5), 42.8 (CH, C-20), 42.4 (C-13), 40.0 (CH, C-4), 39.3 (CH_2 , C-12), 36.1 (C-10), 36.0 (CH_2 , C-1), 34.9 (CH, C-24), 33.1 (CH, C-25), 32.3 (CH_2 , C-7), 31.1 (CH, C-8), 31.1 (CH, C-2), 28.6 (CH_2 , C-16), 28.6 (CH_2 , C-11), 24.2 (CH_2 , C-15), 21.2 (CH_2 , C-6), 21.2 (CH_3 , C-26), 20.9 (CH_3 , C-27), 19.7 (CH_3 , C-19), 18.7 (CH_3 , C-21), 17.6 (CH_3 , C-28), 15.1 (CH_3 , C-29), 12.1 (CH_3 , C-18) (Kokke et al. 1981).

3.3.5. Alismoxide (7):

Colorless crystals, ^1H NMR (CDCl_3) δ_{H} 5.46 (1H, br s, H-6), 1.23, 1.17 (3H each, s, H-14,15), 0.97, 0.96 (3H each, d, $J = 5.5$ Hz, H-12, 13). ^{13}C NMR (CDCl_3): δ_{C} 149.6(C-7), 121.4 (CH, C-6), 80.3 (C-4), 75.3 (C-10), 50.8 (CH, C-5), 50.4 (CH, C-1), 42.7 (CH_2 , C-3), 40.5 (CH_2 , C-9), 37.5 (CH, C-11), 25.1 (CH_2 , C-8), 22.6 (CH_3 , 21.6 (CH_2 , C-2), C-15), 21.5 (CH_3 , C-13), 21.4 (CH_3 , C-12), 21.2 (CH_3 , C-14) (Jin et al., 2012).

3.3.6. 10-O-Methyl alismoxide(8):

Oil, ^1H NMR (200 MHz, CDCl_3) δ_{H} 5.45 (1H, br s, H-6), 3.16 (OCH_3); 1.18, 1.17 (3H each, s, Me-14, 15), 0.97, 0.96 (3H each, d, $J = 4.08$ Hz, Me-12, 13). ^{13}C NMR (CDCl_3): δ_{C} 149.7 (C-7), 121.3 (CH, C-6), 80.3(C-10), 79.2 (C-4), 50.2 (CH, C-5), 48.8 (OCH_3), 48.1 (CH, C-1), 40.6

(CH₂, C-9), 37.3 (CH₂, C-3), 35.6 (CH, C-11), 24.6 (CH₂, C-2), 22.5 (CH₃, C-15), 21.8 (CH₃, C-13), 21.7 (CH₂, C-8), 21.3 (CH₃, C-12), 18.0 (CH₃, C-14). (Jin et al. 2012).

3.3.7. Erythro-N-dodecanoyl-docosasphinga-(4E,8E)-dienine (9):

¹H NMR (600 MHz, CDCl₃): δ_H 5.69 (1H, dd, J = 15.6, 7.6 Hz; H-5), 5.66 (1H, dd, J = 15.6, 7.6 Hz; H-8), 5.46 (1H, dd, J = 15.6, 7.6 Hz; H-9), 5.44 (1H, dt, J = 15.6, 7.6 Hz; H-4), 5.40 (1H, br s, NH), 4.02 (1H, t, J = 7.6 Hz; H-3), 3.82 (1H, m, H-2), 3.65 (2H, dd, J = 11.7, 6.9 Hz; 2H-1), 2.18 (2H, t, J = 10.3 Hz; H-2') 2.05 (2H, m; 2H-6), 2.05 (2H, m; 2H-7), 1.95 (2H, m, 2H-10), 1.57 (4H, m; 2H-3' and 2H-of other position), 1.26 (-(CH₂)_n), 0.87 (6H, t, J_{vic} = 6.84 Hz; Me-22 and Me-16'). ¹³C NMR (100 MHz, CDCl₃): δ_C 174.0 (C-1'), 132.7 (CH, C-5), 130.7 (CH, C-8), 130.1 (CH, C-4), 129.3 (CH, C-9), 72.3 (CH, C-3), 60.9 (CH₂, C-1), 55.4 (CH, C-2), 36.0 (CH₂, C-2'), 32.3 (CH₂, C-19), 32.2 (CH₂, C-14), 32.0 (CH₂, C-10), 29.0–29.4 (all CH₂), 22.4 (CH₂, C-20), 22.3 (CH₂, C-15), 13.0 (2CH₃, C-16, C-21) (Bortolotto et al. 1976).

Figure S1. Selected ^1H - ^1H COSY (---) and HMBC (-->) correlations of **1**, **2**.

Figure S2. ^1H -NMR Spectrum of Compound **1**.

Figure S3. ^{13}C NMR Spectrum of Compound 1.

Figure S4. ^1H -NMR Spectrum of Compound **2**.

Figure S5. ^{13}C NMR Spectrum of Compound **2**.

Figure S6. ^1H -NMR Spectrum of Compound **3**.

Figure S7. ^{13}C NMR Spectrum of Compound **3**.

Figure S8. ^1H -NMR Spectrum of Compound 4.

Figure S9. ^{13}C NMR Spectrum of Compound 4.

Figure S10. ^1H -NMR Spectrum of Compound **5**.

Figure S11. ^{13}C NMR Spectrum of Compound **5**.

Figure S12. ^1H -NMR Spectrum of Compound **6**.

Figure S13. ^{13}C NMR Spectrum of Compound 6.

Figure S14. ^1H -NMR Spectrum of Compound **7**.

Figure S15. ^{13}C NMR Spectrum of Compound **7**.

Figure S16. X-ray spectrum of of Compound **7**.

Figure S17. ^1H -NMR Spectrum of Compound 8.

Figure S18. ^{13}C NMR Spectrum of Compound 8.

Figure S19. ^1H -NMR Spectrum of Compound **9**.

Figure S20. ^{13}C NMR Spectrum of Compound **9**.