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

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**Abstract:** *Nepthea* species are rich in sesquiterpenoids and steroids. The methylene chloride/methanol (1:1) extract of *Nepthea* 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:** *Nepthea* sp., Terpenes, Cytotoxicity, Breast cancer cells MCF-7
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Table S1. $^1$H (600 MHz) and $^{13}$C (150 MHz) NMR Data for 1 and 2.

|    | $^{13}$C | $^1$H  | $^{13}$C | $^1$H  |
|----|---------|--------|---------|--------|
| 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    | 165 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, 180 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) |
| CH$_3$-acetate | ---- | ---- | ---- | ---- |
| C=O | ---- | ---- | ---- | ---- |
| acetate | ---- | ---- | ---- | ---- |
Table S2: Calculated IC$_{50}$ 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)       |

*IC$_{50}$ values shown with their corresponding goodness of the regression fit ($r^2$ value) given between parentheses.
Assignment of known compounds (2-9) (\(^1\)H (600 MHz) and \(^{13}\)C (150 MHz) NMR)

### 3.3.1 24-Methyl-cholesta-5,24(28)-diene-3β,7β,19-triol (3):
Amorphous powder, \(^1\)H-NMR [CD\(_2\)OD] \(\delta_H\) 5.54 (1H, s, H-6), 4.69, 4.63 (1H each, br s, H\(_2\)-28), 3.83, 3.57 (1H each, d, \(J = 11.6\) Hz, H-2-19), 3.61 (1H, d, \(J = 7.6\) Hz, H-7), 3.55 (1H, m, H-3), 102, 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\(_2\)OD] \(\delta_C\) 156.4 (C-24), 138.8 (C-5), 129.7 (CH, C-6), 105.9 (CH\(_2\), C-28), 72.0 (CH, C-7), 70.7 (CH, C-3), 62.2 (CH\(_2\), 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 (CH\(_2\), C-4), 40.1 (CH\(_2\), C-12), 35.7 (CH\(_2\), C-22), 34.8 (CH, C-20), 33.6 (CH, C-25), 33.1 (CH\(_2\), C-1), 31.3 (CH, C-2), 30.9 (CH\(_2\), C-23), 28.3 (CH\(_2\), C-16) 25.9 (CH\(_2\), C-15), 21.6 (CH\(_2\), C-11), 21.2 (CH\(_3\), C-27), 21.0 (CH\(_3\), C-26), 18.1 (CH\(_3\), C-21), 11.4 (CH\(_3\), C-18) (Bortolotto et al. 1976).

### 3.3.2 7β-Acetoxy-24-methyl-cholesta-5,24(28)-diene-3β,19-diol (4):
Amorphous powder, \(^1\)H NMR (CD\(_3\)OD): \(\delta_H\) 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, H\(_2\)-28), 3.83, 3.58 (1H each, d, \(J = 11.6\) Hz, H-2-19), 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, CH\(_3\)-26), 1.00 (3H, d, \(J = 6.5\) Hz, CH\(_3\)-27), 0.99(3H, d, \(J = 4.14\) Hz, CH\(_3\)-21), 0.75 (3H, s, CH\(_3\)-18). \(^{13}\)C NMR(CD\(_3\)OD): \(\delta_C\) 171.7 (C=O acetat), 156.4 (C-24), 141.6 (C-5), 124.4 (CH, C-6), 105.6 (CH\(_2\), C-28), 75.5 (CH, C-7), 70.6 (CH, C-3), 62.0 (CH\(_2\), 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 (CH\(_2\), C-4), 40.0 (CH\(_2\), C-12), 37.6 (CH, C-8), 35.6 (CH\(_2\), C-22), 34.7 (CH, C-20), 33.6 (CH, C-25), 33.1 (CH\(_2\), C-1), 31.2 (CH\(_2\), C-2), 30.8 (CH\(_2\), C-23), 28.2 (CH\(_2\), C-16), 24.9 (CH\(_2\), C-15), 21.2 (CH\(_2\), C-11), 21.1 (CH\(_3\), C-27), 21.0 (CH\(_3\), C-26), 20.3 (CH\(_3\), CH\(_3\)-acetat), 18.0 (CH\(_3\), C-21), 11.2 (CH\(_3\), C-18) (Faheem et al. 2012).

### 3.3.3 24-Methyl-cholesta-5,24(28)-diene-3β-ol (5):
Amorphous powder, \(^1\)H NMR (CDCl\(_3\)) \(\delta_H\) 5.33 (1H, m, H-6), 4.69, 4.63 (1H each, s, H\(_2\)-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: \(\delta_c\) 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\(\alpha\),24\(\alpha\)-Dimethyl-5\(\alpha\)-cholest-22-en-3\(\beta\)-ol (6):
Amorphous powder, \(^1\)H NMR (CDCl\(_3\)), \(\delta_h\) 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: \(\delta_c\) 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, \(^1\)H NMR (CDCl\(_3\)), \(\delta_h\) 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\(_3\)): \(\delta_c\) 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, \(^1\)H NMR (200 MHz, CDCl\(_3\)) \(\delta_h\) 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 (CDCl3): \(\delta_c\) 149.7 (C-7), 121.3 (CH, C-6), 80.3(C-10), 79.2 (C-4), 50.2 (CH, C-5), 48.8 (OCH3), 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, Jvic = 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 $^1$H-$^1$H COSY (—) and HMBC (→) correlations of 1, 2.
Figure S2. $^1$H-NMR Spectrum of Compound 1.
Figure S3. $^{13}$C NMR Spectrum of Compound 1.
Figure S4. $^1$H-NMR Spectrum of Compound 2.
Figure S5. $^{13}$C NMR Spectrum of Compound 2.
Figure S6. $^1$H-NMR Spectrum of Compound 3.
Figure S7. $^{13}$C NMR Spectrum of Compound 3.
Figure S8. $^1$H-NMR Spectrum of Compound 4.
Figure S9. $^{13}$C NMR Spectrum of Compound 4.
Figure S10. $^1$H-NMR Spectrum of Compound 5.
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