Supporting Information

Pyrenosetins A–C, New Decalinoylspirotetramic Acid Derivatives Isolated by Bioactivity-Based Molecular Networking from the Seaweed-Derived Fungus *Pyrenochaetopsis* sp. FVE-001

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Table S1. In vitro anticancer activity (%) of Kupchan subextracts (KH, KC, KM) and SPE fractions against cancer cell lines (A-375, A-549, HT-29, HCT-116, MB-231) and non-cancerous HaCaT cell line.

|        | A-375 (100 µg/ml) | A-549 (100 µg/ml) | HT-29 (100 µg/ml) | HCT-116 (100 µg/ml) | MB-231 (100 µg/ml) | HaCaT (100 µg/ml) |
|--------|-------------------|-------------------|-------------------|---------------------|-------------------|--------------------|
| KH     | 31                | 0                 | 29                | 0                   | 0                 | 31                 |
| KC     | 98                | 99                | 99                | 76                  | 99                | 66                 |
| KM     | 0                 | 0                 | 0                 | 0                   | 0                 | 0                  |
| KC Fr.0| 0                 | 0                 | 0                 | 0                   | 0                 | 0                  |
| KC Fr.1| 0                 | 0                 | 0                 | 0                   | 0                 | 0                  |
| KC Fr.2| 0                 | 0                 | 0                 | 0                   | 0                 | 0                  |
| KC Fr.3| 0                 | 0                 | 0                 | 0                   | 0                 | 0                  |
| KC Fr.4| 0                 | 0                 | 0                 | 0                   | 0                 | 0                  |
| KC Fr.5| 85                | 54                | 99                | 0                   | 63                | 44                 |
| KC Fr.6| 99                | 99                | 99                | 99                  | 99                | 99                 |
| KC Fr.7| 99                | 99                | 99                | 99                  | 99                | 99                 |
| KC Fr.8| 43                | 0                 | 0                 | 0                   | 0                 | 0                  |
| KC Fr.9| 0                 | 0                 | 0                 | 0                   | 0                 | 0                  |
| KC Fr.10| 0               | 0                 | 0                 | 0                   | 0                 | 0                  |

Table S2. The Δδ(δS-δR) data for the S- and R-MTPA esters 6–9 in 1H NMR (CDCl3, 500 MHz).

|       | 6 (S) | 7 (R) | ΔδS-R | 8 (S) | 9 (R) | ΔδS-R |
|-------|-------|-------|-------|-------|-------|-------|
| C     | δH, mult (J in Hz) | δH, mult (J in Hz) | δH, mult (J in Hz) | δH, mult (J in Hz) | δH, mult (J in Hz) | δH, mult (J in Hz) |
| 13    | 3.39, m | 3.35, dd (11.5, 9.5) | 0.04 | 3.27, dd (11.3, 9.7) | 3.28, dd (11.4, 9.4) | -0.01 |
| 14    | 5.92, dd (15.5, 9.5) | 5.84, dd (15.4, 9.8) | 0.08 | 6.09, dd (14.8, 9.7) | 6.10, dd (15.3, 9.7) | -0.01 |
| 15    | 5.65, dd (15.5, 6.4) | 5.58, dd (15.4, 6.4) | 0.07 | 5.39, dd (14.9, 7.8) | 5.52, dd (15.3, 7.6) | -0.13 |
| 16    | 5.46, m | 5.45, m | 0.01 | 5.34, m | 5.38, m | -0.04 |
| 17    | 1.22, d (6.5) | 1.30, d (6.5) | -0.08 | 1.31, d (6.3) | 1.27, d (6.5) | 0.04 |
Table S3. The distance (Å) between protons H-5', H-15 and H-17 in the tetramic acid portion of the compounds 1-3. The red marking indicates the assigned relative stereochemistry based on measured the distances allowing observable NOE correlations (up to 4 Å) between relevant protons.

| Compd  | 2D Structure | 3D model | H-5' orientation | Distance H-5'/H-15 | NOE | Distance H-5'/H-17 | NOE |
|--------|--------------|----------|------------------|-------------------|-----|-------------------|-----|
| 1 (5'-β) | ![2D Structure](image) | ![3D model](image) | β                | 2.96 Å            | YES | 3.28 Å            | YES |
| 1 (5'-α) | ![2D Structure](image) | ![3D model](image) | α                | 4.83 Å            |     | 5.07 Å            |     |
| 2 (5'-β) | ![2D Structure](image) | ![3D model](image) | β                | 2.88 Å            |     | 3.33 Å            |     |
| 2 (5'-α) | ![2D Structure](image) | ![3D model](image) | α                | 5.14 Å            | NO  | 6.84 Å            | NO  |
| 3 (5'-β) | ![2D Structure](image) | ![3D model](image) | β                | 3.80 Å            |     | 5.11 Å            |     |
| 3 (5'-α) | ![2D Structure](image) | ![3D model](image) | α                | 5.41 Å            | NO  | 6.77 Å            | NO  |
Figure S1. $^1$H NMR spectrum of compound 1 (600 MHz, CDCl$_3$).

Figure S2. $^{13}$C NMR spectrum of compound 1 (150 MHz, CDCl$_3$).
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