Supporting Information

Spithioneines A and B, Two New Bohemamine Derivatives Possessing Ergothioneine Moiety from a Marine-derived *Streptomyces spinoverrucosus*

Peng Fu and John B. MacMillan*

Department of Biochemistry, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas 75390, United States

---

**List of Supporting Information**

- **Experimental details**
- **Figure S1.** CD spectra of bohemamine, bohemamine B and bohemamine C
- **Table S1.** $^1$H (600 MHz) and $^{13}$C (100 MHz) NMR data for compounds 1 and 2 in CD$_3$OD
- **Figure S2.** LC/MS profile (280 nm) of the reaction mixture of bohemamine and l-ergothioneine
- **Figure S3.** HRESIMS spectrum of spithioneine A (1)
- **Figure S4.** The $^1$H-NMR spectrum of spithioneine A (1) in DMSO-$d_6$
- **Figure S5.** The $^{13}$C-NMR spectrum of spithioneine A (1) in DMSO-$d_6$
- **Figure S6.** The HSQC spectrum of spithioneine A (1) in DMSO-$d_6$
- **Figure S7.** The $^1$H-$^1$H COSY spectrum of spithioneine A (1) in DMSO-$d_6$
- **Figure S8.** The HMBC spectrum of spithioneine A (1) in DMSO-$d_6$
- **Figure S9.** The $^1$H-NMR spectrum of spithioneine A (1) in CD$_3$OD
- **Figure S10.** The $^{13}$C-NMR spectrum of spithioneine A (1) in CD$_3$OD
- **Figure S11.** The HSQC spectrum of spithioneine A (1) in CD$_3$OD
- **Figure S12.** The $^1$H-$^1$H COSY spectrum of spithioneine A (1) in CD$_3$OD
- **Figure S13.** The HMBC spectrum of spithioneine A (1) in CD$_3$OD
- **Figure S14.** The NOESY spectrum of spithioneine A (1) in CD$_3$OD
- **Figure S15.** HRESIMS spectrum of spithioneine B (2)
- **Figure S16.** The $^1$H-NMR spectrum of spithioneine B (2) in DMSO-$d_6$
- **Figure S17.** The $^{13}$C-NMR spectrum of spithioneine B (2) in DMSO-$d_6$
- **Figure S18.** The HSQC spectrum of spithioneine B (2) in DMSO-$d_6$
- **Figure S19.** The $^1$H-$^1$H COSY spectrum of spithioneine B (2) in DMSO-$d_6$
- **Figure S20.** The HMBC spectrum of spithioneine B (2) in DMSO-$d_6$
Experimental details

**General Procedures.** Specific rotations were recorded with an AUTOPOL AP IV-6W polarimeter equipped with a halogen lamp (589 nm). UV spectra were recorded on a Shimadzu UV-1601 UV–VIS spectrophotometer. CD spectra were measured on JASCO J-815 spectrometer. 1H and 2D NMR data were recorded at 600 MHz in CD3OD and DMSO-d6 solution on Varian System spectrometer, and chemical shifts were referenced to the corresponding residual solvent signal (δH 3.31/δC 49.00 for CD3OD, and δH 2.50/δC 39.52 for DMSO-d6). 13C NMR spectra were acquired at 100 MHz on a Varian System spectrometer. High resolution ESI-TOF mass spectra were provided by The Scripps Research Institute, La Jolla, CA. Low-resolution LC/ESI-MS data were measured using an Agilent 1200 series LC/MS system with a reversed-phase C18 column (Phenomenex Luna, 150 mm × 4.6 mm, 5 μm) at a flow rate of 0.7 mL/min. Preparative HPLC was performed on an Agilent 1200 series instrument with a DAD detector, using a reversed-phase C18 column (Phenomenex Luna, 250 × 10.0 mm, 5 μm). Sephadex LH-20 (GE Healthcare, Sweden) and ODS (50 mm, Merck) were used for column chromatography. Artificial seawater was used in microbial fermentations as described in a previous reference.51

**Collection and phylogenetic analysis of strain SNB-048.** *Streptomyces* sp. strain SNB-048 was isolated from a sediment sample collected from a Bahamian tidal flat. The sediment was desiccated and stamped (sediment grains transferred) onto agar plates using gauze 1 acidic media (10 g starch, 1 g NaNO3, 0.5 g KH2PO4, 0.5 g MgSO4, 0.5 g NaCl, 0.01 g FeSO4, 1 L seawater, 15 g agar, adjust pH to 5.3 with phosphate buffer). Bacterial colonies were selected and streaked to purity using the same agar media. Analysis of the strain by 16S rRNA revealed 99.9% identity to *Streptomyces spinoverrucosus*. The sequence is deposited in GenBank under accession no. KR091963.

**Cultivation and extraction.** Bacterium SNB-048 was cultured in 20 × 2.8 L Fernbach flasks each containing 1 L of a seawater-based medium (10 g starch, 4 g yeast extract, 2 g peptone, 1 g CaCO3, 40 mg Fe2(SO4)3·4H2O, 100 mg KBr) and shaken at 200 rpm at 27 °C. After seven days of cultivation, sterilized XAD-7-HP resin (20 g/L) was added to adsorb the organic products, and the culture and resin were shaken at 200 rpm for 2 h. The resin was filtered through cheesecloth, washed with deionized water, and eluted with acetone. The acetone-soluble fraction was dried *in vacuo* to yield 1.2 g of extract.

**Purification.** The extract (1.2 g) was fractionated by flash column chromatography on ODS (50 μm, 30 g), eluting with a step gradient of MeOH and H2O (10:90–100:0), and 12 fractions (Fr.1–Fr.12) were collected. Fractions 7 and 8 (403.7 mg) were combined and separated into six fractions (Fr.7.1–Fr.7.6) on Sephadex LH-20, eluting with MeOH. Fr.7.2 (24.7 mg) was purified by reversed-phase HPLC (Phenomenex Luna, C18, 250 × 10.0 mm, 5 μm, 2.5
mL/min) using a gradient solvent system from 20% to 50% CH$_3$CN (0.1% formic acid) over 10 min to afford compounds 1 (13.6 mg, $t_R = 6.9$ min) and 2 (1.1 mg, $t_R = 8.1$ min). Fr.7.3 (151.2 mg) was further separated into 16 fractions (Fr.7.3.1–Fr.7.3.16) by flash column chromatography on ODS (50 µm, 30 g), eluting with a step gradient of MeOH and H$_2$O (10:90–100:0). Fr.7.3.7 (37.5 mg) was purified by reversed-phase HPLC (Phenomenex Luna, C$_{18}$, 250 × 10.0 mm, 5 µm, 2.5 mL/min) using a gradient solvent system from 20% to 80% CH$_3$CN (0.1% formic acid) over 20 min to afford two known compounds, bohemamine B (10.3 mg, $t_R = 11.2$ min) and bohemamine (15.8 mg, $t_R = 12.4$ min).

Figure S1. CD spectra of bohemamine, bohemamine B and bohemamine C.

Table S1. $^1$H (600 MHz) and $^{13}$C (100 MHz) NMR data for compounds 1 and 2 in CD$_3$OD
| no. | \( \delta_C \)   | \( \delta_H \), mult. \( (J \text{ in Hz}) \) | \( \delta_C \)   | \( \delta_H \), mult. \( (J \text{ in Hz}) \) |
|-----|-----------------|-----------------|-----------------|-----------------|
| 1   | 204.8, C        |                 | 204.0, C        |                 |
| 2   | ND\(^a\)        | ND              | ND              | 5.69, s         |
| 3   | 169.6, C        |                 | 169.2, C        |                 |
| 4   | 62.4, CH 3.91, qd (6.7, 3.4) | 56.0, CH 4.54, qd (6.7, 2.0) |                 |                 |
| 5   | 65.3, CH 3.68, dd (3.4, 2.0) | 82.9, CH 4.03, brs |                 |                 |
| 6   | 78.8, CH 4.03, d (2.0) | 74.8, CH 3.86, brs |                 |                 |
| 7   | 80.1, C         |                 | 80.5, C         |                 |
| 8   | 24.7, CH3 1.56, s | 23.7, CH3 1.39, s |                 |                 |
| 9   | 19.2, CH3 1.44, d (6.7) | 20.2, CH3 1.49, d (6.7) |                 |                 |
| 1'  | 165.8, C        |                 | 165.7, C        |                 |
| 2'  | 118.2, CH 5.93, m | 118.1, CH 5.94, m |                 |                 |
| 3'  | 159.9, C        |                 | 160.2, C        |                 |
| 4'  | 27.8, CH3 1.96, d (1.3) | 27.8, CH3 1.97, d (1.3) |                 |                 |
| 5'  | 20.6, CH3 2.21, d (1.3) | 20.6, CH3 2.23, d (1.2) |                 |                 |
| 1'' | 138.7, C        |                 | 145.4, C        |                 |
| 2'' | 121.0, CH 7.05, s | ND              | 7.28, s         |                 |
| 3'' | 136.6, C        |                 | 135.8, C        |                 |
| 4'' | 27.0, CH2 3.23, m | 27.3, CH2 3.30, overlap |                 |                 |
| 5'' | 80.0, CH 3.89, dd (9.2, 5.4) | 79.8, CH 3.95, dd (8.6, 5.9) |                 |                 |
| 6'' | 170.8, C        |                 | 170.8, C        |                 |
| 5''-NCH\(_3\) | 52.9, CH3 3.28, s | 52.9, CH3 3.29, s |                 |                 |

\(^a\) ND: not detected.
Reduction of 1 with Raney-Nickel.

A mixture of Raney-Nickel (0.2 mL of suspension) and compound 1 (2.5 mg) in EtOH (0.5 mL) and water (0.5 mL) was stirred at room temperature. After 2 h, the mixture was filtered through Celite and the solvent was evaporated. The crude product was purified by preparative HPLC (Phenomenex Luna, C18, 250 × 10.0 mm, 5 µm, 2.5 mL/min) using a gradient solvent system from 20% to 70% CH3CN (0.1% formic acid) over 15 min to give hercynine (0.7 mg, tR = 3.3 min) and bohemamine C (0.8 mg, tR = 10.9 min).

Sulfoxidation of 1 with Oxone.

Compound 1 (2.0 mg) was dissolved into 0.5 mL of THF and 0.1 mL of water. The mixture was cooled in an ice bath, and 25 µL of aqueous solution of potassium peroxomonosulfate (Oxone) (0.1 g/mL) was added dropwise. The resulting mixture was stirred at 0 °C for 2.5 h. After reaction completion, the solution was allowed to warm to room temperature and 0.2 mL of aqueous solution of NaHCO3 (pH 8) was added. The reaction product was purified by HPLC (Phenomenex Luna, C18, 250 × 10.0 mm, 5 µm, 2.5 mL/min) using a gradient solvent system from 20% to 50% CH3CN (0.1% formic acid) over 10 min to give the semisynthetic product 2 (1.8 mg, tR = 8.1 min). LC/MS and 1H NMR analysis confirmed the identity of this semisynthetic product and spithioneine B (2).

Synthesis of 1 from bohemamine and L-ergothioneine.

A mixture of bohemamine (1.0 mg), L-ergothioneine (2.0 mg), and Na2CO3 (20 mg) in water (0.6 mL) was stirred at room temperature for 48 h. The reaction mixture was analyzed by LC-MS (Phenomenex Luna, C18, 150 mm × 4.6 mm, 5 µm, 0.7 mL/min) using a gradient solvent system from 10% to 99% CH3CN (0.1% formic acid) over 17 min. In this reaction, compound 1 was produced (Figure S2).
Antibiotic assays. The antibiotic activities against *Pseudomonas aeruginosa* and *Bacillus subtilis* were evaluated by an agar dilution method. The tested strains were cultivated in LB agar plates at 37 °C. Compounds 1 and 2, and positive control (erythromycin) were dissolved in MeOH at different concentrations from 100 to 0.1 µg/mL by the continuous 10-fold dilution methods. A 10 µL quantity of test solution was absorbed by a paper disk (5 mm diameter) and placed on the assay plates. After 24 h incubation, zones of inhibition (mm in diameter) were recorded.

Cytotoxicity assays. Cell lines were cultivated in 10 cm dishes (Corning, Inc.) in NSCLC cell-culture medium: RPMI/L-glutamine medium (Invitrogen, Inc.), 1000 U/mL penicillin (Invitrogen, Inc.), 1 mg/mL streptomycin (Invitrogen, Inc.), and 5% fetal bovine serum (Atlanta Biologicals, Inc.). Cell lines were grown in a humidified environment in the presence of 5% CO₂ at 37 °C. For cell viability assays, HCC366, A549, HCC44 and HCC1171 cells (60 µL) were plated individually at a density of 1200, 750 and 500 cells/well, respectively, in 384-well microtiter assay plates (Bio-one; Greiner, Inc.). After incubating the assay plates overnight under the growth conditions described above, purified compounds were dissolved and diluted in DMSO and subsequently added to each plate with final compound concentrations ranging from 50 µM to 1 nM and a final DMSO concentration of 0.5%. After an incubation of 96 h under growth conditions, Cell Titer Glo reagent (Promega, Inc.) was added to each well (10 mL of a 1:2 dilution in NSCLC culture medium) and mixed. Plates were incubated for 10 min at room temperature, and luminescence was determined for each well using an Envision multimodal plate reader (Perkin-Elmer, Inc.). Relative luminescence units were normalized to the untreated control wells (cells plus DMSO only). Data were analyzed using the Assay Analyzer and Condoseo modules of the Screener Software Suite (GeneData, Inc.) as described previously.²

(S1) Keller, M. D.; Selvin, R. C.; Claus, W.; Guillard, R. R. L. J. Phycol. 1987, 23, 633–638.

(S2) Kim, H. S.; Mendiratta, S.; Kim, J.; Pecot, C. V.; Larsen, J. E.; Zubovych, I.; Seo, B. Y.; Kim, J.; Eskiocak, B.; Chung, H.; McMillan, E.; Wu, S.; De Brabander J; Komurov, K.; Toombs, J. E.; Wei, S.; Peyton, M.; Williams, N.; Gazdar, A. F.; Posner, B. A.; Brekken, R. A.; Sood, A. K.; Deberardinis, R. J.; Roth, M. G.; Minna, J. D.; White, M. A. Cell 2013, 155, 552–566.
Figure S3. HRESIMS spectrum of spithioneine A (1)

Polarity/Scan Type: Positive
Sample Name: U6JM8181
Acq. File: 040215014.wff
Acq. Date: Thursday, April 02, 

MH⁺
492.2274

246.6180

493.2297

514.2087
Figure S4. The $^1$H-NMR spectrum of spithioneine A (1) in DMSO-$d_6$
Figure S5. The $^{13}$C-NMR spectrum of spithioneine A (1) in DMSO-$d_6$
Figure S6. The HSQC spectrum of spithioneine A (1) in DMSO-$d_6$
Figure S7. The $^1$H-$^1$H COSY spectrum of spithioneine A (1) in DMSO-$d_6$. 
Figure S8. The HMBC spectrum of spithioneine A (1) in DMSO-$d_6$. 
Figure S9. The $^1$H-NMR spectrum of spithioneine A (1) in CD$_3$OD
Figure S10. The $^{13}$C-NMR spectrum of spithioneine A (1) in CD$_3$OD.
Figure S11. The HSQC spectrum of spithioneine A (1) in CD$_3$OD
Figure S12. The $^1$H-$^1$H COSY spectrum of spithioneine A (1) in CD$_3$OD
Figure S13. The HMBC spectrum of spithioneine A (1) in CD$_3$OD
Figure S14. The NOESY spectrum of spithioneine A (1) in CD$_3$OD
Figure S15. HRESIMS spectrum of spithioneine B (2)

Polarity/Scan Type: Positive

Sample Name: U6JM8183

Acq. File: 040215016.wiff
Acq. Date: Thursday, April 02, 2020
Max. 3.7e5 counts

+TOF MS: 0.140 min from 040215016.wiff Agilent

MH+ 508.2224
MNa+ 530.2046
Figure S16. The $^1$H-NMR spectrum of spithione B (2) in DMSO-$d_6$. 

![NMR spectrum of spithione B (2) in DMSO-$d_6$]
Figure S17. The $^{13}$C-NMR spectrum of spithioneine B (2) in DMSO-$d_6$
Figure S18. The HSQC spectrum of spithioneine B (2) in DMSO-$d_6$.
Figure S19. The $^1$H-$^1$H COSY spectrum of spithioneine B (2) in DMSO-$d_6$
Figure S20. The HMBC spectrum of spithioneine B (2) in DMSO-$d_6$
Figure S21. The $^1$H-NMR spectrum of spithione B (2) in CD$_3$OD
Figure S22. The $^{13}$C-NMR spectrum of spithioneine B (2) in CD$_3$OD
Figure S23. The HSQC spectrum of spithioneine B (2) in CD$_3$OD
Figure S24. The $^1$H-$^1$H COSY spectrum of spithioneine B (2) in CD$_3$OD
Figure S25. The HMBC spectrum of spithioneine B (2) in CD$_3$OD
Figure S26. The NOESY spectrum of spithioneine B (2) in CD$_3$OD