Chaetocochins A−C, Epipolythiodioxopiperazines from *Chaetomium cochliodes*

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Three new epipolythiodioxopiperazines, chaetocochins A (**1**), B (**2**), and C (**3**), along with dethio-tetra (methylthio) chetomin (**4**) and chetomin (**5**), were isolated from the ethyl acetate extract of the solid-state fermented rice culture of the fungus *Chaetomium cochliodes*. Their structures were elucidated on the basis of spectroscopic analysis. Compounds **1**, **3**, and **4** exhibited significant cytotoxicity in vitro against cancer cell lines Bre-04, Lu-04, and N-04.

Epipolythiodioxopiperazines are widely found as mold secondary metabolites with bioactivities including antitumor, antimicrobial, antinematodal, and cytotoxicity effects.[1−6] Several species of fungi belonging to the genus *Chaetomium* produce compounds of this class, such as chetomin and chaetozin.[7,8] In this study, three new epipolythiodioxopiperazines, chaetocochins A (**1**), B (**2**), and C (**3**), were isolated, along with dethio-tetra (methylthio) chetomin (**4**) and chetomin (**5**), from the solid-state fermented rice culture of the fungus *Chaetomium cochliodes*. We herein report the isolation and structure elucidation of compounds **1**−**3** and cytotoxic activities of compounds **1**, **3**, and **4**.

Compound **1** was isolated as a colorless, amorphous powder. Its molecular formula, $C_{36}H_{42}N_6O_6S_4$, was provided by the quasi-molecular ion peak at *m*/*z* 805.1947 $[M + Na]^+$ (calcd 805.1941) in the HRESIMS. The ^{13}C NMR spectrum showed 36 carbon signals (Table 1). From the ^{13}C NMR signals at δ 167.7, 166.5, 165.6, and 165.0 and the IR peaks at ν_{max}1659 cm^{-1}, four amide carbonyls were postulated. Four S-methyls and three N-methyls were recognized from the NMR data. The IR spectrum indicated the presence of a hydroxyl group (3434 cm^{-1}). The evidence mentioned above, together with UV absorptions at λ_{max} 205, 220, 287, and 297 nm, indicated that compound **1** should be an analogue of dethio-tetra (methylthio) chetomin (**4**). However, the molecule of **1** ($C_{36}H_{42}N_6O_6S_4$) contained one more carbon atom than that of **4** ($C_{35}H_{42}N_6O_6S_4$). This was confirmed by the NMR spectra of **1**, in which the signal of H-6 was absent, but signals for the methylene (C-1″) at δ_H 4.95 and 5.28 and at δ_C 82.6 were observed. The connection of this methylene with N-6 and O-3′ (3′-CH_2O−) was determined by the HMBC correlations of H-5/C-1″, H-1″/C-6a and C-3′a, and H-3′a/C-3′ and C-4′ (Figure 1). The relative stereochemistry of compound **1** was determined by a NOESY experiment (Figure 2).

Compound **2** was isolated as a colorless, amorphous powder. The quasi-molecular ion peak at *m*/*z* 775.1453 $[M + Na]^+$ in the HRESIMS provided the molecular formula of $C_{34}H_{36}N_6O_6S_4$ for **2**. The NMR spectra of compound **2** were very similar to those of **1**. However, **2** had two methyls less than **1**, in accordance with the formulas of **1** and **2**. Thus, a S−S bridge at C-3 and C-11a was determined after the two S-methyls were located at C-3′ and C-6′ by the HSQC and HMBC experiments (Figure 1). The relative stereochemistry was determined from the NOESY correlations (Figure 2).

Compound **3** was obtained as a colorless, amorphous powder. The quasi-molecular ion peak at *m*/*z* 763.1497 $[M + Na]^+$ (calcd 763.1471) in the HRESIMS gave the molecular formula $C_{33}H_{36}N_6O_6S_4$. The IR, UV, and NMR data and formula suggested that **3** was an analogue of chetomin (**5**), but **3** contained two methyls resonating at δ_H 2.12 and 2.30 and δ_C 13.4 and 14.3 (Table 1) rather than a S−S bridge at C-3′ and C-6′ as in **5** from the HMBC correlations of SCH_3-3′ (δ 2.12)/C-3′ (δ 71.4) and SCH_3-6′ (δ 2.30)/C-6′ (δ 72.8). The structure of compound **3** was finally elucidated by detailed analysis of the HSQC and HMQC spectra (Figure 1). The stereochemistry was determined by comparing the NMR data of **3** and **5** and confirmed by the NOESY correlations (Figure 2).

Compounds **1** and **2** possessing a 14-memebered ring represent a novel type of epipolythiodioxopiperazine. Compounds **1**, **3**, and **4** were evaluated for their cytotoxicity in vitro against cancer cell

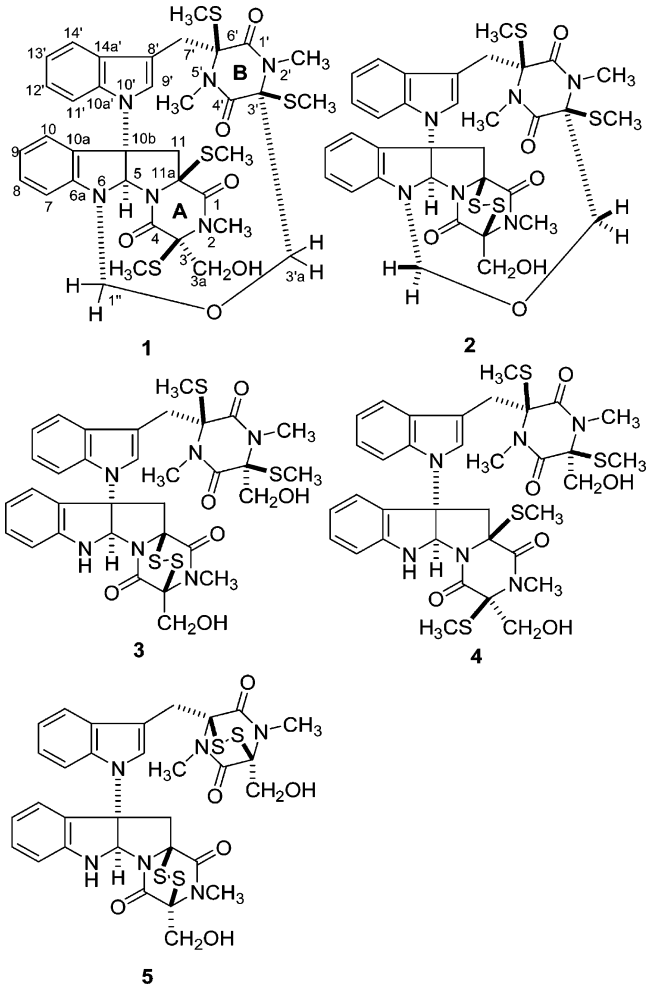

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Table 1. NMR Data of Compounds **1** (in C_5D_5N) and **2** and **3** (in $CDCl_3$)[a]

	1 (mult., J = Hz)		**2** (mult., J = Hz)		**3** (mult., J = Hz)	
no.	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		167.7		165.7		165.7
2-N*CH*$_3$	3.40 (3H, s)	29.8	3.23 (3H, s)	27.6	3.17 (3H, s)	30.5
3		74.6		76.2		76.2
3-S*CH*$_3$	2.28 (3H, s)	14.2				
3a-*CH*$_2$OH	4.23, 4.83 (each 1H, d, 11.2)	65.3	4.24 (1H, dd, 12.6, 5.5) 4.35 (1H, dd, 12.6, 9.0)	60.6	4.25 (1H, dd, 12.5, 8.9) 4.35 (1H, dd, 12.5, 5.1)	60.6
4		165.6		162.5		163.2
5	6.22 (1H, s)	88.9	5.62 (1H, s)	86.5	6.13 (1H, s) 5.31 (1H, s)	80.3
6						
6a		151.4		148.6		148.5
7	7.14 (1H, d, 7.6)	115.0	7.00 (1H, d, 7.8)	114.3	6.80 (1H, d, 7.6)	111.3
8	7.43 (1H, t, 7.6)	131.9	7.47 (1H, t, 7.8)	131.4	7.32 (1H, t, 7.6)	131.4
9	7.04 (1H, t, 7.6)	123.7	7.25 (1H, t, 7.8)	124.1	7.04 (1H, t, 7.6)	120.8
10	7.63 (1H, d, 7.6)	125.9	7.48 (1H, d, 7.8)	125.6	7.41(1H, d, 7.6)	126.0
10a		134.5		132.3		126.8
10b		73.5		73.1		73.8
11	3.67, 4.21 (each 1H, d, 15.0)	43.7	3.04, 4.79 (each 1H, d, 15.8)	41.0	3.02, 4.40 (each 1H, d, 15.7)	42.4
11a		70.4		73.7		73.6
11a-S*CH*$_3$	2.12 (3H, s)	16.5				
1′		166.5		165.5		165.9
2′-N*CH*$_3$	2.87 (3H, s)	30.5	2.62 (3H, s)	29.0	2.75 (3H, s)	29.2
3′		70.8		70.0		71.4
3′-S*CH*$_3$	2.27 (3H, s)	14.8	2.16 (3H, s)	13.7	2.12 (3H, s)	13.4
3′a-*CH*$_2$O-	3.61, 4.26 (each 1H, d, 9.2)	69.8	3.32, 4.01 (each 1H, d, 9.1)	68.7	3.17 (over lapped) 3.83 (1H, dd, 11.3, 7.9)	63.9
4′		165.0		163.7		165.4
5′-N*CH*$_3$	3.22 (3H, s)	32.0	3.06 (3H, s)	31.3	3.18 (3H, s)	29.2
6′		73.6		71.8		72.8
6′-S*CH*$_3$	2.39 (3H, s)	15.6	2.39 (3H, s)	14.9	2.30 (3H, s)	14.3
7′	3.50, 4.18 (each 1H, d, 15.1)	33.3	3.24, 3.94 (each 1H, d, 15.1)	31.5	3.16, 3.87 (each 1H, d, 14.9)	32.5
8′		109.4		107.6		107.4
9′	7.06 (1H, s)	127.9	6.42 (1H, s)	127.1	6.88 (1H,s)	126.2
10a′		135.6		134.0		133.9
11′	7.84 (1H, d, 7.6)	113.3	7.26 (1H, d, 7.3)	111.1	7.08 (1H, d, 7.8)	111.3
12′	7.06 (1H, t, 7.6)	124.0	7.22 (1H, t, 7.3)	122.7	7.14 (1H, t, 7.8)	122.7
13′	7.01 (1H, t, 7.6)	120.9	7.17 (1H, t, 7.3)	120.4	7.13 (1H, t, 7.8)	120.4
14′	7.67 (1H, d, 7.6)	120.6	7.54 (1H, d, 7.3)	119.4	7.63 (1H, d, 7.8)	120.0
14a′		131.7		130.4		129.9
1″	4.95, 5.28 (each 1H, d, 9.2)	82.6	4.69, 4.78 (each 1H, d, 10.1)	82.0		

[a] Assignments are based on HSQC and HMBC experiments.

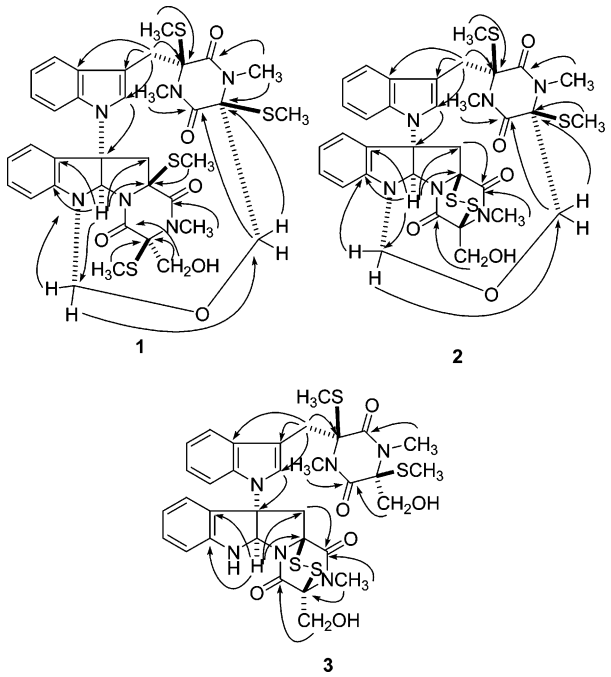

Figure 1. Key HMBC correlations of compounds **1**−**3**.

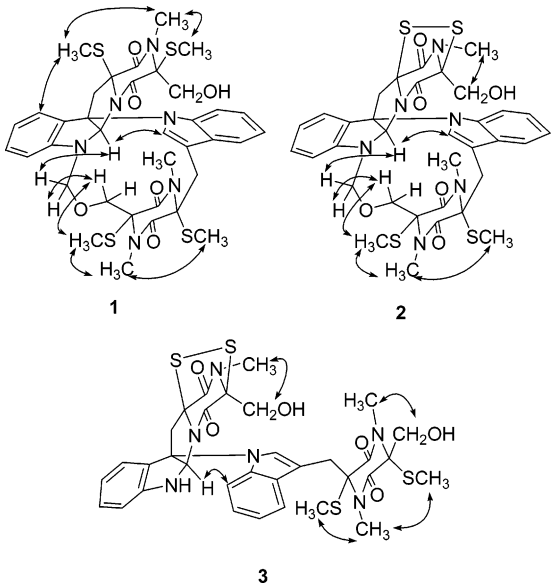

Figure 2. Selected NOESY correlations of **1**−**3**.

These compounds appear to show positive correlations with the number of S-methyls present.

lines Bre-04 (MDA-MB-231), Lu-04 (NCI-H460), and N-04 (SF-268) (Table 2). Compound **4** showed the strongest cytotoxicity.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. CD spectra were measured

Table 2. GI_{50} Values (μg/mL) of Compounds **1**, **3**, and **4**

compound	Bre-04	Lu-04	N-04
1	4.1	3.4	7.0
3	0.4	1.9	0.4
4	0. 06	0.05	0.2
Taxol	0.04	0.03	0.04

on a Jasco Model J-500C spectropolarimeter. UV spectra and IR spectra were carried out on a Perkin-Elmer Lambda 35 UV/vis spectrometer and a Perkin-Elmer Spectrum One FT-IR spectrometer, respectively. NMR spectra were recorded on a Bruker Avance 600 spectrometer. Electrospray ionization mass spectra (ESIMS) and high-resolution electrospray ionization mass spectra (HRESIMS) were obtained on a BioTOF-Q mass spectrometer. HPLC isolation was performed using a Perkin-Elmer Series 200 LC pump and a Perkin-Elmer Series 200 UV/vis detector. A Kromasil 100-10 C_{18} column (10 mm × 250 mm, 5 μm) was used for semipreparative HPLC with MeOH−H_2O as solvent at a flow rate of 3 mL/min. Silica gel (200−300 mesh) for column chromatography (CC) and silica gel GF254 (10−40 μm) for TLC were obtained from Qingdao Haiyang Chemical Company, China. All solvents including petroleum ether (60−90 °C) were distilled prior to use.

Microorganism and Fermentation. *Chaetomium cochliodes* (C. 3.198) was isolated from soil collected in Sichuan Province, China, and identified at the Institute of Microbiology, the Chinese Academy of Sciences (CAS). It was maintained on a potato dextrose agar slant (PDA) at 4 °C and was stocked in the Chengdu Institute of Biology (CAS). The seed culture medium was comprised of dextrose (20 g/L), yeast extract (1 g/L), KH_2PO_4 (3 g/L), $MgSO_4{\cdot}7H_2O$ (1.5 g/L), and potato extract prepared by extracting 200 g of potato with 1 L of boiling water for 20 min. The pH of the medium was adjusted to 6.0 with 1 mol/L NaOH(aq). The solid culture medium was comprised of rice and 0.3% peptone. The sterilization was carried out at 121 °C under 15 psi for 30 min.

The fresh mycelium grown on a PDA slant at 28 °C for 3 days was inoculated into 500 mL flasks containing 100 mL of sterilized seed medium. Flasks with inoculated medium were placed in a rotary shaker at 28 °C and incubated at 180 rpm for 2 days. The seed culture was inoculated into sterilized solid medium for further fermentation at 28 °C for 25 days.

Extraction and Isolation. The fermented solid medium (10 kg) was soaked with ethyl acetate (10 L × 3, 2 days for each time) at room temperature. The solvent was evaporated under reduced pressure to afford a residue (25 g). The residue was separated on a silica gel column (600 g, ϕ 150 mm × 330 mm) eluted with petroleum ether−acetone (5:1, 2:1, 1:1, 0:1, each 4000 mL) to yield four fractions: A (13.0 g), B (3.2 g), C (1.0 g), and D (4.5 g). Fraction B was separated over a silica gel column (60 g, 22 mm × 300 mm) eluted with $CHCl_3$−acetone (25, 15:1, 10:1, 5:1 each 500 mL) to afford fractions BA (630 mg), BB (80 mg), BC (110 mg), BD (240 mg), and BE (750 mg). Fraction BB was separated by semipreparative HPLC (70%, MeOH−H_2O) several times to yield **2** (12 mg) and **5** (chetomin, 35 mg). Separation of fraction BC by semipreparative HPLC (65%, MeOH−H_2O) yielded **1** (32 mg). Compound **3** (55 mg) was obtained by separating fraction BD over a silica gel column (17 g, ϕ 15 mm × 200 mm) eluted with petroleum ether−acetone (3:1, 400 mL). Fraction BE was subjected to a silica column (35 g, ϕ 20 mm × 240 mm) eluted with $CHCl_3$−MeOH (30:1, 930 mL) to afford **4** (dethio-tetra (methylthio) chetomin, 150 mg).

Bioassay. Cancer cell lines Bre-04 (MDA-MB-231), Lu-04 (NCI-H460), and N-04 (SF-268) were obtained from the American Type Culture Collection (ATCC) and cultured according to the supplier's instruction. The cells were seeded into 96-well plates, incubated for 16 h at 37 °C, and treated with compounds **1**, **3**, and **4** at different concentrations for 48 h. Taxol was used as a positive control. The cytotoxic activities were examined by means of a colorimetric chemosentivity assay with SRB (sulforodhamine B). The GI_{50} value (the drug concentration required to inhibit the cell growth by 50%) was used as a parameter for cytotoxicity.[9,10]

Chaetocochin A (1): colorless, amorphous powder; $[\alpha]_D^{20}$ +121.3, $[\alpha]_{365}^{20}$ +690.0, $[\alpha]_{436}^{20}$ +300.3, $[\alpha]_{546}^{20}$ +147.5, $[\alpha]_{578}^{20}$ +127.5 (*c* 0.10, MeOH); CD (*c* 0.10, CH_3CH_2OH) λ_{max} nm ($\Delta\epsilon$) 229 (−11.9), 260 (+1.3), 302 (+2.4); UV (MeOH) λ_{max} nm (log ϵ) 205 (3.64), 220 (3.49), 287 (2.78), 297 (2.77); IR (KBr) ν_{max} 3434, 2922, 2847, 1659, 1602, 1486, 1382, 745 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; (+)-HRESIMS (positive mode) *m*/*z* 805.1947 $[M + Na]^+$ (calcd for $C_{36}H_{42}N_6O_6S_4Na$, 805.1941).

Chaetocochin B (2): colorless, amorphous powder; $[\alpha]_D^{20}$ +204.0, $[\alpha]_{365}^{20}$ +960.0, $[\alpha]_{436}^{20}$ +453.0, $[\alpha]_{546}^{20}$ +238.0, $[\alpha]_{578}^{20}$ +162.0 (*c* 0.10, $CHCl_3$); CD (*c* 0.11, CH_3CH_2OH) λ_{max} nm ($\Delta\epsilon$) 240 (+10.4), 266 (−2.5), 300 (+4.4); UV (MeOH) λ_{max} nm (log ϵ) 205 (3.64), 220 (3.53), 287 (2.82), 294 (2.83) nm; IR (KBr) ν_{max} 3434, 2923, 1691, 1652, 1606, 1456, 1379, 742 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; (+)-HRESIMS (positive mode) *m*/*z* 775.1453 $[M + Na]^+$ (calcd for $C_{34}H_{36}N_6O_6S_4Na$, 775.1471).

Chaetocochin C (3): colorless, amorphous powder; $[\alpha]_D^{20}$ +206.0, $[\alpha]_{365}^{20}$ +965.0, $[\alpha]_{436}^{20}$ +456, $[\alpha]_{546}^{20}$ +240.0, $[\alpha]_{578}^{20}$ +168.0 (*c* 0.10, $CHCl_3$); CD (*c* 0.10, CH_3CH_2OH) λ_{max} nm ($\Delta\epsilon$) 240 (+11.5), 265 (−5.0), 300 (+2.0); UV (MeOH) λ_{max} nm (log ϵ) 205 (3.67), 220 (3.56), 287 (2.85), 294 (2.85) nm; IR (KBr) ν_{max} cm^{-1} 3421, 3362, 2922, 1694, 1674, 1646, 1609, 1459, 1384, 1055, 743; ^1H and ^{13}C NMR data, see Table 1; (+)-HRESIMS *m*/*z* 763.1497 $[M + Na]^+$ (calcd for $C_{33}H_{36}N_6O_6S_4Na$, 763.1471).

Supporting Information Available: HRESIMS and ^1H and ^{13}C NMR spectra of compounds **1**−**3** are available free of charge via the Internet at http://pubs.acs.org.

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