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Cytotoxic Metabolites from the Antarctic Psychrophilic Fungus *Oidiodendron truncatum*

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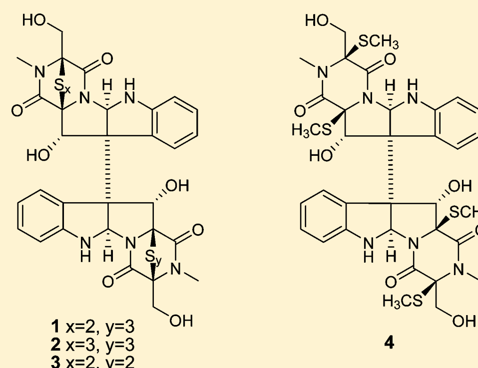
Supporting Information

ABSTRACT: Two new epipolythiodioxopiperazines, named chetracins B and C (**1** and **2**), and five new diketopiperazines, named chetracin D (**4**) and oidioperazines A–D (**5**, **10**, **12**, and **13**), were isolated from the fungus *Oidiodendron truncatum* GW3-13, along with six known compounds (**3**, **6**, **7**, **8**, **9**, and **11**). Their structures were elucidated by extensive NMR, MS, and CD analyses, as well as chemical transformation. An in vitro MTT cytotoxicity assay revealed potent biological activity for **1** in the nanomolar range against a panel of five human cancer lines.



Epipolythiodioxopiperazines (ETPs) constitute an important class of biologically active compounds characterized by a bridged polysulfide piperazine ring. These metabolites have been found to possess a wide variety of biological activities, including antiproliferative (glionitrin A),[1] cytotoxic (chaetocochins A),[2] immunomodulatory (chetoseminudins A),[3] antiviral (gliotoxin analogues),[4] and antibacterial and antifungal activities (sirodesmin PL).[5] The crucial structural element responsible for the observed biological properties has been shown to be the sulfide linkage.[6,7] Recent investigations on the intracellular target (hypoxia inducible factor 1) of ETPs have confirmed this particular structure–activity relationship.[8,9]

The Antarctic fungus *Oidiodendron truncatum* GW3-13 was isolated from soil under lichens near the Great Wall station (Chinese Antarctic station). As part of our investigations aimed at exploring new secondary metabolites with anticancer activities, we found that the ethyl acetate extract of the whole culture of fungal strain GW3-13 showed significant in vitro cytotoxicity against P-388 lymphocytic leukemia cells. Bioassay-guided fractionation of the culture extract yielded two new epipolythiodioxopiperazines, named chetracins B and C (**1** and **2**), and five new diketopiperazines, named chetracin D (**4**) and oidioperazines A–D (**5**, **10**, **12**, and **13**), along with the known natural products melinacidin IV (**3**),[10] T988 B (**6**),[6] T988 C (**7**),[6] T988 A (**8**),[6] chetoseminudin C (**9**),[3] and cyclo-L-Trp-L-Ser (**11**).[11] Herein, we report the isolation, structural determination, and cytotoxicity exhibited by these secondary metabolites against a panel of five human tumor cell lines (HCT-8, Bel-7402, BGC-823, A-549, and A-2780).

RESULTS AND DISCUSSION

Chetracin B (**1**) was obtained as a pale yellow, amorphous powder. HRESIMS showed the molecular formula to be $C_{30}H_{28}N_6O_8S_5$, consistent with 20 degrees of unsaturation. The IR spectrum of **1** exhibited absorption bands at 3395, 1669, and 1607 cm^{-1}, characteristic of alcohol, amide, and aromatic ring functionalities. The physicochemical properies and NMR data obtained for **1** showed a striking resemblance to that of the known epipolythiodioxopiperazines melinacidin IV (**3**)[10] and chetracin A,[12] which differ from **1** by one and three sulfur atoms, respectively. Twinned resonances in the ^{1}H NMR spectrum of **1** were indicative of its dimeric nature, as the metabolite appeared to comprise two slightly different monomers. In the ^{13}C NMR spectrum, half of the resonances were assigned to 1,2-disubstituted benzene carbons (C-6a′ to C-10a′) at δ_C 148.4, 110.9, 130.1, 120.7, 128.6, and 128.9, two amide carbons (C-1′ and C-4′) at δ_C 166.4 and 162.7, two methine carbons (C-5a′ and C-11′) at δ_C 81.5 and 83.0, three quaternary sp^3 carbons (C-3′, C-10b′, and C-11a′) at δ_C 75.5, 63.5, and 76.1, one hydroxy methylene carbon (C-13′) at δ_C 60.2, and one N-methyl carbon (C-12′) at δ_C 27.0. These chemical shifts were almost indistinguishable from those of melinacidin IV (**3**), thus suggesting that half of **1** was structurally identical to melinacidin IV. This was further supported by the appearance of a MS/MS fragment at m/z 364 [d]$^+$ (Figure 2). The molecular formula of **1** led us to place a trisulfide bridge in the second monomeric subunit C-1–C-13 on the basis of the downfield chemical shift observed for C-11a (δ_C 84.4) caused by a deshielding effect of the trisulfide

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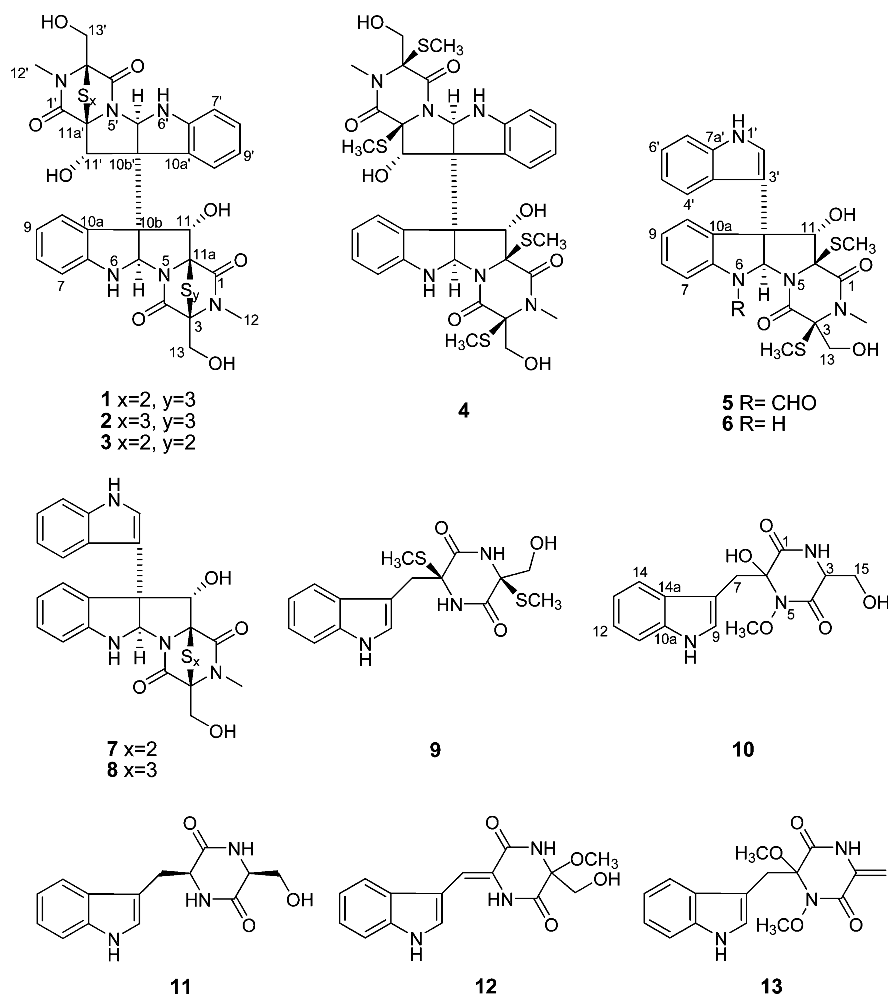

Figure 1. Structures of 1−13.

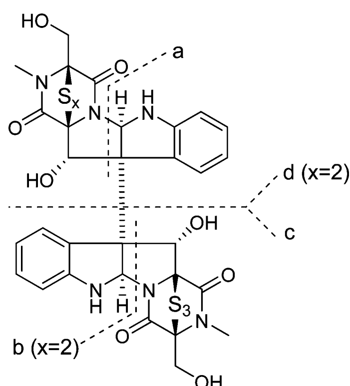

Figure 2. Major fragment ions in the mass spectra of 1 and 2.

group.[13] In addition, the MS/MS analysis of 1 revealed a fragment peak at m/z 396 $[c]^+$ expected for the proposed monomer, together with a peak at m/z 647 $[M + H - 3S - H_2O]^+$, arising from the loss of the polysulfide bridge in the C-1−C-13 dioxopiperazine ring followed by dehydration. This concluded the planar structure elucidation for metabolite 1, which was named chetracin B.

The relative configuration of 1 was deduced from an extensive analysis of the NOESY spectrum. NOE correlations from H-10 to H-11 and from H-10′ to H-11′ indicated that H-11 and H-11′ were oriented cis to C-10a−C-10b and C-10a′−C-10b′, respectively. Since the N-6−C-5a bond must be on the same side of the C-10a−C-10b bond, H-5a and the C-10b−C10b′ must be trans to H-11, and H-5a′ was oriented trans to H-11′. In order to confirm the relative configuration of 1, the tetrakis(methylsulfanyl) derivative (4) (Figures S20, S21), which was also isolated from this strain, was produced by treatment of 1 with $NaBH_4$ and MeI. Cross-peaks from 11a-SCH_3 to H-11 and 3-SCH_3 observed in the NOESY spectrum of 4 demonstrated that H-11 was cis to the disulfide (or trisulfide) bridge in 1.

The absolute configuration of 1 could not be determined unambiguously by comparison of its CD spectrum to that of melinacidin IV because it lacked two characteristic absorptions [a negative Cotton effect (S→CO charge transfer transition) at about 270 nm and a positive Cotton effect (disulfide $n\sigma^*$ transition) at 230 nm] (Figure S1). Its CD spectrum is probably influenced by the trisulfide bridge as seen for leptosin B.[14] Interestingly, when a solution of 1 in DMSO was allowed to stand for about two weeks at room temperature, 2 and 3 were produced (Figure S10, S10.1−10.6). This reaction could be explained by decomposition of polysulfides via a free radical mechanism, which has been previously reported.[15]

Compound 2 was also isolated from the extract of the fungus grown on medium III. Its HRESIMS showed an $[M + H]^+$ ion corresponding to the molecular formula $C_{30}H_{28}N_6O_8S_6$ and containing two sulfurs more than that of melinacidin IV. The ^1H and ^{13}C NMR spectra of 2 exhibited only half of the

Table 1. ^{1}H and ^{13}C NMR Data for Compounds 1−3

	1[a]		2[b]		3[b]	
position	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1	169.1, qC		167.9, qC		165.5, qC	
3	74.4, qC		75.9, qC		78.2, qC	
4	165.5, qC		163.3, qC		161.2, qC	
5a	84.8, CH	5.74 s	83.7, CH	5.38 d (5.5)	81.5, CH	5.47 d (4.6)
6a	150.8, qC		153.0, qC		150.4, qC	
7	111.2, CH	6.75 d (7.7)	109.9, CH	6.50−6.60[c]	109.7, CH	6.54 d (7.8)
8	131.0, CH	7.21 t (7.1, 7.7)	130.7, CH	7.04 t (7.1, 7.7)	128.0, CH	7.03 t (7.8, 7.3)
9	120.3, CH	6.81 t (7.1, 7.1)	118.1, CH	6.50−6.60[c]	118.7, CH	6.65 t (7.3, 7.3)
10	128.6, CH	7.72 d (6.8)	129.5, CH	7.50 d (7.7)	129.7, CH	7.70 d (7.8)
10a	126.6, qC		126.9, qC		129.9, qC	
10b	65.7, qC		64.1, qC		66.6, qC	
11	81.8, CH	4.99 s	80.9, CH	4.72 s	82.1, CH	4.88 s
11a	84.4, qC		85.4, qC		78.0, qC	
12	27.4, CH_3	3.23 s	27.6, CH_3	3.11 s	27.8, CH_3	3.01 s
13	62.2, CH_2	4.16[c]	60.2, CH_2	3.66 dd (4.9, 12.7)	58.8, CH_2	4.06 dd (6.4, 12.8)
		3.93 d (13.2)		4.01 dd (6.1, 12.7)		4.21 dd (5.0, 12.4)
1′	166.4, qC					
3′	75.5, qC					
4′	162.7, qC					
5a′	81.5, CH	5.64 s				
6a′	148.4, qC					
7′	110.9, CH	6.66 d (7.7)				
8′	130.1, CH	7.15 t (6.6, 7.1)				
9′	120.7, CH	6.84 t (6.6, 7.7)				
10′	128.6, CH	7.73 d (6.6)				
10a′	128.9, qC					
10b′	63.5, qC					
11′	83.0, CH	5.07 s				
11a′	76.1, qC					
12′	27.0, CH_3	3.09 s				
13′	60.2, CH_2	4.16[c]				
		4.25[c]				
6-NH				6.88 s		6.73 s
11-OH				6.52 d (4.9)		6.51 d (4.1)
13-OH				5.68 t (5.5, 6.0)		5.84 t (5.5, 6.0)

[a]Spectra were recorded at 400 MHz for ^{1}H and at 100 MHz for ^{13}C using $CDCl_3$ as solvent. [b]Spectra were recorded at 600 MHz for ^{1}H and at 150 MHz for ^{13}C using DMSO-d_6 as solvent. [c]Signals were overlapped.

expected signals, indicating a symmetrical dimer. Comparison of the ^{1}H and ^{13}C NMR data for **2** and melinacidin IV (**3**) showed similar chemical shifts. The molecular formula of **2** suggested that each monomeric subunit had a trisulfide bridge, which was confirmed by ESIMS/MS fragments at m/z 679 [M + H − 3S − H_2O]$^+$, 583 [M + H − 6S − H_2O]$^+$, 513 [a + H]$^+$, 396 [c]$^+$ (Figure 2). Thus, the planar structure of the new metabolite, named chetracin C, was elucidated. Furthermore, the absolute configuration of both **1** and **2** was presumed to be the same as melinacidin IV (**3**),[10,16,17] an aspect additionally supported by their common biogenetic origin.[18]

Chetracin D (**4**) was isolated as a pale yellow, amorphous powder. The HRESIMS showed an [M + Na]$^+$ ion corresponding to the molecular formula $C_{34}H_{40}N_6O_8S_4$. Its UV and IR spectra were similar to those obtained for melinacidin IV. The ^{1}H and ^{13}C NMR spectra combined with the molecular formula indicated the presence of four S-methyl groups in **4**. On the basis of ^{1}H NMR analysis at 20 °C in DMSO-d_6, compound **4** existed as a 1:3.3 mixture of two symmetric rotational isomers formed by rotation of two monomers about the C-10b−C-10′b bond (Table 2).[13] The relative configuration of **4** was found to be the same as **1** according to NOESY data. Because **4** was the reduction product of **1** (Figure S20−21), its absolute configuration is also likely to be the same.

Oidioperazine A (**5**) was isolated as a pale yellow, amorphous powder exhibiting an [M + H]$^+$ ion corresponding to the molecular formula $C_{26}H_{26}N_4O_5S_2$. The comparison of the ^{1}H and ^{13}C NMR data between **5** and T988 B (**6**)[6] suggested that they shared similar structural features. The only difference was the presence of one additional aldehyde group (δ_H 9.16, δ_C 162.6) in **5**, which was connected to the nitrogen of the dihydroindole system based on HMBC correlations (Figure 3).

The relative configuration of **5** was deduced by analyzing its NOESY spectrum. Correlations between H-5a and H-2′ as well as H-11 and H-10 observed in the NOESY spectrum indicated that H-5a was cis to the C-10b−C-3′ bond while trans to H-11. A correlation between 11a-SCH_3 and H-11 suggested they also had a cis relationship. No information could be obtained on the relative orientation of the 3-SCH_3 group. Nevertheless, both 3-SCH_3 and 11a-SCH_3 in **5** were proposed to have a cis

Table 2. ^{1}H and ^{13}C NMR Data for Compound 4

	major symmetric conformor[a]			minor symmetric conformor[a]		
position	δ_C, type	δ_H (J in Hz)	HMBC[b]	δ_C, type	δ_H (J in Hz)	HMBC[b]
1	164.6, qC			165.5, qC		
3	71.5, qC			71.5, qC		
4	162.5, qC			162.4, qC		
5a	79.7, CH	6.34 s	6a, 10a, 11a, 10b′	80.5, CH	5.05 brs	6a, 11a, 10b′
6a	151.2, qC			151.5, qC		
7	107.9, CH	6.09 d (7.7)	9, 10a	108.1, CH	6.31 d (7.7)	9, 10a
8	128.8, CH	6.64 t (7.7, 7.7)	6a, 10	128.8, CH	6.90 t (7.1, 7.1)	6a, 10
9	116.7, CH	6.19 t (7.7, 7.7)	7, 10a	117.9, CH	6.40 t (7.7, 7.1)	7, 10a
10	121.9, CH	7.19 d (7.1)	6a, 8	127.2, CH	7.69 d (7.7)	6a, 8
10a	130.2, qC			130.6, qC		
10b	63.6, qC			66.6, qC		
11	82.1, CH	4.79 s	5a, 10a	81.8, CH	5.15 d (7.1)	5a
11a	72.2, qC			72.9, qC		
12	28.4, CH_3	2.91 s	1, 3	28.5, CH_3	2.96 s	1, 3
13	63.2, CH_2	4.19 d (11.0) 3.69 d (11.6)	4	63.2, CH_2	4.00 dd (6.6, 11.0) 3.69 dd	4
3-SCH_3	13.5, CH_3	2.18 s	3	13.4, CH_3	2.09 s	3
11a-SCH_3	16.0, CH_3	1.84 s	11a	15.9, CH_3	1.82 s	11a
6-NH		6.84 s	6a, 10a, 10b		6.57 brs	10a, 10b
11-OH		7.25 brs			5.64 d (6.6)	10b
13-OH		5.11[c]			5.11[c]	

[a]Spectra were recorded at 600 MHz for ^{1}H and at 150 MHz for ^{13}C using DMSO-d_6 as solvent. [b]HMBC correlations from H to C. [c]Signals were overlapped.

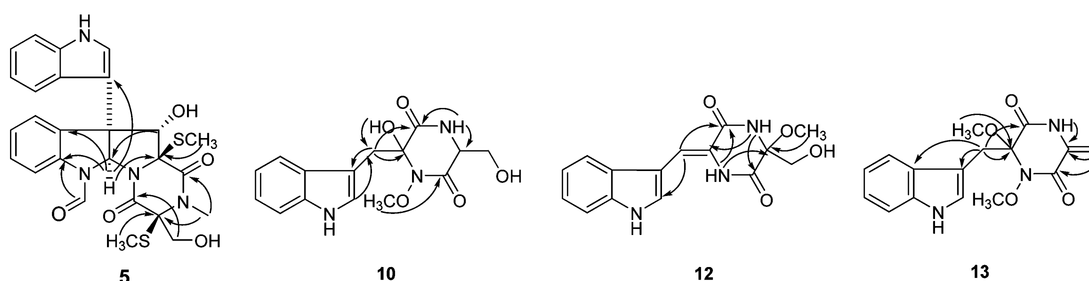

Figure 3. Key HMBC correlations for **5**, **10**, **12**, and **13**.

orientation due to the presence of sulfide bridges between C-3 and C-11a in T988 C (**7**) and T988 A (**8**), thus completing the relative stereochemistry of **5**. The absolute configuration of T988 C (**7**) was determined to be (3*S*, 11a*S*) by analyzing its CD spectrum (Figure S6).[13] Treatment of **7** with $NaBH_4$ and MeI afforded a bis methylsulfanyl derivative identified as T988 B (**6**) (Figure S44−45), thus the absolute configuration of **5** and **6** was determined to be the same as T988 C.

Oidioperazine B (**10**) was isolated as a yellow, amorphous powder displaying an $[M + Na]^+$ ion corresponding to the molecular formula $C_{15}H_{17}N_3O_5$. The IR spectrum of **10** showed absorptions for hydroxyl (3420 cm^{-1}), carbonyl (1682 cm^{-1}), and aromatic functional groups (1602, 1548 cm^{-1}). ^{1}H NMR signals at δ_H 10.88 (NH), 7.70, 7.29, 7.10, 7.02, and 6.94 (Table 4) indicated the presence of a 3-substituted indole moiety. In addition to eight carbon signals for the indole system, the ^{13}C NMR showed two amide carbonyl carbons (δ_C 164.1 and 165.8), one sp^3 quaternary carbon (δ_C 82.1), two methylenes (δ_C 34.1 and 58.3), one methine (δ_C 63.3), and one methoxyl carbon (δ_C 60.8). The ^{1}H NMR (Table 4) data of **10** were similar to those of cyclo-L-Trp-L-Ser (**11**),[11] except for the absence of the H-6 signal at δ_H 4.21 and the presence instead of two additional resonances at δ_H 5.81 (OH-6) and 3.29 (CH_3O-5), indicating that **10** was also a diketopiperazine derivative containing an indole moiety. The positions of methoxyl and hydroxyl groups were located at N-5 and C-6 by HMBC correlations from MeO-N to C-4 and from OH-6 to C-6 and C-7 (Figure 3). Cyclo-L-Trp-L-Ser (**11**) was the cyclization product of L-Trp and L-Ser;[11] thus the absolute configuration at C-3 is *S*. For **10**, the absolute configuration at C-3 is likely to be the same as cyclo-L-Trp-L-Ser based on their common biosynthetic origin,[19] but due to the lack of NOE correlations for OH-6, the configuration of the stereocenter remains unknown.

Oidioperazine C (**12**) was a pale yellow, amorphous powder. The molecular formula was determined as $C_{15}H_{15}N_3O_4$ by HRESIMS. Comparison of ^{1}H NMR data indicated that **12** had the same scaffold as cyclo-L-Trp-L-Ser. The absence of the two amino α-H signals at δ_H 4.21 and 3.81 and methylene signals at δ_H 3.30 and 2.88, together with the presence of an additional methoxyl group (δ_H 3.15) and an sp^2 methenyl (δ_H 7.07), led us to assemble the structure of **12** as shown (Figure 1; see Figure 3 for HMBC correlations). The geometry of the double bond at C-7 was assigned as *Z* on the basis of the downfield chemical shift of H-7 (δ_H 7.07), caused by the deshielding effect of the neighboring carbonyl group.[20]

Oidioperazine D (**13**) was obtained as a pale yellow, amorphous powder. The molecular formula was established as

Table 3. ^{1}H and ^{13}C NMR Data for Compounds 5 and 6

	5[a]		6[a]	
position	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1	164.5, qC		165.3, qC	
3	71.8, qC		72.0, qC	
4	164.6, qC		163.5, qC	
5a	81.3, CH	6.92 s	81.5, CH	6.27 s
6a	140.6, qC		149.9, qC	
7	116.1, CH	7.85 d (8.2)	108.7, CH	6.46 d (7.8)
8	128.7, CH	7.22 t (7.6, 7.7)	128.3, CH	6.91 t (7.4, 7.4)
9	124.8, CH	7.06 t (7.3, 7.7)	117.3, CH	6.51 t (7.4, 7.4)
10	124.1, CH	7.68 d (7.8)	123.2, CH	7.28 d (7.7)
10a	136.6, qC		133.2, qC	
10b	58.7, qC		59.5, qC	
11	79.3, CH	5.22 d (6.9)	79.8, CH	5.07 d (7.1)
11a	73.9, qC		73.5, qC	
12	28.6, CH_3	2.99 s	28.5, CH_3	2.97 s
13	63.0, CH_2	3.77 dd (6.4, 11.5)	63.3, CH_2	3.70 dd (6.6, 11.5)
		4.24 dd (5.9, 11.9)		4.21 dd (5.6, 11.4)
14	162.6, CH	9.16 s		
2′	124.4, CH	7.26 d (2.7)	123.6, CH	7.08 d (2.4)
3′	114.8, qC		116.3, qC	
3a′	126.2, qC		126.6, qC	
4′	121.8, CH	7.77 d (8.3)	121.9, CH	7.79 d (8.0)
5′	119.4, CH	6.97 t (7.3, 7.8)	119.0, CH	6.90 t (7.1, 7.1)
6′	121.6, CH	7.06 t (7.3, 7.8)	121.3, CH	7.01 t (7.5, 7.5)
7′	112.0, CH	7.31 d (8.2)	111.8, CH	7.28 d (7.7)
7a′	137.5, qC		137.2, qC	
3-SCH_3	13.5, CH_3	2.18 s	13.4, CH_3	2.22 s
11a-SCH_3	16.0, CH_3	2.01 s	16.0, CH_3	2.00 s
1′-NH		11.02 s		
11-OH		5.95 d (6.9)		5.51 d (7.1)
13-OH		5.45 t (6.0)		5.28 t (6.0)

[a]Spectra were recorded at 600 MHz for ^{1}H and at 150 MHz for ^{13}C using DMSO-d_6 as solvent.

$C_{16}H_{17}N_3O_4$ on the basis of the HRESIMS. In comparing the ^{1}H NMR data for **13** and **10** (Table 4), it was shown that the signals for OH-15 (δ_H 5.90), OH-6 (δ_H 5.81), H-3 (δ_H 3.93), and H-15 (δ_H 3.60−3.73) of **10** were absent in **13**, while an additional O-methyl signal at δ_H 3.14 and two singlets at δ_H 5.21 and 4.88 appeared in the ^{1}H NMR spectrum of **13**. The ^{13}C NMR spectrum of **13** indicated the replacement of the two carbons of **10** at δ_C 63.3 (C-3) and 58.3 (C-15) by two olefinic carbons at δ_C 133.6 and 99.9, in addition to a methoxyl carbon (δ_C 51.1). The C-15 carbon at 99.9 ppm, found to be an sp^2 methylene via HMQC, was assigned as a terminal olefinic carbon. Metabolite **13** corresponded to the dehydration and methylation product of **10**. This was supported by HMBC correlations (Figure 3). The position of the additional methoxyl group (δ_H 3.14) was assigned to C-6 by an HMBC correlation from O-Me to C-6. Although the remaining methoxyl group (δ_H 3.27) could not be unequivocally assigned at N-2 or N-5 due to lack of HMBC correlations, it was probably attached to N-5 as in **10**, given their similar chemical shifts. Thus, the structure of **13** was proposed as shown (Figure 1). The conformation of the stereocenters of **13** remains undetermined.

The known compounds melinacidin IV (**3**),[10] T988 B (**6**),[6] T988 C (**7**),[6] T988 A (**8**),[6] chetoseminudin C (**9**),[3] and cyclo-L-Trp-L-Ser (**11**)[11] were identified by comparison of their spectroscopic data with that in the literature.

Biological evaluation of **1**−**9** (Table 5) using an MTT method[21] showed that **1** and **3** exhibited potent cytotoxicity against five human cancer lines in the nanomolar range, whereas metabolites **2**, **4**, **7**, and **8** showed significant cytotoxicity at a micromolar concentration. Compounds **5**, **6**, and **9**−**13** showed no significant cytotoxicity at 10 μM. Comparison of the activity data suggested that the sulfide bridge was a determinant factor for their cytotoxicity. The number of sulfur atoms in the bridge did not seem to influence activity. In the case of **2**, the low level of cytotoxicity obtained was probably caused by high instability (**2** proved to be quite unstable under the bioassay conditions used).

Diketopiperazines are common secondary metabolites produced by a variety of marine and terrestrial microorganisms. On the other hand, diketopiperazines such as compounds **10**, **12**, and **13** having OH/OR groups at the α-carbon of the amino acid residues appear to be unusual.[22] It has been reported that the bishydroxylation at the α-carbon of substrates (diketopiperazines) is a key enzymatic reaction in the gliotoxin biosynthesis, and hydroxylation takes place from the same face in the molecule.[19] This reaction is catalyzed by the *gliC* gene product, a putative cytochrome P450 monooxygenase. The sulfurization steps in gliotoxin biosynthesis are catalyzed by glutathione S-transferase (GST) encoded by the *gliG* gene. Phylogenetic analysis revealed that genes coding for gliG homologues are widespread in the genomes of ETP producers.[19] According to the biogenetic hypothesis above, in the fungus *O. truncatum* compound **11** is probably the biosynthetic intermediate of **1**, **2**, and **3**, whereas **10**, **12**, and **13** are likely shunt products in ETP's biosynthesis. In addition, the presence of metabolites **10**, **12**, and **13** suggests that bishydroxylation may occur in two steps (first hydroxylating at C-6, then C-3). Nevertheless, because the mechanism of hydroxylation has not been elucidated yet, the configuration at C-3 or C-6 in **10**, **12**, and **13** requires further confirmation. The strain *O. truncatum* is a psychrophilic fungus that can be cultured only under 20 °C, and ETPs (**1**, **2**, and **3**) are its main metabolites. To our knowledge, this is the first report on ETP secondary metabolites isolated from a low-temperate Antarctic fungus.

■ EXPERIMENTAL SECTION

General Experimental Procedures. Specific rotations were obtained on a JASCO P-1020 digital polarimeter. UV spectra were recorded on a Cary 300 spectrophotometer. IR spectra were recorded on a Nicolet NEXUS 470 spectrophotometer in KBr discs. ^{1}H and ^{13}C NMR, DEPT, and 2D NMR spectra were recorded on a JEOL JNM-ECP 600 spectrometer using TMS as internal standard, and chemical shifts were recorded as δ values. ESIMS was measured on a Micromass Q-TOF Ultima Global GAA076 LC mass spectrometer. Semi-preparative HPLC was performed using an ODS column (YMC-Pack ODS-A, 10 × 250 mm, 5 μm, 4 mL/min).

Fungus Source. The Antarctic fungus *Oidiodendron truncatum* GW3-13 was isolated from soil collected under lichens near the Great Wall station (Chinese Antarctic station), in Jan 2005. The isolate was identified by rDNA sequence analysis. The ITS1-5.8S-ITS2 rDNA sequence of the GW3-13 fungus has been submitted to GenBank with the accession numbers HM565134.1.

Cultivation and Extraction. The fungus was cultivated in three different culture media (two liquid media and one solid medium). The culture medium ingredients are described below. Liquid medium I: maltose 2.0%, sorbitol 2.0%, monosodium glutamate 1.0%, KH_2PO_4 0.05%, $MgSO_4$ 0.03%, yeast extract 0.3%, tryptophan 0.05%, pH 6.5;

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Table 4. ^{1}H and ^{13}C NMR Data for Compounds 10, 12, and 13

	10[a]		12[a]		13[a]	
position	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1	164.1, qC		161.1, qC		159.0, qC	
3	63.3, CH	3.93 brs	87.3, qC		133.6, qC	
4	165.8, qC		163.5, qC		157.6, qC	
6	82.1, qC		121.9, qC		88.7, qC	
7	34.1, CH_2	3.01 d (13.2) 3.52 d (14.3)	107.7, CH	7.07 s	35.6, CH_2	3.06 d (13.7) 3.50 d (13.8)
8	107.4, qC		107.6, qC		106.3, qC	
9	125.1, CH	7.10 d (2.2)	126.5, CH	8.02 d (2.2)	125.9, CH	7.01 d (2.3)
10a	135.7, qC		135.7, qC		136.3, qC	
11	111.0, CH	7.29 d (7.7)	111.8, CH	7.43 d (8.8)	111.8, CH	7.27 d (7.7)
12	120.7, CH	7.02 t (7.7, 7.7)	122.0, CH	7.18 t (6.7, 7.7)	121.5, CH	7.01 t (6.9, 6.9)
13	118.2, CH	6.94 t (6.6, 7.7)	119.9, CH	7.11 t (6.6, 7.7)	119.1, CH	6.95 t (6.8, 7.8)
14	119.2, CH	7.70 d (7.7)	118.0, CH	7.64 d (7.7)	119.4, CH	7.56 d (7.8)
14a	127.6, qC		127.0, qC		127.9, qC	
15	58.3, CH_2	3.63 m	65.0, CH_2	3.37 dd (4.4, 9.8)	99.9, CH_2	4.88 s
		3.71 m		3.78 dd (6.6, 9.9)		5.21 s
3-OCH_3			50.2, CH_3	3.15 s		
5-OCH_3	60.8, CH_3	3.29 s			61.7, CH_3	3.27 s
6-OCH_3					51.1, CH_3	3.14 s
2-NH		8.96 s		8.65 s		9.34 s
5-NH				9.70 s		
10-NH		10.88 s		11.70 s		10.96 s
6-OH		5.81 s				
15-OH		5.90 t (4.4)		5.29 t (5.5)		

[a]Spectra were recorded at 600 MHz for ^{1}H and at 150 MHz for ^{13}C using DMSO-d_6 as solvent.

Table 5. Cytotoxicity of 1−13 against Five Human Cancer Cell Lines

	IC_{50} (μM)				
compound	HCT-8	Bel-7402	BGC-823	A549	A2780
1	0.013 ± 0.06	0.003 ± 0.04	0.011 ± 0.01	0.022 ± 0.01	0.028 ± 0.11
2	0.49 ± 0.09	0.38 ± 0.03	0.70 ± 0.04	0.72 ± 0.01	0.58 ± 0.03
3	0.052 ± 0.07	0.005 ± 0.03	0.047 ± 0.01	0.022 ± 0.01	0.054 ± 0.11
4	1.06 ± 0.10	0.81 ± 0.01	1.83 ± 0.07	0.14 ± 0.03	1.65 ± 0.07
5	>10	>10	>10	>10	>10
6	>10	>10	>10	>10	>10
7	0.70 ± 0.23	0.55 ± 0.01	0.67 ± 0.23	1.24 ± 0.01	0.48 ± 0.06
8	1.18 ± 0.06	0.75 ± 0.27	1.46 ± 0.01	1.53 ± 0.01	0.42 ± 0.04
9	>10	>10	>10	>10	>10
10	>10	>10	>10	>10	>10
11	>10	>10	>10	>10	>10
12	>10	>10	>10	>10	>10
13	>10	>10	>10	>10	>10
paclitaxel	0.051	0.006	<0.001	0.016	<0.001

liquid medium II: yeast extract 0.3%, malt extract 0.3%, peptone 0.5%, glucose 2%; solid medium III: wheat bran 80%, water 120%. Spores of fungus GW3-13 growing on PDA were inoculated into 1 L Erlenmeyer flasks, each containing 300 mL of liquid culture medium or 200 g of solid substrate, followed by static incubation for 30 days at 15 °C. The whole broth was filtered through cheesecloth to separate the broth supernatant and mycelia. The former was extracted with ethyl acetate, while the latter was extracted with acetone. The acetone extract was evaporated under reduced pressure to afford an aqueous solution and then extracted with ethyl acetate. The two ethyl acetate extracts were combined and concentrated under reduced pressure to give crude extracts (medium I (90 L), 35.0 g; medium II (90 L), 15.6 g). The solid culture (medium III, 12 kg) was extracted with acetone. The acetone extract was evaporated under reduced pressure to afford an aqueous solution and then extracted with ethyl acetate. The ethyl acetate extract was concentrated under reduced pressure to give a crude extract (5.6 g).

Purification. The crude extract (35.0 g) of medium I was applied to a silica gel (200−300 mesh) column and was separated into seven fractions (Fr.1−Fr.7) using a step gradient elution of petroleum ether−acetone and $CHCl_3$−CH_3OH. Fr.4, eluting in $CHCl_3$, was fractionated on Sephadex LH-20 using $CHCl_3$−CH_3OH (1:1) as the eluting solvent. Fr.4-1 and Fr.4-2 were further separated on a silica gel column using elution of $CHCl_3$−CH_3OH. The subfractions 4-1-1, 4-1-5, and 4-2-1 were further purified respectively by semipreparative HPLC (35:65 CH_3OH−H_2O, 35:65 CH_3OH−H_2O, 53:47 CH_3OH−H_2O, 4 mL/min) to give compound **13** (8.0 mg, t_R 20.2 min), **9** (12.0 mg, t_R 15.1 min), **5** (9.0 mg, t_R 28.9 min), and **6** (2.0 mg, t_R 25.7 min). The active fraction, Fr.5, contained pale yellow material that could not be dissolved in $CHCl_3$−CH_3OH. Filtering Fr.5 through filter paper produced pale yellow powder Fr.5-1 and the filtrate Fr.5-2. Fr.5-1 was dissolved in THF and further separated on a silica gel column using $CHCl_3$−MeOH (200:1) to afford compound **1** (210.0 mg) and compound **3** (66.0 mg). A solution of **1** in dimethyl sulfoxide gave **2**

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and **3** after approximately two weeks at room temperature. Fr.5-2 was applied on a silica gel column using elution of $CHCl_3$−MeOH (100:1) and then separated by semipreparative HPLC (40:60 CH_3OH−H_2O, 4 mL/min) to give compound **10** (2.0 mg, t_R 6.8 min) and **12** (6.0 mg, t_R 20.5 min). Fr.6, eluting in $CHCl_3$−MeOH (49:1), was separated on Sephadex LH-20 using $CHCl_3$−CH_3OH (1:1) as the eluting solvent and semipreparative HPLC (30:70 CH_3OH−H_2O, 4 mL/min) to afford compound **11** (20.0 mg, t_R 7.3 min).

The crude extract (15.6 g) of culture grown on medium II was applied to a silica gel (200−300 mesh) column and was separated into seven fractions (Fr.1−Fr.7) using a step gradient elution of petroleum ether−acetone and CH_2Cl_2−CH_3OH. Fr.1, eluting in CH_2Cl_2−CH_3OH (500:1), was fractionated on Sephadex LH-20 and semipreparative HPLC (65:35 CH_3OH−H_2O, 4 mL/min) to afford compound **7** (6.2 mg, t_R 9.6 min). Fr.2, eluting in CH_2Cl_2−CH_3OH (100:1), was fractionated on Sephadex LH-20 using CH_2Cl_2−CH_3OH (1:1) as the eluting solvent and further fractionated on semipreparative HPLC (65:35 CH_3OH−H_2O, 4 mL/min) to afford compound **4** (10.0 mg, t_R 12.8 min) and **8** (5.5 mg, t_R 16.2 min).

The crude extract (5.6 g) of culture grown on medium III was fractionated on Sephadex LH-20 using CH_2Cl_2−CH_3OH (1:1) as the eluting solvent. The active Fr.5 was applied on a C_{18} column using CH_3OH−H_2O and semipreparative HPLC (70:30 CH_3OH−H_2O, 4 mL/min) to give compound **2** (28.0 mg, t_R 10.7 min).

Cytotoxicity Assay. Cytotoxic activities of **1**−**13** were evaluated by an MTT method using HCT-8 (human colon cancer cell line), BEL-7402 (human hepatocellular carcinoma cell line), BGC-823 (human gastric carcinoma cell line), A-549 (human lung cancer cell line), and A-2780 (human ovarian carcinoma cell line). The detailed methodology for biological testing has already been described in a previous report.[21]

Chetracin B (1): pale yellow powder; $[\alpha]^{25}_{D}$ +806.0 (*c* 0.066, $CHCl_3$); CD (6.58 × 10^{-4} M in DMSO) λ_{max} ($\Delta\varepsilon$) 322 (+0.27), 283 (+0.12), 265 (+28.2) nm; IR (KBr) ν_{max} 3394, 1669, 1607 cm^{-1}; UV (MeOH) λ_{max} (log ε) 205 (4.71), 302 (3.70) nm; ^1H and ^{13}C NMR data see Table 1; ESIMSMS *m/z* 697 $[M + H - 2S]^+$, 679 $[M + H - 2S - H_2O]^+$, 647 $[M + H - 3S - H_2O]^+$, 583 $[M + H - 5S - H_2O]^+$, 513 $[a + H]^+$, 481 $[b + H]^+$, 417 $[a + H - 3S]^+$, 399 $[a + H - 3S - H_2O]^+$, 378 $[c - H_2O]^+$, 364 $[d]^+$, 300 $[c - 3S]^+$ or $[d - 2S]^+$, 282 $[c - 3S - H_2O]^+$ or $[d - 2S - H_2O]^+$, 233 [bis-indol-3-yl]$^+$; HRESIMS *m/z* 761.0656 $[M + H]^+$ (calcd for $C_{30}H_{29}N_6O_8S_5$, 761.0650).

Chetracin C (2): pale yellow powder; $[\alpha]^{25}_{D}$ +597.1 (*c* 0.1, $CHCl_3$); CD (6.31 × 10^{-4} M in MeOH) λ_{max} ($\Delta\varepsilon$) 309 (+14.2), 279 (+7.79), 250 (+34.8), 235 (+22.4) nm; IR (KBr) ν_{max} 3430, 1678, 1611 cm^{-1}; UV (MeOH) λ_{max} (log ε) 204 (4.51), 305 (3.57) nm; ^1H and ^{13}C NMR data see Table 1; ESIMS/MS *m/z* 697 $[M + H - 3S]^+$, 679 $[M + H - 3S - H_2O]^+$, 583 $[M + H - 6S - H_2O]^+$, 513 $[a + H]^+$, 416 $[a - 3S]^+$, 399 $[a + H - 3S - H_2O]^+$, 396 $[c]^+$, 378 $[c - H_2O]^+$, 300 $[c - 3S]^+$, 282 $[c - 3S - H_2O]^+$, 233 [bis-indol-3-yl]$^+$; HRESIMS *m/z* 793.0363 $[M + H]^+$ (calcd for $C_{30}H_{29}N_6O_8S_6$, 793.0371).

Chetracin D (4): pale yellow powder; $[\alpha]^{25}_{D}$ +419.3 (*c* 0.1, $CHCl_3$); CD (6.35 × 10^{-4} M in MeOH) λ_{max} ($\Delta\varepsilon$) 314 (+16.0), 281 (+6.90), 254 (+27.8), 237 (+18.6) nm; IR (KBr) ν_{max} 3443, 1675, 1609 cm^{-1}; UV (MeOH) λ_{max} (log ε) 204 (4.75), 305 (3.70) nm; ^1H and ^{13}C NMR data see Table 2; HREIMS *m/z* 811.1666 $[M + Na]^+$ (calcd for $C_{34}H_{40}N_6O_8S_4Na$, 811.1688).

Oidioperazine A (5): pale yellow powder; $[\alpha]^{25}_{D}$ +103.1 (*c* 0.1, $CHCl_3$); CD (9.29 × 10^{-4} M in MeOH) λ_{max} ($\Delta\varepsilon$) 291 (+2.08), 278 (+1.00), 261 (+7.93), 243 (−1.19), 230 (+8.43) nm; IR (KBr) ν_{max} 3475, 1650, 1556, 1509, 1457 cm^{-1}; UV (MeOH) λ_{max} (log ε) 201 (4.55), 255 (3.98) nm; ^1H and ^{13}C NMR data see Table 3; HRESIMS *m/z* 539.1419 $[M + H]^+$ (calcd for $C_{26}H_{27}N_4O_5S_2$, 539.1423).

Oidioperazine B (10): yellow powder; $[\alpha]^{25}_{D}$ −40.5 (*c* 0.1, CH_3OH); IR (KBr) ν_{max} 3420, 1682, 1602, 1548, 710 cm^{-1}; UV (MeOH) λ_{max} (log ε) 204 (4.32), 218 (4.42), 280 (3.67) nm; ^1H and ^{13}C NMR data see Table 4; HRESIMS *m/z* 342.1065 $[M + Na]^+$ (calcd for $C_{15}H_{17}N_3O_5Na$, 342.1066).

Oidioperazine C (12): pale yellow powder; $[\alpha]^{25}_{D}$ −38.1 (*c* 0.1, CH_3OH); IR (KBr) ν_{max} 3480, 1675, 1578, 1512, 695 cm^{-1}; UV (MeOH) λ_{max} (log ε) 226 (4.05), 354 (3.96) nm; ^1H and ^{13}C NMR data see Table 4; HRESIMS *m/z* 324.0974 $[M + Na]^+$ (calcd for $C_{15}H_{15}N_3O_4Na$, 324.0960).

Oidioperazine D (13): pale yellow powder; $[\alpha]^{25}_{D}$ −34.1 (*c* 0.1, CH_3OH); IR (KBr) ν_{max} 1699, 1540, 1521, 1509, 668 cm^{-1}; UV (MeOH) λ_{max} (log ε) 217 (4.45) nm; ^1H and ^{13}C NMR data see Table 4; HRESIMS *m/z* 338.1113 $[M + Na]^+$ (calcd for $C_{16}H_{17}N_3O_4Na$, 338.1117).

Formation of Tetrakis(methylsulfanyl) Derivatives from Compound 1. Compound **1** (10.2 mg) was dissolved in pyridine (0.1 mL) and MeOH (0.16 mL). MeI (0.2 mL) and $NaBH_4$ (4 mg) were added, and the mixture was stirred for 30 min at room temperature. The reaction mixture was then diluted with water and extracted with diethyl ether. The solvent was evaporated under reduced pressure, and the residue was purified by HPLC (65:35 CH_3OH−H_2O, 3 mL/min) to afford compound **4** (2.9 mg, t_R 16.2 min).

Formation of Bis(methylsulfanyl) Derivatives from Compound 7. Compound **7** (2.0 mg) was dissolved in pyridine (0.1 mL) and MeOH (0.16 mL). MeI (0.05 mL) and $NaBH_4$ (2 mg) were added, and the mixture was stirred for 30 min at room temperature. The reaction mixture was then diluted with water and extracted with diethyl ether. The solvent was evaporated under reduced pressure, and the residue was purified by HPLC (65:35 CH_3OH−H_2O, 3 mL/min) to afford compound **6** (0.9 mg, t_R 8.5 min).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

MS and NMR spectra of **1**, **2**, **4**, **5**, **10**, **12**, and **13**. These materials are available free of charge *via* the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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