Nematicidal Epipolysulfanyldioxopiperazines from *Gliocladium roseum*

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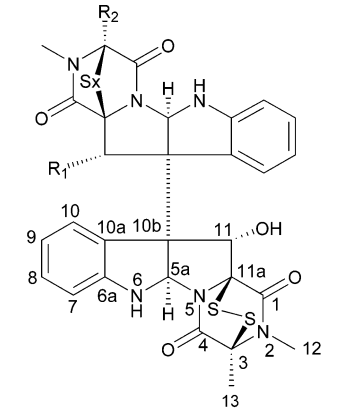
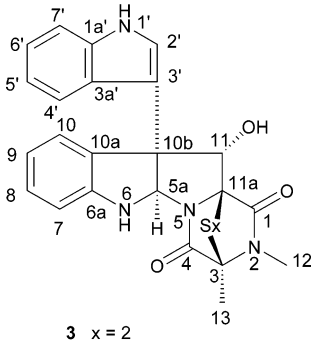
Five new verticillin-type epipolysulfanyldioxopiperazines, gliocladine A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**), were isolated from wheat solid-substrate fermentation of *Gliocladium roseum* 1A, along with four known compounds, verticillin A (**6**), 11′-deoxyverticillin A (**7**), Sch52900 (**8**), and Sch52901 (**9**). Their structures were elucidated by extensive 1D and 2D NMR studies, MS, and chemical transformations. In vitro immersion tests showed that all nine compounds exhibited antinematodal activity against *Caenorhabditis elegans* and *Panagrellus redivivus*. The monomeric epipolysulfanydioxopiperazines (**3**−**5**), with the indole moiety, were found to be less active than the dimeric compounds (**1**, **2**, **6**−**8**).

Fungi of the *Gliocladium* genus are commonly used as biocontrol agents against plant pathogenic fungi, such as *Pythium ultimum* and *Rhizoctonia solani*. The phytochemical studies undertaken by different groups have resulted in the isolation of various diketopiperazines,[2−4] terpenoids,[5,6] peptides,[7,8] and polyketides.[9,10] In previous screening of fungi obtained from submerged woody substrates collected in various freshwater habitats in Yunnan Province, China, for nematicidal activity, aliphatic extracts of wheat solid-substrate fermentation of *G. roseum* 1A were found to strongly inhibit the mobility of the pine wood nematode, *Bursaphelenchus xylophilus*, and the two nonpathogenic nematodes *Caenorhabditis elegans* and *Panagrellus redivivus.*[11] Fractionation of the aliphatic extracts led to the isolation and structural elucidation of five new compounds, gliocladines A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**), along with four known compounds (**6**−**9**),[3,15] which belong to a series of epipolysulfanyldioxopiperazine fungal antibiotics. Among these are leptosins,[12,13] chaetocin and chetoracin A.[14] Compounds **1**−**9** were examined for nematicidal activity against *C. elegans*, *P. redivivus*, and *B. xylophilus.* We describe herein the isolation, structural elucidation, and nematicidal activity of these mebabolites from *G. roseum* 1A.



1 R_1= OH R_2 = CH_3 x = 3
2 R_1= OH R_2 = CH_3 x = 4
6 R_1= OH R_2 = CH_3 x = 2
7 R_1= H R_2 = CH_3 x = 2
8 R_1= OH R_2 = CH_2CH_3 x = 2
9 R_1= OH R_2 = $CH(OH)CH_3$ x = 2



3 x = 2
4 x = 3
5 x = 4

Results and Discussion

The MeOH extract of solid-substrate fermentation of *G. roseum* 1A on wheat was purified by bioassay-directed fractionation employing a combination of silica gel vacuum-liquid chromatography (VLC), Sephadex LH-20 column chromatography, middle-performance liquid chromatography (MPLC), and high-performance liquid chromatography (HPLC) to afford compounds **1**−**9**.

The UV, IR, and ^{1}H and ^{13}C NMR data indicated that compounds **1**−**9** belong to the epipolysulfanyldioxopiperazine class of fungal antibiotics by direct comparison with the compounds **6**−**9** (verticillin A,[3] 11′-deoxyverticillin A,[15] Sch52900,[3] and Sch52901,[3] respectively), as reported in the literature (see Tables 1 and 2 for NMR data).

Compound **1** was isolated as a white amorphous powder. Its HRFABMS showed an $[M + H]^+$ ion at *m/z* 729.0419, corresponding to the molecular formula $C_{30}H_{28}N_6O_6S_5$, which contained one sulfur atom more than that of **6**. The ^{13}C NMR spectrum of **1** revealed an unsymmetrical dimeric structure because some carbon signals appeared to be split due to the presence of two slightly different subunits in comparison with **6**. Assignments of the ^{1}H (Table 1) and ^{13}C NMR (Table 2) signals for **1** using ^{1}H−^{1}H COSY and HMBC correlations revealed that chemical shifts of the signals for one monomeric subunit (C-1−C-13) were almost identical to those of **6**, and hence one-half of **1** is the same as that of verticillin A with two disulfide bridges. This was supported by the appearance of a FABMS fragment at *m/z*

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Table 1. ^{1}H NMR Chemical Shift Assignments (δ) and Coupling Data of **1**−**9** (pyridine-d_5)

C	1	2	3	4	5	6	7	8	9
5a	5.72(s)	6.08(s)	5.98(s)	6.84(s)	7.13(s)	5.78(s)	5.82(s)	5.79(s)	5.77(s)
7	6.67(d,7.8)	6.65(d,7.8)	6.64(d,8.0)	6.90(d,8.0)	6.88(d,8.0)	6.70(d,7.8)	6.58(m)	6.70(d,7.8)	6.72(d,7.8)
8	7.12(t,7.5,7.8)	7.05(t,7.5,7.8)	6.90(t,7.6)	7.13(t,7.6)	7.10(t,7.6)	7.12(t,7.5,7.8)	6.98(m)	7.10(t,7.5,7.8)	7.15(t,7.5,7.8)
9	6.77(t,7.5)	6.61(m)	6.57(t,7.4)	6.72(t,7.5)	6.73 (7.5)	6.79(t,7.5)	6.66(m)	6.80(t,7.5)	6.79(t,7.5)
10	8.34(7.5)	8.37(d,7.5)	7.56(d,7.5)	7.68(d,7.6)	7.66(7.6)	8.28(d,7.5)	6.98(m)	8.28(d,7.5)	8.27(d,7.5)
11	6.27(s)	6.38(s)	6.68(s)	6.23(s)	6.15(s)	6.26(s)	6.93 (s)	6.29(s)	6.26(s)
12	2.79(s)	2.86(s)	2.69(s)	3.14(s)	2.98(s)	2.83(s)	2.89(s)	2.82(s)	2.88(s)
13	1.77(s)	1.73(s)	1.72(s)	1.76(s)	1.86(s)	1.75(s)	1.97(s)	1.74(s)	1.81(s)
2′			7.49(s)	7.63(brs,2.2)	7.65(brs,2.3)				
4′			8.25(d,7.1)	8.36(d,8.0)	8.44(d,8.0)				
5′			7.09(m)	7.21(t,8.0)	7.19(t,8.0)				
5a′	5.78(s)	5.85(s)				5.78(s)	6.95(s)	5.86(s)	5.77(s)
6′			7.09(m)	7.26(t,8.0)	7.28(t,8.0)				
7′	6.72(d,7.8)	6.69(d,7.8)	7.42(d,7.2)	7.53(d,8.1)	7.55(d,8.1)	6.70(d,7.8)	6.58(m)	6.68(d,7.8)	6.72(d,7.8)
8′	7.09 (t,7.7)	6.93 (t,7.7)				7.12(t,7.5,7.8)	6.98(m)	7.07(t,7.5,7.8)	7.15(t,7.5,7.8)
9′	6.69(7.5)	6.61(m)				6.79(t,7.5)	6.66(m)	6.80(t,7.5)	6.79(t,7.5)
10′	8.17(7.6)	8.12 (7.7)				8.28(d,7.5)	6.98(m)	8.31(d,7.5)	8.27(d,7.5)
11′	6.39(s)	6.08(s)				6.26(s)	3.33(brs,15.0)	6.33(s)	6.24(s)
							5.03(brs,15.0)		
12′	3.01(s)	2.87(s)				2.83(s)	2.87(s)	2.82(s)	2.88(s)
13′	1.57(s)	1.61(s)				1.75(s)	1.92(s)	4.99(q)	2.12(m)
14′								1.52(d,6.2)	1.25(t,7.4)

Table 2. ^{13}C NMR Chemical Shift Assignments (δ) of **1**−**9** (pyridine-d_5)

C	1	2	3	4	5	6	7	8	9
1	166.3s	166.1s	166.1s	168.5s	167.5s	166.1s	166.6s	166.1s	166.3s
3	74.1s	74.0s	74.5s	72.8s	75.5s	74.3s	74. 6s	74.3s	74.5s
4	162.3s	162.2s	163.0s	165.6s	165.2s	162.3s	162.7s	162.3s	162.6s
5a	83.6d	83.4d	83.5d	82.5d	84.3d	83.3d	83.3d	83.3d	83.5d
6a	150. 8s	150.9s	148.7s	151.7s	149.6s	150.9s	150.6s	150.9s	151.0s
7	110.5d,	110.3d	110.5d	110.7d	110.2d	110.3d	109.0d	110.3d	110.5d
8	130.9d	130.0d	129.2d	129.9d	128.6d	130.1d	129.9d	130.1d	130.3d
9	119.5d	119.4d	119.4d	118.8d	119.1d	119.4d	118.3d	119.3d	119.5d
10	128.8d	128.6d	124.6d	125.6d	124.9d	128.6d	129.3d	128.6d	128.7d
10a	130.9s	130.8s	133.3s	130.9 s	133.7s	130.8s	129.6s	130.8s	130.9s
10b	67.4s	67.5s	62.4s	60.5s	60.7s	67.5s	65.6s	67.5s	67.6s
11	82.6d,	82.8d	80.6d	83.1d	81.6d	82.8d	81.3d	82.8d	83.0d
11a	78.5s,	78.8s	78.8s	86.9s	81.7s	78.5s	77.7s	78.5s	78.7s
12	26.9q	27.2q	27.1q	28.1q	28.8q	26.9q	27.2q	26.9q	27.2q
13	17.5q	17.5q	17.9q	21.5q	23.5q	17.6q	18.3q	17.6q	17.8q
1	168.6s	167.4s				166.1s	165.5s	167.4s	167.2s
1a′			138.3s	138.4s	138.4s				
2′			123.5d	123.4d	123.6d				
3′	72.5s	75.3s	115.6s	116.2s	116.4s	74.3s	74.7s	74.3s	74.5s
3a′			126.9s	126.7s	126.9s				
4′	164.9s	164.5s	122.0d	121.9d	121.7d	162.3s	162.5s	162.1s	161.8s
5′			119.6d	119.6d	119.6d				
5a′	82.0d	84.2d				83.3d	82.0d	83.5d	83.6d
6′			121.9d	121.8d	121.6d				
6a′	153.8s	151.6s				150.9s	150.8s	150.8s	150.1s
7′	110.6d	110.7d	112.3d	112.4d	112.5d	110.3d	109.2d	110.3d	110.5d
8′	130.1d	128.8d				130.1d	129.9d	130.1d	130.3d
9′	118.8d	119.0d				119.4d	118.2d	119.5d	119.7d
10′	129.7d	129.1d				128.6d	129.3	128.7d	128.8d
10a′	128.2s	131.1s				130.8s	131.1s	130.9s	130.9s
10b′	65.51s					67.5s	61.3s	67.5s	67.6s
11′	85.6d					82.8d	45.8t	82.8d	83.0d
11a′	86.6d					78.5s	74.9s	78.5s	79.1s
12′	27.9q	28.6q				26.9q	26.9q	29.2q	28.2q
13′	21.2q	23.2q				17.6q	17.7	67.2d	25.1t
14′								20.0q	9.9q

348 [c]$^+$ (see Figure 1 for the fragment ions). The molecular formula of **1** showed the other monomeric subunit (C-1′−C-13′) to have a trisulfide bridge. In addition, the FABMS of **1** revealed a fragment peak at *m/z* 380 [a]$^+$, corresponding to this monomeric subunit of **1** together with peaks at *m/z* 633 [M + H − 3S]$^+$ and 615 [M + H − 3S − H_2O]$^+$ arising from the loss of sulfur atoms from the polysulfide bridge in the C-1′−C-13′ dioxopiperazine ring and successive dehydration, respectively. Desulfurization of **1** with triphenylphosphine gave **6**. The results indicated that **1** is the sulfur homologue of **6**, where one of the two disulfide bridges in **6** has been replaced by a trisulfide in **1**. Thus, **1** was identified as shown and named gliocladine A.

Compound **2** was obtained as a white amorphous powder. Its HRFABMS showed an [M + H]$^+$ ion at *m/z* 761.0479, corresponding to the molecular formula $C_{30}H_{28}N_6O_6S_6$, which contained one sulfur atom more than that of **1**. The ^{1}H and ^{13}C NMR spectrum of **2** showed two slightly different sets of signals, also indicating that it had an asymmetrical structure. The UV, IR, and NMR spectra were very similar to **1**, indicating that they are structurally related. The ^{13}C NMR signals for the C-1−C-13 half of **2**,

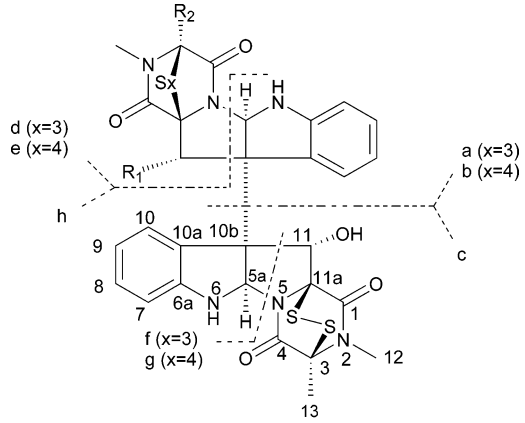

Figure 1. Major fragment ions in the mass spectra of compounds **1**, **2**, and **6**−**9**.

assigned by ^{1}H−^{1}H COSY and HMBC correlations, also showed close correspondence with those of **6** and **1** with the same disulfide bridge. The FABMS of **2** revealed a fragment peak at *m/z* 348 [c]$^+$, correspondencing to the C-1−C-13 half of **2**. This indicated that the monomeric subunit of **2** was the same as that of **6** and **1** with a common disulfide bridge. Treatment of **2** with triphenylphosphine gave **1** and **6**. Formation of **1** and **6** from **2** together with the molecular formula of **2** showed that the other monomeric subunit (C-1′−C-13′) had a tetrasulfide bridge. This was confirmed by FABMS fragments at *m/z* 633 [M + H − 4S]$^+$, arising from the loss of sulfur atoms from the polysulfide bridge in the above dioxopiperazine ring, and *m/z* 530 [g + H]$^+$, 402 [g − 4S]$^+$, 384 [g + H − 4S − H_2O]$^+$, 412 [b]$^+$. The information allowed assignment of structure **2** with the same configuration as that of **1** and **6**. Thus, **2** was identified as shown and named gliocladine B.

Compound **3** was isolated as a white amorphous powder. Its HRFABMS showed an [M + H]$^+$ ion at *m/z* 465.1062, corresponding to the molecular formula $C_{23}H_{20}N_4O_3S_2$. The UV, IR, and NMR spectra were similar to those of **6**. Close inspection of its ^{1}H and ^{13}C NMR spectra revealed that one of the two monomeric subunits of **6** was replaced by a 3-substituted indole moiety in **3**. A chemical shift difference of the ^{13}C NMR signals for C-5a, C-10a, and C-10b of **3**, relative to those of **6**, revealed that C-10b was linked at C-3 of the indole moiety in **3**. This was supported by HMBC correlations between δ 6.68 (corresponding to the carbon δ 83.5 in the HMQC experiment) and δ 78.8, 62.4, 115.6, 133.3, and 148.6 and between δ 7.49 (corresponding to the carbon δ 123.5 in the HMQC experiment) and δ 62.4, 115.6, 126.9, and 138.3. In addition, the FABMS of **3** exhibited a fragment ion at *m/z* 401 [M + H − 2S]$^+$ arising from the desulfurization of [M + H]$^+$, together with other fragments at *m/z* 383 [M + H − 2S − H_2O]$^+$, 232 [bis-indol-3-yl]$^+$, and 151 [M + H − 2S − 232 − H_2O]$^+$. Treatment of **6** with triphenylphosphine gave **3**. Formation of **3** from **6** showed the absolute configuration of **3** to be the same as that of **6**. Thus, **3** was identified as shown and named gliocladine C.

Compounds **4** and **5** had molecular formulas of $C_{23}H_{20}N_4O_3S_3$ and $C_{23}H_{20}N_4O_3S_4$, respectively, by HRFABMS. Their UV, IR, and NMR spectra closely resembled those of **3**, except that some ^{13}C NMR signals for their dioxopiperazine rings exhibited chemical shift differences relative to those of **3**. Desulfurization of **5** and **4** with triphenylphosphine afforded **4** and **3**, and **3**, respectively. In addition, the FABMS of both **4** and **5** showed a fragment peak at *m/z* 401 [MH − 3S]$^+$ or [MH − 4S]$^+$, arising from desulfurization of MH$^+$, together with the same fragments (*m/z* 383 and 232) as those of **3**. This evidence allowed assignment of stereostructures to **4** and **5**, with the same configuration as that of **6**. Thus, **4** and **5** were identified as shown and named gliocladine D and E, respectively.

Table 3. Nematicidal Activities of **1**−**9** toward *C. elegans*, *P. redivivus*, and *B. xylophilus*

	ED_{50} (μg/mL)[a]		
compd	*C. elegans*	*P. redivivus*	*B. xylophilus*
1	25	50	>400
2	30	25	>400
3	200	250	>400
4	200	250	>400
5	200	250	>400
6	30	80	>400
7	10	40	>400
8	50	80	>400
9	50	50	>400

[a] ED_{50}: concentrations causing more than 50% mortality after 24 h.

The nematicidal effects of **1**−**9**, listed in Table 3, show that *C. elegans* and *P. redivi*vus are sensitive to all compounds, with compound **7** being most potent. Compared to the dimeric epipolysulfanyldioxopiperazines (**1**, **2**, and **6**−**9**), the monomeric epipolysulfanydioxopiperazines (**3**−**5**) with the indole moiety were less active. The results also indicated that the number of sulfur atoms in the dioxopiperazine rings had no influence on the nematicidal activity. However, no distinct mortalities were observed on *B. xylophilus* at concertrations of compounds **1**−**9** up to 400 μg/mL.

The analogues (verticillins A−F) of gliocladine A−E have been reported to have antitumor, antibacterial, and antifungal activities.[3,4] Also **8** and **9** were reported together with **6** as inhibitors of c-*fos* proto-oncogene induction from an unidentified *Gliocladium* species.[3] The closely related antibiotic leptosins produced by *Leptosphaeria* sp. possess hydroxymethyl or isopropyl side chains and varying numbers of sulfur atoms in the bridges across the dioxopiperazine rings.[12,13] Some of these compounds also show in vivo antitumor effects.[12,13]

Experimental Section

General Experimental Procedures. IR spectra were measured on a Perkin-Elmer-577 spectrophotometer. UV spectra were taken on a Shimadzu double-beam 210A spectrophotometer. Optical rotations were measured with a Horiba SEPA-300 polarimeter. MS was performed on an Autospec-3000 spectrometer. The 1D and 2D NMR spectra were recorded on Bruker AM-400 and DRX-500 spectrometers, respectively.

Fungal Identification, Culturing, and Isolation of Metabolites. The strain of *G. roseum* 1A was initially isolated from submerged wood, collected from fresh water in Yunnan Province, China, identified by the Institute of Microbiology, Academic Sinica Beijing, China, and deposited in Yunnan Province Key Laboratory of Microbe Fermentation, Yunnan, China (culture collection number YMF1.00133). The fungal strain was grown on wheat at 26 °C for a period of 20 days. The cultures were lyophilized and extracted with CH_3OH. The combined extracts were concentrated under vacuum to give a residue (120 g), which was chromatographed on a silica gel column (200−300 mesh, 4 kg), eluted with a 0−100% CH_3COCH_3 gradient in petroleum (bp 60−90 °C). The active fractions (20−30%) were combined to obtain a residual light yellow-gray powder (23 g) based on the nematicidal bioassay

data. This was further subjected to CC on silica gel by normal-phase MPLC using a gradient from $CHCl_3$/EtOAc 1% to $CHCl_3$/EtOAc 25% to furnish six fractions [1 (296 mg), 2 (2556 mg), 3 (643 mg), 4 (324 mg), 5 (853 mg), 6 (941 mg)] based on TLC behavior. Fraction 3, obtained on elution with $CHCl_3$/EtOAc 8%, afforded pure **7** (212 mg). Fraction 1, obtained on elution with $CHCl_3$/EtOAc 4%, was purified by HPLC using MeOH/H_2O (7:3) as the eluent to afford **8** (53 mg) and **9** (12 mg). Fraction 2, obtained on elution with $CHCl_3$/EtOAc 12%, was further subjected to VLC using a gradient from $CHCl_3$/CH_3COCH_3 1% to $CHCl_3$/CH_3COCH_3 10% to give three subfractions [2a (315 mg), 2b (456 mg), and 2c (115 mg)]. Each subfraction was finally separated on a Sephadex LH-20 gel column eluting with $CHCl_3$/CH_3COCH_3 50% to yield pure **6** (253 mg), **1** (294 mg), and **2** (26 mg). Fraction 5 ($CHCl_3$/EtOAc 15%) and fraction 6 ($CHCl_3$/EtOAc 20%) afforded **3** (256 mg), and **4** (213 mg) and **5** (52 mg), respectively, after purification by MPLC using $CHCl_3$/CH_3COCH_3 (2%−10%) as the eluent.

Gliocladine A (1): white amorphous powder ($CHCl_3$-CH_3COCH_3); $[\alpha]_D^{18.0}$ +553.6° (*c* 0.45, pyridine); UV (pyridine) λ_{max} (log ϵ) 261.3 (0.3166), 255.6 (0.2879), 250.1 (0.3704), 202.4 (0.7033) nm; IR (film) ν_{max} 3441, 1664, 1608, 1557, 1490, 1480, 753 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; FABMS *m/z* (rel int) 729 $[M + H]^+$ (14), 633 $[M + H - 3S]^+$ (4), 615 $[M + H - 3S - H_2O]^+$ (2), 496 $[f + H]^+$ (3), 400 $[f + H - 3S]^+$ (2), 382 $[f + H - 3S - H_2O]^+$ (2), 380 $[a]^+$ (5), 348 $[c]^+$ (1), 284 $[c - 2S]^+$ or $[a - 3S]^+$ (5), 232 [bis-indol-3-yl]$^+$ (67), 85 (47); HRFABMS *m/z* 729.0758 $[M + H]^+$ (calcd for $C_{30}H_{29}N_6O_6S_5$, 729.0752).

Gliocladine B (2): white amorphous powder ($CHCl_3$-CH_3COCH_3); $[\alpha]_D^{18.0}$ +555.8° (*c* 0.44, pyridine); UV (pyridine) λ_{max} (log ϵ) 261.2 (0.4166), 255.6 (0.5879), 250.2 (0.5700), 202.4 (0.8036) nm; IR (film) ν_{max} 3430, 1678, 1611, 1552, 1483, 1470, 749 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; FABMS *m/z* (rel int) 761 $[M + H]^+$ (4), 633 $[M + H - 4S]^+$ (3), 530 $[g + H]^+$ (12), 402 $[g - 4S]^+$ (2), 384 $[g + H - 4S - H_2O]^+$ (1), 412 $[b]^+$ (2), 348 $[c]^+$ (1), 284 $[c - 2S]^+$ or $[b - 4S]^+$ (5), 232 [bis-indol-3-yl]$^+$ (67), 85 (47); HRFABMS *m/z* 761.0479 $[M + H]^+$ (calcd for $C_{30}H_{29}N_6O_6S_6$, 761.0472).

Gliocladine C (3): white amorphous powder ($CHCl_3$-CH_3COCH_3); $[\alpha]_D^{18.7}$ +512.6° (*c* 0.33, pyridine); UV (pyridine) λ_{max} (log ϵ) 289.6 (0.1550), 282.6 (0.1571), 218.8 (0.9282), 203.8 (0.9450); IR (film) ν_{max} 3424, 3335, 1692, 1675, 1610, 1551, 1483, 1460, 1343, 1104, 752, 666 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; FABMS *m/z* (rel int) 465 $[M + H]^+$ (23), 401 $[M + H - 2S]^+$ (3), 383 $[M + H - 2S - H_2O]^+$ (1), 232 [bis-indol-3-yl]$^+$ (100), 151 (12), 123 (29); HRFABMS *m/z* 465.1062 $[M + H]^+$ (calcd for $C_{23}H_{21}N_4O_3S_2$, 465.1055).

Gliocladine D (4): white amorphous powder ($CHCl_3$-CH_3COCH_3); $[\alpha]_D^{18.4}$ +604.1° (*c* 0.47, in pyridine); UV (pyridine) λ_{max} (log ϵ) 289.6 (0.1084), 219.2 (0.6414), 204.0 (0.5451); IR (film) ν_{max} 3411, 1674, 1608, 1547, 1481, 1460, 743 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; FABMS *m/z* (rel int) 497 $[M + H]^+$ (28), 401 $[M + H - 3S]^+$ (8), 383 $[M + H - 3S - H_2O]^+$ (2), 380 $[M + H -$ indol-3-yl$]^+$ (3), 284 $[M + H -$ 3S-indol-3-yl$]^+$ (2), 232 [bis-indol-3-yl]$^+$ (100), 80 (12); HRFABMS *m/z*: 497.0786 $[M + H]^+$ (calcd for $C_{23}H_{21}N_4O_3S_3$, 497.0776).

Gliocladine E (5): white amorphous powder ($CHCl_3$-CH_3COCH_3); $[\alpha]_D^{18.6}$ +562.6° (*c* 0.43, pyridine); UV (pyridine) λ_{max} (log ϵ) 289.6 (0.1672), 218.6 (0.7423), 205.7 (0.6413); IR (film) ν_{max} 3419, 1684, 1608, 1557, 1471, 1460, 753 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; FABMS *m/z* (rel int) 529 $[M + H]^+$ (20), 401 $[M + H - 4S]^+$ (8), 383 $[M + H - 4S - H_2O]^+$ (24), 232 [bis-indol-3-yl]$^+$ (100), 80 (12); HRFABMS *m/z*: 529.0504 $[M + H]^+$ (calcd for $C_{23}H_{21}N_4O_3S_4$, 529.0497).

Formation of 6 from 1.[14] Triphenylphosphine (12 mg) was added to a $CHCl_3$ solution (5 mL) of **1** (25 mg), and the reaction mixture was left to stand at room temperature for 1.5 h. The solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography using CH_2Cl_2 to afford **6** (11 mg), which was identified by ^1H NMR, and TLC.

Formation of 6 and 1 from 2. According to the procedure described for **1**, **2** (18 mg) was treated with triphenylphosphine (10 mg) at room temperature for 1.5 h to yield **6** (3 mg) and **1** (1.6 mg), which were identified by ^1H NMR and TLC.

Formation of 3 from 6.[14] According to the procedure described for **1**, **6** (25 mg) was treated with triphenylphosphine (15 mg) at 60 °C for 5 h to yield **3**, which was identified by ^1H NMR and TLC.

Formation of 3 and 4 from 5. According to the procedure described for **1**, **5** (18 mg) was treated with triphenylphosphine (10 mg) at room temperature for 1.5 h to yield **3** (1.8 mg) and **4** (1.4 mg), which were identified by ^1H NMR and TLC.

Formation of 3 from 4. According to the procedure described for **1**, **4** (14 mg) was treated with triphenylphosphine (8 mg) at room temperature for 1.5 h to yield **3** (4.2 mg), which were identified by IR, ^1H NMR, and TLC.

Determination of Nematicidal Activity. The nematode cultures, *C. elegans*, *P. redivivus*, and *B. xylophilus*, were maintained in our laboratory. *C. elegans* was grown on NG agar media containing a strain of *Escherichia coli* in disposable Petri dishes, which were wet with 2−4 mL of physiological saline. *P. redivivus* was maintained in aseptic, liquid Basal Heme media (5 mL) in scintillation vials. *B. xylophilus* was grown on PDB agar media containing a strain of *Botrytis cinerea* in disposable Petri dishes wet with 2−4 mL of physiological saline. The cultures were stored at room temperature and subcultured prior to the assay. The assay was conducted in Corning polystyrene 96-well plates. The nematodes were added to 1 mL of physiological saline in a scintillation vial. This was diluted until the nematode counts were 20−25 in a 48 μL aliquot. A solution (48 μL) containing nematodes was delivered to each of three wells per treatment. Two microliters of DMSO (50%) or DMSO (50%) and test compounds was added to each well. The plates were covered, parafilmed, and kept in a humid chamber. The number of dead nematodes was recorded in 24 h by microscopic observation.

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