Celogentins A−C, New Antimitotic Bicyclic Peptides from the Seeds of *Celosia argentea*

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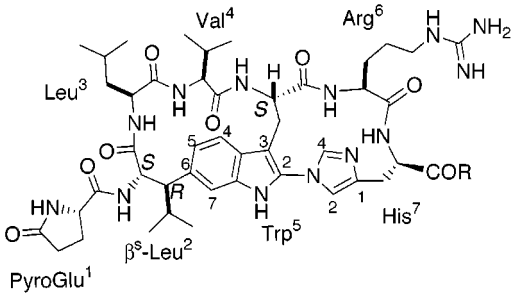
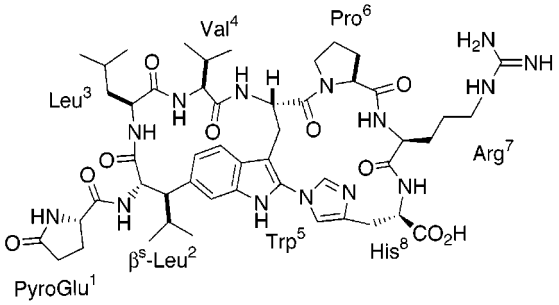
Three new bicyclic peptides, celogentins A (**1**), B (**2**), and C (**3**), have been isolated together with a known-related peptide, moroidin (**4**), from the seeds of *Celosia argentea*, and their structures including absolute stereochemistry were determined by using extensive NMR methods and chemical means. Celogentins A (**1**), B (**2**), and C (**3**) inhibited the polymerization of tubulin, and celogentin C (**3**) was four times more potent than moroidin (**4**) in the inhibitory activity. Structure−activity relationship study using moroidin derivatives **5**−**7** and analogue **8** as well as celogentins A−C (**1**−**3**) and moroidin (**4**) indicates that the bicyclic ring system including unusual non-peptide connections among β^s-Leu, Trp, and His residues characteristic of celogentins and moroidin, with ring size and conformations suitable for interaction with tubulin would be important for their biological activity.

Microtubules play a pivotal role in mitotic spindle assembly and cell division.[1] These cytoskeletal elements are formed by the self-association of the $\alpha\beta$ tubulin heterodimers.[1] There are a number of natural compounds that inhibit the microtuble formation and the mitotic arrest of eucaryotic cells.[2] The antimitotic agents have potential applications in drug development. The seeds of *Celosia argentea* (Amaranthaceae) are Chinese herbal medicines used as a therapeutic drug for eye and hepatic diseases in China and Japan.[3] During our search for bioactive compounds from medicinal plants,[4] we have found that moroidin (**4**) obtained from the seeds of *C. argentea*, which is a unique bicyclic peptide originally isolated from *Laportea moroides* (Labiatae),[5] remarkably inhibits the tubulin polymerization.[6] Further investigation of the extract of *C. argentea* resulted in the isolation of three new moroidin-type bicyclic peptides, celogentins A (**1**), B (**2**), and C (**3**). In this paper, we describe the isolation, structure elucidation, and antimitotic activity of **1**−**3**.



1: R=OH
2: R=His^8



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The seeds of *C. argentea* were extracted with MeOH, and the MeOH extract was in turn partitioned with hexane, EtOAc, and *n*-BuOH. *n*-BuOH-soluble materials were subjected to a Diaion HP-20 column ($MeOH/H_2O$, 0:1 → 1:0), in which fractions eluted with 60% and 80% MeOH were purified by an amino silica gel column ($CHCl_3/MeOH/H_2O$, 7:3:0.5 → 6:4:1) followed by C_{18} HPLC (CH_3CN/0.1% CF_3CO_2H, 22:78) to afford celo-

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Table 1. ^{1}H and ^{13}C NMR Data of Celogentin A (1) in DMSO-d_6 at 320 K

δ_H [int mult, J (Hz)]		δ_C		NOE relationship
PyroGlu[1]				
α	4.15 (1H, dd, 3.1, 8.8)	α	54.89	PyroGlu[1]: Hβ, Hγ, NH; β[s]-Leu[2]: NH, Hβ
β	2.26 (2H, m)	β	28.80	PyroGlu[1]: Hγ
γ	1.75 and 2.10 (2H, m)	γ	25.28	
NH	7.78 (1H, s)	δ	177.13	
		C=O	172.28	
β[s]-Leu[2]				
α	5.01 (1H, dd, 9.2, 11.3)	α	54.81	β[s]-Leu[2]: NH, Hβ, Hγ, Hδ; Trp[5]: H4, H5
β	3.00 (1H, dd, 3.7, 11.3)	β	52.61	β[s]-Leu[2]: NH, Hδ, Hγ; Trp[5]: H7
γ	2.12 (1H, m)	γ	27.07	β[s]-Leu[2]: NH, Hδ; Trp[5]: H7
δ	0.85 (3H, d, 6.8)	δ	17.39	Trp[5]: H7
	0.88 (3H, d, 6.8)		21.58	
NH	8.47 (1H, d, 8.6)	C=O	170.77	
Leu[3]				
α	4.12 (1H, m)	α	51.13	Leu[3]: NH, Hγ, Hδ; Val[4]: NH
β	1.33 and 1.42 (1H, m)	β	43.50	Leu[3]: NH; Val[4]: NH
γ	1.42 (1H, m)	γ	23.48	Leu[3]: Hδ
δ	0.74 (3H, d, 6.0)	δ	20.68	
	0.79 (3H, d, 6.0)		22.99	
NH	8.54 (1H, d, 10.1)	C=O	172.94	β[s]-Leu[2]: Hα; Trp[5]: H4, H5; Val[4]: NH
Val[4]				
α	4.10 (1H, dd, 4.7, 8.6)	α	58.56	Val[4]: Hβ, Hγ, NH; Trp[5]: NH
β	2.42 (1H, m)	β	29.25	Val[4]: Hγ, NH
γ	0.94 (3H, d, 6.9)	γ	16.62	Val[4]: NH, Trp[5]: H4, H5
	1.10 (3H, d, 6.9)		18.93	
NH	6.53 (1H, d, 8.6)	C=O	169.24	Trp[5]: NH, H4, H5
Trp[5]				
α	4.85 (1H, ddd, 1.9, 6.2, 8.3)	α	51.90	Trp[5]: NH, H4, Hβ; Arg[6]: NH
β	2.86 (1H, d, 13.7)	β	25.56	Arg[6]: NH; His[8]: H4; Trp[5]: H4
	3.28 (1H, dd, 6.2, 13.7)	C2	121.82	
NH1	11.51 (1H, s)	C3	103.17	Trp[5]: H7; His[8]: H2, H4
H4	7.39 (1H, d, 8.4)	C4	118.10	
H5	7.16 (1H, d, 8.4)	C5	119.90	
H7	6.94 (1H, s)	C6	132.08	
NH	6.34 (1H, d, 8.3)	C7	114.36	
		C8	132.63	
		C9	125.41	
		C=O	168.95	
Arg[6]				
α	4.32 (1H, brdd, 7.1, 9.2)	α	51.03	Arg[6]: Hβ; His[7]: NH, H2
β	1.39 (1H, m)	β	29.71	Arg[6]: NH; His[7]: NH
	1.53 (1H, m)	γ	24.91	
γ	1.39 (2H, m)	δ	40.14	
δ	3.09 (2H, m)	ϵ	156.57	
ϵ(NH)	7.44 (1H, br t, 5.4)	C=O	170.27	
NH	8.25 (1H, d, 9.2)			Arg[6]: Hα
His[7]				
α	4.70 (1H, ddd, 3.2, 9.7, 13.0)	α	51.13	His[7]: Hβ
β	2.71 (1H, t, 13.0)	β	31.03	His[7]: H2
	3.16 (1H, dd, 3.2, 13.0)	C1	137.49	
H2	7.09 (1H, s)	C2	121.17	Trp[5]: NH
H4	8.15 (1H, brs)	C4	137.76	
NH	8.35 (1H, d, 9.7)	C=O	172.07	His[7]: Hα, Hβ, H2

gentins A (**1**, 0.0002% yield), B (**2**, 0.0001%), and C (**3**: 0.001%) as colorless solids together with moroidin (**4**, 0.02%).[6]

FABMS data of **1**[[α]$^{23}_D$ −43° (*c* 0.3, 50% MeOH)] showed the pseudomolecular ion at *m*/*z* 930 $(M + H)^+$, and the molecular formula, $C_{45}H_{63}N_{13}O_9$, was established by HRFABMS [*m*/*z* 930.4991, $(M + H)^+$, Δ +4.1 mmu]. IR absorptions implied the presence of amide carbonyl group (1660 cm^{-1}), while the UV absorption at 283 nm indicated the presence of aromatic chromophore.

Though the peptide nature of celogentin A (**1**) was readily inferred from its ^{1}H and ^{13}C NMR spectral features, **1** was negative to ninhydrin, implying blockade of the N-terminus or a cyclic peptide. Standard amino acid analysis of the hydrolysates of **1** showed the presence of 1 mol each of glutamic acid (Glu), leucine (Leu), valine (Val), arginine (Arg), and histidine (His). The ^{1}H NMR (Table 1) spectrum of **1** in DMSO-d_6 at 320K contained 58 proton resonances, nine of which were assigned as either OH or NH groups. Seven proton resonances (δ 4.10−5.01) were indicative of α-protons of amino acid residues. These data combined with observation of seven carbonyl signals (δ 168.95−172.28) in the ^{13}C NMR spectrum suggested that **1** was a heptapeptide. Additional ^{13}C NMR data showed the presence of six methyl groups, eight methylenes, 11 methines, five olefins, and a carbonyl carbon (δ 177.13) assignable to PyroGlu[1].

Detailed analysis of ^{1}H−^{1}H COSY, HOHAHA, HMQC, and HMBC data of celogentin A (**1**) in DMSO-d_6 showed the presence of five amino acid residues, PyroGlu, Leu, Val, Arg, and His, in addition to two unusual amino acids, β-substituted Leu (β[s]-Leu) and 2,6-substituted Trp (Trp). The connection between Cβ of β[s]-Leu and C-6 of Trp was deduced from HMBC correlations (Figure 1) of Hβ of β[s]-Leu to C-6 of Trp and Hγ of β[s]-Leu to C-6 of Trp. NOESY correlations (Figure 2) of H-5 of Trp/Hα of β[s]-Leu and

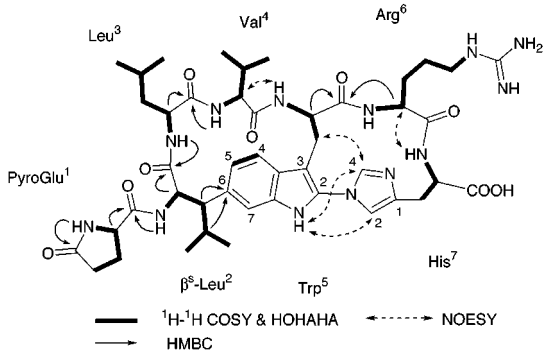

Figure 1. Selected 2D NMR correlations of celogentin A (**1**).

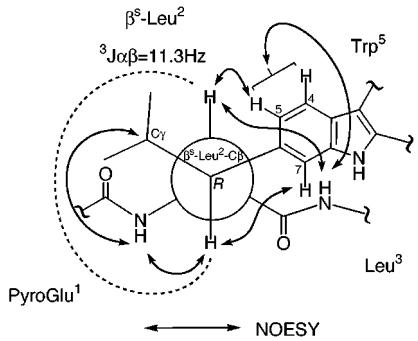

Figure 2. Rotamer for β^s-Leu^2-Cα−β^s-Leu^2-Cβ of **1**. Configuration and conformation of β^s-Leu^2 on the basis of $^3J\alpha\beta$ and key NOESY correlations.

H-7 of Trp/Hβ of β^s-Leu also corroborated the β^s-Leu^2-(Cβ)-Trp^5(C-6) connection. The connection between C-2 of Trp and N-3 of His was suggested by NOESY correlations (Figure 1) of H-4 of His/NH-1 and H$_2\beta$ of Trp, and H-2 of His/NH-1 of Trp, indicating that the indole and imidazole rings were not coplanar.

The sequence of PyroGlu^1-β^s-Leu^2-Leu^3-Val^4 in **1** was elucidated by HMBC correlations from the amide proton to the carbonyl carbon of each adjacent amino acid residue and from the α-proton to carbonyl carbon of each one. The HMBC correlation of Hα of Arg to the carbonyl carbon of Trp^5 (δ 171.7) provided the sequence of Trp^5-Arg^6. Furthermore, the NOESY correlation of amide NH of Trp^5/ Hα of Val^4 allowed the sequence to be extended to PyroGlu^1-β^s-Leu^2-Leu^3-Val^4-Trp^5-Arg^6. The remaining amino acid, His, was connected to Arg^6 from the NOESY correlation of NH of His^7/ Hα of Arg^6 to complete the whole sequence of **1**. The remaining NOESY correlations (Table 1) observed for **1** also supported the proposed sequence.

The absolute configurations of the PyroGlu^1, Leu^3, Val^4, Arg^6, and His^8 residues in celogentin A (**1**) were assigned as all L-configurations by chiral HPLC analysis of the hydrolysates of **1**. The Trp^5 residue was transformed into Asp by treatment of **1** with O_3/AcOH and then H_2O_2 followed by acid hydrolysis.[7] Chiral HPLC analysis of the Asp in the degradation products revealed it to be L-form, indicating *S*-configuration at Cα of the Trp^5 residue. Since the large vicinal coupling (11.3 Hz) between Hα and Hβ of β^s-Leu^2 established an antiperiplanar arrangement of these two protons consistent with the observation of almost the same NOESY correlations (Hα of β^s-Leu^2/ NH of Leu^3, Hβ of β^s-Leu^2/H-7 of Trp^5, Hβ of β^s-Leu^2/NH of β^s-Leu^2, NH of β^s-Leu^2/Hγ of β^s-Leu^2, and NH of Leu^3/ H-4 and H-5 of Trp^5) around β^s-Leu^2 residue of **1** as those of moroidin (**4**),[5] the absolute configurations at Cα and Cβ of the β^s-Leu^2 residue were elucidated to be *S* and *R*, respectively (Figure 2).

HRFABMS data [*m*/*z* 1067.5590, $(M+H)^+$, Δ +5.0 mmu] of celogentin B (**2**) indicated the molecular formula, $C_{51}H_{70}N_{16}O_{10}$. The IR absorption (1660 cm^{-1}) implied the presence of amide carbonyl functionality. Amino acid analysis of the hydrolysates of **2** showed the presence of 1 mol each of Glu, Leu, Val, and Arg, and 2 mol of His. The ^{1}H and ^{13}C NMR (Table 2) spectra of **2** revealed signals due to 9 carbonyl carbons, 12 sp^3 methines, 9 methylenes, and 6 methyl, implying that **2** was an octapeptide. Detailed analyses of 2D NMR (^{1}H-^{1}H COSY, HOHAHA, HMQC, and HMBC) spectra of **2** and comparison of the ^{13}C chemical shifts of the bicyclic part with those of **1** indicated the presence of the same bicyclic skeleton from PyroGlu^1 to His^7 for **2**. The remaining His residue was suggested to be connected to His^7 from NOESY correlations of Hα of His^7/amide NH of His^8 and H$_2\beta$ of His^7/amide NH of His^8 (Figure 3).

The absolute configurations of the PyroGlu^1, Leu^3, Val^4, Arg^6, His^7, and His^8 residues in **2** were assigned as all L-configurations by chiral HPLC analysis of the hydrolysates of **2**. The Trp^5 residue was transformed into Asp by treatment of **2** with O_3/AcOH and then H_2O_2 followed by acid hydrolysis.[7] Chiral HPLC analysis of the Asp in the degradation products revealed it to be L-form, indicating *S*-configuration at Cα of the Trp^5 residue. Treatment of celogentin A (**1**) with His, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSC), and 1-hydroxy-7-azabenzotriazole (HOAt)[8] yielded an octapeptide, of which spectral data were identical with those of natural celogentin B (**2**). Thus, the structure of celogentin B (**2**) was concluded to be as shown in Figure.

HRFABMS data [*m*/*z* 1027.5450, $(M + H)^+$, Δ −2.7 mmu] of celogentin C (**3**) revealed the molecular formula, $C_{50}H_{70}N_{14}O_{10}$. Amino acid analysis of the hydrolysates of **3** showed the presence of 1 mol each of Glu, Leu, Val, Arg, His, and proline (Pro). The ^{1}H and ^{13}C NMR (Table 3) spectra of **3** revealed the presence of nine carbonyl carbons, 12 sp^3 methines, 11 methylenes, and six methyls. The structure of **3** was elucidated by 2D NMR (^{1}H−^{1}H COSY, HOHAHA, HMQC, and HMBC) data. NOESY correlations indicating its sequence were shown in computer-generated 3D drawing (Figure 4). Almost the same NOESY correlations as those of **1** and **2** were observed for the left-hand part of the molecule. However, intrinsic NOESY correlations of Hα of Trp^5/H$_2\delta$ of Pro, Hα of Pro/NH of Arg, and Hα of Arg/NH of His were observed, indicating the partial sequence of Trp-Pro-Arg-His. In addition, NOESY correlations of H-2 of His/NH-1 of Trp and H-4 of His/ H$_2\beta$ of Trp strongly supported the connection between C-2 of Trp and N-3 of His. Further evidence supporting the proposed structure of **3** was provided by tandem mass spectrometry through examination of the collision-induced dissociation (CID) mass spectrum of the $(M-H)^-$ ions.[9] Negative ion FABMS/MS spectra of **3** showed characteristic patterns for charge-remote fragmentation,[10] probably due to the presence of

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Table 2. ^{1}H and ^{13}C NMR Data of Celogentin B (2) in DMSO-d_6 at 310 K

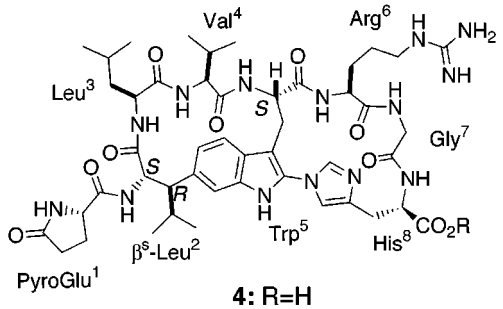
δ_H [int mult, J (Hz)]		δ_C		NOE relationship
PyroGlu[1]				
α	4.14 (1H, dd, 2.0, 8.6)	α	54.84	PyroGlu[1]: Hβ, Hγ, NH; β^s-Leu[2]: NH, Hα, Hβ
β	2.09 (2H, m)	β	28.80	PyroGlu[1]: Hγ
γ	1.72 and 2.26 (each 1H, m)	γ	25.31	
NH	7.83 (1H, s)	δ	177.13	β^s-Leu[2]: NH
		C=O	172.30	
β^s-Leu[2]				
α	5.00 (1H, t, 10.0)	α	54.72	β^s-Leu[2]: NH, Hβ, Hγ, Hδ; Trp[5]: H4, H5
β	2.99 (1H, dd, 2.9, 11.3)	β	52.51	β^s-Leu[2]: NH, Hδ, Hγ; Trp[5]: H7
γ	2.11 (1H, m)	γ	26.99	β^s-Leu[2]: NH, Hδ; Trp[5]: H7
δ	0.83 (3H, d, 6.6)	δ	17.31	Trp[5]: H7
	0.86 (3H, d, 6.6)		21.58	
NH	8.55 (1H, d, 8.7)	C=O	170.27	
Leu[3]				
α	4.12 (1H, m)	α	51.11	Leu[3]: NH, Hβ, Hγ, Hδ; Val[4]: NH
β	1.32 and 1.40 (each 1H, m)	β	43.46	Leu[3]: NH; Val[4]: NH
γ	1.41 (1H, m)	γ	23.44	Leu[3]: Hδ
δ	0.73 (3H, d, 5.3)	δ	20.61	
	0.78 (3H, d, 5.3)		23.01	
NH	8.58 (1H, d, 9.9)	C=O	173.00	β^s-Leu[2]: Hα; Trp[5]: H4, H5; Val[4]: NH
Val[4]				
α	4.09 (1H, dd, 4.8, 8.4)	α	58.55	Val[4]: Hβ, Hγ, NH; Trp[5]: NH
β	2.41 (1H, m)	β	29.22	Val[4]: Hγ, NH
γ	0.93 (3H, d, 6.8)	γ	16.54	Val[4]: NH, Trp[5]: H4
	1.09 (3H, d, 6.8)		18.91	
NH	6.54 (1H, d, 8.4)	C=O	169.24	Trp[5]: NH, H4, H5; β^s-Leu[2]: Hα
Trp[5]				
α	4.85 (1H, br s)	α	51.86	Trp[5]: NH, H4, Hβ; Arg[6]: NH, Hβ
β	2.86 (1H, d, 14.1)	β	25.56	Arg[6]: NH; Trp[5]: NH, H4
	3.29 (1H, dd, 5.4, 14.1)	C2	121.44	
NH1	11.65 (1H, s)	C3	103.37	Trp[5]: H7; His[7]: H2; β^s-Leu[2]: Hβ
H4	7.39 (1H, d, 8.4)	C4	118.12	
H5	7.16 (1H, d, 8.4)	C5	119.99	
H7	6.94 (1H, s)	C6	132.13	
NH	6.33 (1H, d, 8.2)	C7	114.39	
		C8	132.64	
		C9	125.28	
		C=O	168.99	
Arg[6]				
α	4.35 (1H, m)	α	51.05	Arg[6]: NH, Hβ, Hδ; His[7]: NH
β	1.40 (1H, m)	β	29.69	Arg[6]: NH, Hγ, Hδ; His[7]: NH
	1.50 (1H, m)	γ	24.85	Arg[6]: Hδ
γ	1.38 (2H, m)	δ	40.11	Arg[6]: Hϵ
δ	3.04 (2H, m)	ϵ	156.60	
ϵ(NH)	7.60 (1H, br s)	C=O	170.18	
NH	8.29 (1H, d, 8.9)			
His[7]				
α	4.73 (1H, br t, 9.8)	α	51.76	His[7]: NH, Hβ; His[8]: NH, Hβ
β	2.62 (1H, t, 12.6)	β	30.08	His[7]: NH, H2; His[8]: NH
	3.09 (1H, m)	C1	129.31	
H2	7.14 (1H, br s)	C2	121.44	His[8]: NH
H4	8.45 (1H, br s)	C4	137.49	
NH	8.32 (1H, d, 9.4)	C=O	171.54	His[8]: NH
His[8]				
α	4.60 (1H, m)	α	51.15	His[8]: NH, Hβ
β	3.21 (1H, dd, 5.1, 15.3)	β	25.91	His[8]: NH
	3.06 (1H, m)	C1	127.28	
H2	7.38 (1H, s)	C2	116.73	
H4	8.97 (1H, brs)	C4	133.71	
NH	8.47 (1H, d, 7.7)	C=O	171.08	

the carboxylate group at His. Product ion peaks generated by fissions at peptide bonds or cleavage of a carbon−nitrogen connection between Trp and His residues were prominently observed (Figure 5). Thus, celogentin C (**3**) was elucidated to possess the sequence, in which was a Pro residue was inserted between the Trp and Arg residues of celogentin A (**1**). The absolute configuration of each amino acid in **3** was assigned as all L-configurations by chiral HPLC analysis of the hydrolysates of **3**. Therefore, the structure of **3** was assigned as shown in Figure.

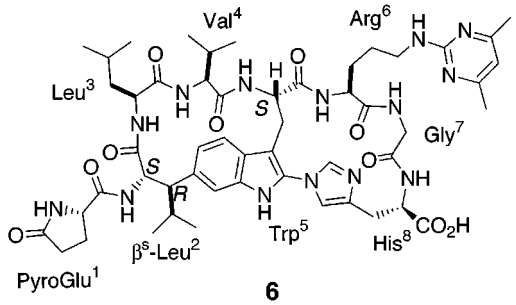
Generally antimitotic agents bind to either colchicine binding site or vinca alkaloid binding site. In the previous study, moroidin (**4**) has been found to inhibit the polymerization of tubulin.[6] In this study, such inhibitory activity was examined for celogentins A−C (**1**−**3**) (Figure 6). Celogentins A (**1**) and B (**2**), lacking the Gly residue of moroidin (**4**), showed less potent (IC_{50}, **1**, 20 μM; **2**, 30 μM) than moroidin (**4**, 3.0 μM) in inhibition of the tubulin polymerization. The presence of additional His[8] residue in **2** seems not to influence severely the inhibitory activity of celogentin A (**1**), judging from the inhibitory activity of **1** and **2**. Celogentin C (**3**) was more potent (IC_{50} 0.8 μM) than moroidin (**4**). On the other hand, the methyl

(10) Naoki, H. *LC/MS no Jissai*; Harada, K., Oka, H., Eds.; Kodansha: Tokyo, 1996; pp 225−252.

ester (**5**) and the pyrimidine derivative (**6**) of moroidin (**4**), which was prepared by treatment of **4** with 2,4-pentanedione, were found to be 2 times less potent (IC_{50}, 7.0 μM and 6.0 μM, respectively) than moroidin (**4**), indicating that the charges of Arg and His are not so important for the activity. Whereas, a monocyclic analogue (**7**) of **4** produced by treatment of **4** with α-chymotrypsin showed less inhibitory activity (IC_{50} 20.0 μM).[11] Stephanotic acid (**8**),[12] corresponding to the left-hand part of celogentins (**1**−**3**) and moroidin (**4**), did not show such inhibition. These results suggest that the bicyclic ring system including unusual non-peptide connections among β^s-Leu, Trp, and His residues characteristic of celogentins and moroidin, with ring size and conformations suitable for interaction with tubulin would be important for their biological activity.



4: R=H
5: R=CH_3



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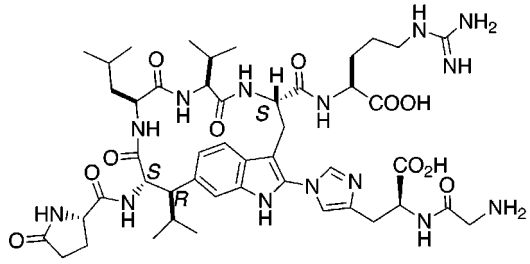

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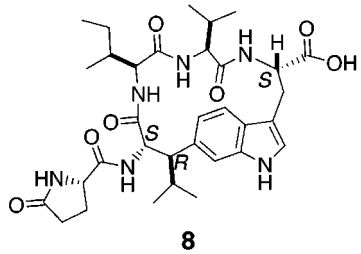

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(11) The fact that NOESY correlations of **7** are similar to those observed for moroidin (**4**) suggests that **7** adopts a conformation similar to moroidin (**4**).

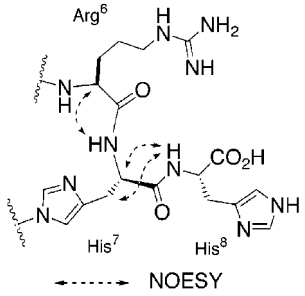

Figure 3. Selected NOESY correlations of Arg[6]-His[7]-His[8] moiety of celogentin B (**2**).

Celogentins A (**1**)−C (**3**) are a new type bicyclic peptides related to moroidin (**4**) with different ring size of right-hand backbone skeleton.[13] The right-hand 17-membered ring of celogentin C (**3**) and moroidin (**4**) may be important for the biological activity in addition of the left-hand 17-membered ring as common structural element of celogentins A − C (**1** − **3**) and moroidin (**4**). Celogentins might be useful as a tool for investigating the interaction with tubulin.

Experimental Section

General Procedures. ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 on a 600 MHz spectrometer (Bruker AMX600) equipped with an X32 computer and an Eurotherm temperature control unit. 1D NMR spectra were measured at 300−330 K, which were multiplied by a Gaussian filter and zero filled to 32K data points before Fourier transformation. 2D NMR spectra were measured at 300−330 K. NOESY and HOHAHA spectra in the phase sensitive mode were recorded using the TPPI method. HOHAHA spectra were recorded by spin-lock field preceded and followed by 2.5 ms trim pulses. NOESY spectra were measured with mixing times of 400, 600, and 800 ms. Since NOESY spectra gave no indications of spin diffusion at 600 ms, NOE intensities at this mixing time were used in the calculations. Typically, 256 FIDs of 2K data points, and 32 scans each were employed. Chemical shifts were presented using residual DMSO-d_6 (δ_H 2.50 and δ_C 39.5) as internal standards. Standard pulse sequences were employed for 2D NMR experiments. HMBC spectra were recorded using a 50 ms delay time for long-range C−H coupling with *Z*-axis PFG.

MS Experiments. FABMS was measured on a JEOL JMS−HX110 by using glycerol as matrix. FABMS/MS spectra were recorded on a JEOL JMS-700TZ tandem mass spectrometer equipped with a CCD array detector using magic bullet as matrix. The mass spectrometer was operated at an accelerating voltage of 10 kV xenon beam and in the negative mode.

Material. The seeds of *C. argentea* were purchased from Uchida Wakannyaku Co. in 1996. The botanical identification was made by Mr. N. Yoshida, Graduate School of Pharmaceutical Sciences, Hokkaido University. A voucher specimen has been deposited in the herbarium of Hokkaido University.

Isolation. The seeds (13.5 kg) of *C. argentea* were crushed and extracted with MeOH (18 L × 3), and the MeOH extract was in turn partitioned with hexane, EtOAc, and *n*-BuOH. The

(12) Yoshikawa, K.; Tao, S.; Arihara, S. *J. Nat. Prod.* **2000**, *63*, 540−542.

(13) Celogentins A−C (**1**−**3**) and moroidin (**4**) were elucidated to adopt only a single solution conformation in DMSO-d_6 solution. The proline amide bond of **3** was shown to be trans by the chemical shifts (δ 29.11 and 25.02, respectively) of β and γ carbons of Pro[6] residue,[14] and the triplet signal of Hα of Pro[6].[15] The temperature coefficients ($\Delta\delta/\Delta T$)[16] of Arg[7] (0.8) and Gly[7] (−0.4) of **3** and **4** indicated that these NHs were involved in intramolecular hydrogen bonds. These observations suggest that the right-hand portions of **3** and **4** take a similar backbone conformation to each other regardless of different sequence.

Table 3. ^{1}H and ^{13}C NMR Data of Celogentin C (3) in DMSO-d_6 at 300 K

δ_H [int mult, J (Hz)]		δ_C		NOE relationship
PyroGlu[1]				
α	4.11 (1H, brd, 8.1)	α	55.24	PyroGlu[1]: Hβ, Hγ, NH; β^s-Leu[2]: NH, Hβ
β	2.09 (2H, m)	β	29.11	PyroGlu[1]: Hγ; β^s-Leu[2]: NH
γ	1.70 and 2.24 (each 1H, m)	γ	25.61	
NH	7.89 (1H, s)	δ	177.68	β^s-Leu[2]: NH
		C=O	171.59	
β^s-Leu[2]				
α	4.84 (1H, t, 10.3)	α	54.92	β^s-Leu[2]: NH, Hβ, Hγ, Hδ; Trp[5]: H4, H5
β	3.07 (1H, m)	β	51.53	β^s-Leu[2]: NH, Hδ, Hγ; Trp[5]: H7
γ	2.16 (1H, m)	γ	26.76	β^s-Leu[2]: NH, Hδ; Trp[5]: H7
δ	0.73 (3H, d, 6.2)	δ	17.16	Trp[5]: H7
	0.84 (3H, d, 6.2)		21.82	
NH	8.53 (1H, d, 8.7)	C=O	171.24	
Leu[3]				
α	3.98 (1H, m)	α	52.23	Leu[3]: NH, Hβ, Hδ; Val[4]: NH
β	1.18 and 1.45 (each 1H, m)	β	41.68	Leu[3]: NH; Val[4]: NH
γ	1.41 (1H, m)	γ	24.02	Leu[3]: Hδ
δ	0.67 (3H, d, 6.0)	δ	20.97	
	0.77 (3H, d, 6.0)		23.12	
NH	8.33 (1H, d, 9.3)	C=O	172.28	β^s-Leu[2]: Hα; Trp[5]: H4, H5; Val[4]: NH
Val[4]				
α	3.62 (1H, t, 7.3)	α	57.42	Val[4]: Hβ, Hγ, NH; Trp[5]: NH
β	1.82 (1H, m)	β	31.13	Val[4]: Hγ, NH
γ	0.71 (3H, d, 6.1)	γ	18.35	Val[4]: NH, Trp[5]: H4, H5
	0.72 (3H, d, 6.1)		18.57	
NH	6.96 (1H, d, 7.9)	C=O	169.52	Trp[5]: NH, H4
Trp[5]				
α	5.63 (1H, m)	α	47.26	Trp[5]: NH, H4, H5, Hβ; Pro[6]: Hδ, Hγ
β	2.60 (1H, t, 13.0)	β	25.52	Pro[6]: Hδ; Arg[7]: NH; His[8]: H2; Trp[5]: H4
	3.34 (1H, dd, 5.8, 15.0)	C2	126.67	
NH1	11.89 (1H, s)	C3	103.46	Trp[5]: H7; His[8]: H2, H4
H4	7.57 (1H, d, 8.3)	C4	120.07	
H5	7.03 (1H, d, 8.3)	C5	119.90	
H7	6.96 (1H, s)	C6	133.08	
NH	8.14 (1H, d, 8.5)	C7	114.37	
		C8	132.40	
		C9	124.87	
		C=O	169.15	
Pro[6]				
α	4.14 (1H, t, 6.6)	α	61.61	Pro[6]: Hβ, Hγ; Arg[7]: NH
β	1.84 (1H, m)	β	29.11	Pro[6]: Hδ
	2.23 (1H, m)	γ	25.02	Pro[6]: Hδ
γ	2.01 (2H, m)	δ	46.99	
δ	3.80 (1H, m)	C=O	171.22	
	3.99 (1H, m)			
Arg[7]				
α	4.22 (1H, m)	α	52.05	Arg[7]: Hβ, Hγ; Arg[7]: NH; His[8]: NH
β	1.62 (1H, m)	β	29.94	Arg[7]: Hδ
	1.76 (1H, m)	γ	24.19	Arg[7]: Hδ
γ	1.47 (2H, m)	δ	40.55	Arg[7]: Hϵ(NH)
δ	3.08 (2H, m)	ϵ	156.94	
ϵ(NH)	7.70 (1H, br s)	C=O	171.50	
NH	6.83 (1H, d, 5.9)			His[8]: NH
His[8]				
α	4.92 (1H, t, 10.3)	α	50.19	His[8]: NH, Hβ, H2
β	2.96 (1H, t, 13.5)	β	28.19	
	3.43 (1H, d, 15.8)	C1	131.56	
H2	7.79 (1H, brs)	C2	120.07	His[8]: NH
H4	9.41 (1H, brs)	C4	136.80	
NH	8.85 (1H, d, 8.6)	C=O	171.35	

n-BuOH-soluble materials were subjected to a Diaion HP-20 column (MeOH/H_2O, 0:1 → 1:0), in which a fraction eluted with 60% MeOH was purified by an amino silica gel column ($CHCl_3$/MeOH/H_2O, 7:3:0.5 → 6:4:1) followed by C_{18} HPLC (CH_3CN/0.1% CF_3CO_2H, 22:78) to afford celogentin A (**1**, 0.0002% yield) and moroidin (**4**, 0.02%) as a colorless solid. The fraction eluted with 80% MeOH through the Diaion HP-20 column was purified by the same methods as described above to afford celogentins B (**2**, 0.0001%) and C (**3**, 0.001%) as colorless solid.

Celogentin A (1): colorless solid; $[\alpha]^{23}_D$ −43° (*c* 0.3, 50% MeOH); UV (MeOH) λ_{max} (log ϵ) 283 (3.8) and 226 (4.3) nm; IR (KBr) ν_{max} 3400, 2960, 1660, and 1545 cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); FABMS *m*/*z* 930 (M + H)$^+$; HRFABMS *m*/*z* 930.4991 (M + H; calcd for $C_{45}H_{64}N_{13}O_9$, 930.4950).

Celogentin B (2): colorless solid; $[\alpha]^{23}_D$ −32° (*c* 0.5, 50% MeOH); UV (MeOH) λ_{max} (log ϵ) 282 (3.6) and 225 (4.2) nm; IR (KBr) ν_{max} 3390, 2930, 1660, and 1380 cm^{-1}; ^{1}H and ^{13}C NMR (Table 2); FABMS *m*/*z* 1067 (M + H)$^+$; HRFABMS *m*/*z* 1067.5590 (M + H; calcd for $C_{51}H_{71}N_{16}O_{10}$, 1067.5585).

Celogentin C (3): colorless solid; $[\alpha]^{23}_D$ −54° (*c* 0.5, 50% MeOH); UV (MeOH) λ_{max} (log ϵ) 283 (3.7) and 226 (4.2) nm; IR (KBr) ν_{max} 3280, 2960, 1660, 1530, and 1200 cm^{-1}; ^{1}H and ^{13}C NMR (Table 3); FABMS *m*/*z* 1027 (M + H)$^+$; HRFABMS *m*/*z* 1027.5450 (M + H; calcd for $C_{50}H_{71}N_{14}O_{10}$, 1027.5477).

Amino Acid Analysis of 1−3. Each solution of **1**−**3** (0.1 mg each) in 6 N HCl was heated at 110 °C for 24 h in a sealed tube. After cooling, each solution was concentrated to dryness.

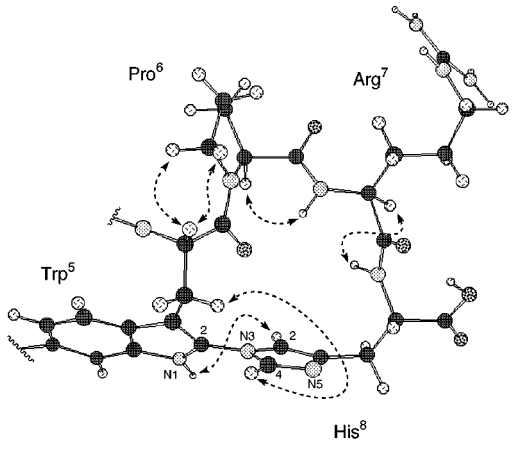

Figure 4. Selected NOESY correlations (dotted arrows) for celogentin C (**3**). Right-hand part consists of a 17-membered ring. To clarify the backbone structure, the left-hand part was omitted.

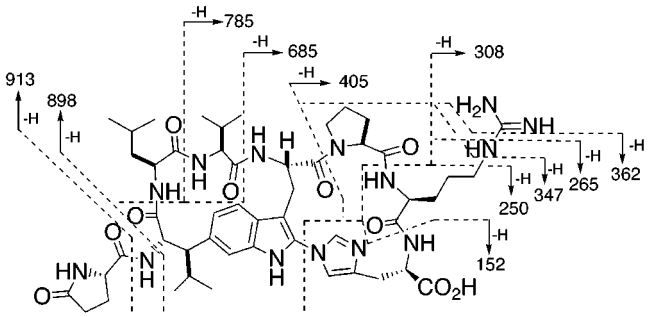

Figure 5. Fragmentation patterns observed in negative-ion FABMS/MS spectrum of celogentin C (**3**) (precursor ion *m*/*z* 1025.5)

The hydrolysates were dissolved in 0.02 N HCl and were subjected to amino acid analyzer.

Absolute Configuration of Amino Acids. Each solution of **1**−**3** (each 0.1 mg) in 6 N HCl (0.2 mL) was heated at 110° for 24 h. The solution was concentrated to dryness. The residue was dissolved in H_2O (50 μL), and chiral HPLC analyses were carried out using a SUMICHIRAL OA-5000 column (Sumitomo Chemical Industry; 150 mm; 25 °C, detection at 254 nm). Retention times (min) of authentic amino acids were as follows: L-Glu (19.2), D-Glu (24.2), L-Val (6.1), D-Val (9.0), L-Arg (2.2), D-Arg (2.4), L-His (9.4), D-His (7.8), L-Leu (13.6), and D-Leu (20.2) [eluent: MeOH/H_2O (15:85) containing 2.0 mM $CuSO_4$, flow rate 1.0 mL/min]. Retention times of the hydrolysates of **1**−**3** were as follows: **1**, L-Glu (19.1), L-Val (6.1), L-Arg (2.2), L-His (9.4), and L-Leu (13.6); **2**, L-Glu (19.1), L-Val (6.1), L-Arg (2.2), L-His (9.4), and L-Leu (13.6); **3**, L-Glu (19.1), L-Val (6.1), L-Arg (2.2), L-His (9.4), and L-Leu (13.6).

Absolute Configuration of Trp. Each celogentin A−C (each 0.1 mg) in AcOH (0.2 mL) was treated with ozone at −78 °C for 1 min. After removal of excess ozone by a stream of nitrogen, the mixture was treated with 30% H_2O_2 (200 μL) at room temperature for 3 h. The reaction mixture was concentrated and was hydrolyzed with 6 N HCl (100 μL) at 110 °C for 6 h. The hydrolysate was subjected to chiral HPLC analyses [SUMICHIRAL OA-5000, 4 × 150 mm; 40 °C, flow rate, 1.0 mL/min; eluent; MeOH/H_2O (15:85) containing 2.0 mM $CuSO_4$]. The retention times of authentic L- and D-Asp were found to be 12.9 and 17.0 min, respectively. The retention time of Asp in the degradation products of **1**−**3** was found to be 12.9 min (L-Asp).

Conversion of Celogentin A (1) to Celogentin B (2). Coupling reaction was carried out by preparing a solution of 1.29 μmol of histidine hydrochloride (0.2 mg), 2.21 μmol of HOAt (0.3 mg), and 0.86 μmol of celogentin A (**1**, 0.8 mg) in 20 μL of DMF. The mixture was cooled in an ice bath and treated with 1.56 μmol of WSC (0.3 mg). After 20 h, solvent was removed under reduced pressure and the residue was dissolved in MeOH and purified by C_{18} HPLC (CH_3CN/0.1% CF_3CO_2H, 22:78) to afford a peptide (0.1 mg), whose spectral data were identical with those of natural celogentin B (**2**).

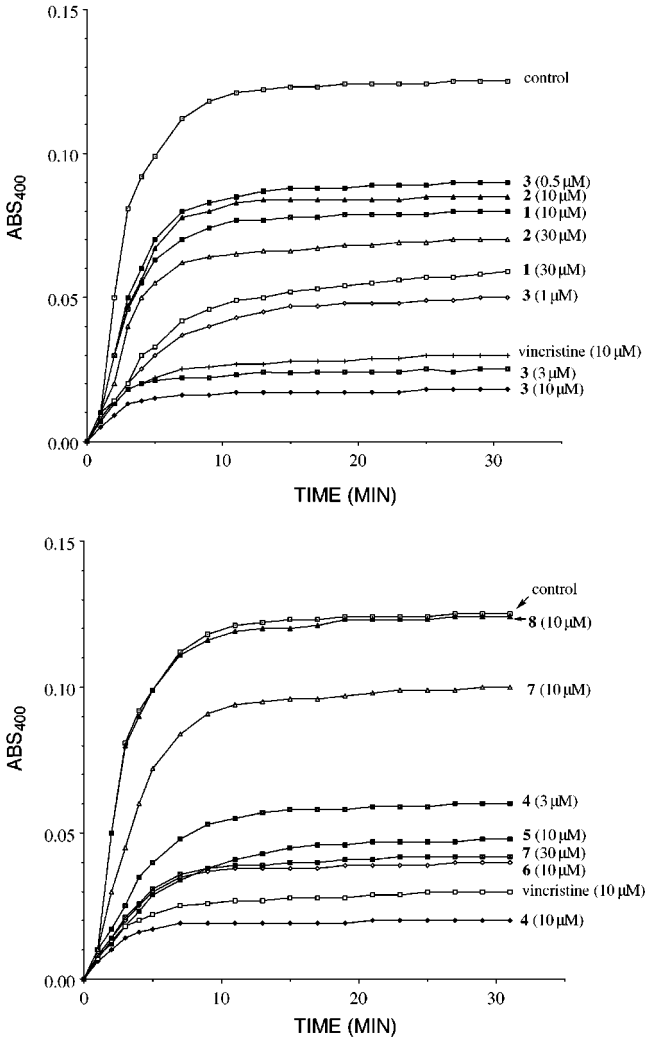

Figure 6. Inhibitory effects of celogentins A (**1**), B (**2**), and C (**3**), moroidin (**4**), moroidin methylate (**5**), pyrimidine derivative of moroidin (**6**), moroidin hydrolysate (**7**), stephanotic acid (**8**), and vincristine on the in vitro polymerization of microtubule protein. Various concentrations of compounds were mixed with microtubule protein (1.0 mg/mL) at 0 °C and incubated at 37 °C.

Methylation of Moroidin (4). Trimethylsilyldiazomethane (2.0 M hexane solution, 100 μL) was added to a stirred solution of moroidin (**4**, 1 mg) in methanol (0.5 mL) at room temperature. The mixture was stirred at room temperature for 5 min, and concentrated in vacuo. The residue was subjected to an amino silica gel column chromatography to give the methyl derivative (**5**) as colorless solid; IR (KBr) ν_{max} 3420, 1730, 1680, 1435 cm^{-1}; ^1H NMR (600 MHz in DMSO-d_6, 320 K) δ 0.71 and 0.79 (each 3H, d, 6.0, Leu^3-Hδ), 0.79 and 0.86 (each 3H, d, 6.0, β^s-Leu^2-Hδ), 0.83 and 0.86 (each 3H, d, 6.0, Val^4 −Hγ), 1.73 and 2.10 (each 1H, m, PyroGlu^1-Hγ), 2.03 (1H, m, Val-Hβ), 2.16 (1H, m, β^s-Leu^2-Hγ), 2.26 (2H, m, PyroGlu^1-Hβ), 2.70 (1H, dd, 7.7, 15.1, Trp^5-Hβ), 2.93 (1H, dd, 9.9, 15.6, His^8-Hβ), 3.02 (1H, dd, 3.4, 11.8, β^s-Leu^2-Hβ), 3.06 (1H, dd, 1.0, 7.2, His^8-Hβ), 3.34 (1H, m, Trp^5-Hβ), 3.62 (1H, dd, 5.2, 16.0, Gly^7-Hα), 3.69 (3H, s, OCH_3), 3.73 (1H, dd, 3.4, 16.0, Gly^7-Hα), 3.87 (1H, m, Val^4-Hα), 4.04 (1H, m, Leu^3-Hα), 4.13 (1H, dd, 3.3, 8.9, PyroGlu^1-Hα), 4.26 (1H, m, Arg^6-Hα), 4.69 (1H,

m, His[8]-Hα), 4.88 (1H, m, β[s]-Leu[2]-Hα), 5.28 (1H, m, Trp[5]-Hα), 6.80 (1H, br s, Val[4]-NH), 6.89 (1H, s, Trp[5]-H7), 7.04 (1H, d, 8.4, Trp[5]-H5), 7.38 (1H, br s, Trp[5]-NH), 7.47 (1H, d, 8.4, Trp[5]-H4), 7.73 (1H, br s, His[8]-H2), 7.77 (1H, s, PyroGlu[1]-NH), 7.91 (1H, br s, Gly[7]-NH), 8.35 (1H, br s, Leu[3]-NH), 8.38 (1H, br s, Arg[6]-NH), 8.39 (1H, br s, β[s]-Leu[2]-NH), 8.40 (1H, br s, His[8]-NH), 11.41 (1H, s, Trp[5]-NH1); FABMS *m*/*z* 1001 $(M + H)^+$; HRFABMS *m*/*z* 1001.5310 (M + H; calcd for $C_{48}H_{69}N_{14}O_{10}$, 1001.5321).

Conversion of Moroidin (4) to Pyrimidine Derivative (6). A solution of moroidin (**4**, 2 mg) in pyridine (0.4 mL) and 2,4-pentanedione (0.1 mL) was heated at 110 °C for 12 h in a sealed tube. The solution was evaporated in vacuo, and the residue was separated by C_{18} HPLC (CH_3CN/0.1% CF_3CO_2H, 1:3) to give **6** (1.5 mg) as a colorless solid: UV (MeOH) λ_{max} (log ϵ) 292 (3.8), 284 (3.8) and 228 (4.3) nm; IR (KBr) ν_{max} 3400, 1650, 1530, and 1440 cm^{-1}; ^1H NMR (600 MHz in DMSO-d_6, 300K) δ 0.69 and 0.76 (each 3H, d, 6.2, Leu[3]-Hδ), 0.78 and 0.84 (each 3H, d, 6.0, β[s]-Leu[2]-Hδ), 0.78 (6H, d, 6.0, Val[4] −Hγ), 1.42 (1H, m, Leu[3]-Hγ), 1.70 and 2.09 (each 1H, m, PyroGlu[1]-Hγ), 1.89 (1H, m, Val-Hβ), 2.17 (6H, s, pyrimidine-Me), 2.25 (2H, m, PyroGlu[1]-Hβ), 2.63 (1H, m, Trp[5]-Hβ), 2.86 (1H, m, His[8]-Hβ), 3.03 (1H, br d, 11.2, β[s]-Leu[2]-Hβ), 3.10 (1H, br d, 14.7, His[8]-Hβ), 3.33 (1H, m, Trp[5]-Hβ), 3.62 (1H, br d, 14.0, Gly[7]-Hα), 3.69 (1H, br d, 14.0, Gly[7]-Hα), 3.73 (1H, br t, 7.0, Val[4]-Hα), 3.99 (1H, br t, 8.9, Leu[3]-Hα), 4.10 (1H, dd, 2.0, 7.0, PyroGlu[1]-Hα), 4.16 (1H, m, Arg[6]-Hα), 4.71 (1H, m, His[8]-Hα), 4.85 (1H, br t, 10.0, β[s]-Leu[2]-Hα), 5.39 (1H, m, Trp[5]-Hα), 6.30 (1H, s, pyrimidine-H5), 6.85 (1H, s, Trp[5]-H7), 6.91 (1H, d, 7.6, Val[4]-NH), 6.98 (1H, d, 8.4, Trp[5]-H5), 7.29 (1H, br s, His[8]-H2), 7.48 (1H, br s, Gly[7]-NH), 7.49 (1H, d, 8.4, Trp[5]-H4), 7.78 (1H, br s, Trp[5]-NH), 7.86 (1H, s, PyroGlu[1]-NH), 8.37 (1H, d, 8.9, Leu[3]-NH), 8.49 (2H, m, Arg[6]-NH and His[8]-NH), 8.53 (1H, d, 8.8, β[s]-Leu[2]-NH), 11.43 (1H, s, Trp[5]-NH1); FABMS *m*/*z* 1049 $(M - H)^-$.

Enzymatic Hydrolysis of Moroidin (4). α-Chymotrypsin (0.25 mg dissolved in 50 μL of 0.001% HCl, Merck) was added to NH_4HCO_3 solution (1%, 0.45 mL) of moroidin (**4**, 0.5 mg) and the digestion was performed at 37 °C with the pH maintained at 8.0 by addition of 0.1 N HCl. After 2 days, the reaction was stopped by adjusting the solution to pH 2.2 with 1 N HCl and the digestion mixture was lyophilized to dryness and subjected to HPLC (Develosil ODS-HG-5 column, 10 mm i.d. × 250 mm, Nomura Chemical, eluted with 17% $CHCN_3$/0.05%TFA, flow rate 2 mL/min) to give the hydrolysate (**7**) as an amorphous powder: UV (MeOH) λ_{max} (log ϵ) 283 (3.7) and 226 (4.2) nm; IR (KBr) ν_{max} 3430, 1680, 1435, 1210 cm^{-1}; ^1H NMR (600 MHz in DMSO-d_6, 320K) δ 0.70 and 0.76 (each 3H, d, 6.5, Leu[3]-Hδ), 0.77 and 0.85 (each 3H, d, 6.5, β[s]-Leu[2]-Hδ), 0.79 and 0.82 (each 3H, d, 6.6, Val[4]-Hγ), 1.43 (1H, m, β[s]-Leu[2]-Hγ), 1.77 and 2.25 (each 1H, m, PyroGlu[1]-Hγ), 1.87 (1H, m, Val[4]-Hβ), 2.10 (2H, m, PyroGlu[1]-Hβ), 2.94 (1H, m, Trp[5]-Hβ), 2.93 (1H, dd, 9.9, 15.6, His[8]-Hβ), 3.02 (1H, dd, 3.4, 11.8, β[s]-Leu[2]-Hβ), 3.06 (1H, dd, 1.0, 7.2, His[8]-Hβ), 3.25 (1H, m, Trp[5]-Hβ), 3.62 (1H, d, 11.3, Gly[7]-Hα), 3.73 (1H, dd, 3.4, 16.0, Gly[7]-Hα), 3.75 (1H, t, 7.5, Val[4]-Hα), 4.02 (1H, m, Leu[3]-Hα), 4.14 (1H, dd, 3.8, 8.0, PyroGlu[1]-Hα), 4.20 (1H, m, Arg[6]-Hα), 4.60 (1H, m, His[8]-Hα), 4.82 (1H, t, 10.0, β[s]-Leu[2]-Hα), 5.48 (1H, br s, Trp[5]-Hα), 7.11 (1H, d, 7.5, Val[4]-NH), 6.91 (1H, s, Trp[5]-H7), 6.96 (1H, d, 8.4, Trp[5]-H5), 8.17 (1H, d, 8.6, Trp[5]-NH), 7.56 (1H, d, 8.4, Trp[5]-H4), 7.90 (1H, br s, His[8]-H2), 7.27 (1H, br s, His[8]-H4), 7.80 (1H, s, PyroGlu[1]-NH), 7.42 (1H, d, 7.5, Gly[7]-NH), 8.29 (1H, d, 9.0, Leu[3]-NH), 7.99 (1H, d, 6.8, Arg[6]-NH), 8.39 (1H, d, 10.0, β[s]-Leu[2]-NH), 8.65 (1H, d, 7.0, His[8]-NH), 11.50 (1H, s, Trp[5]-NH1); FABMS *m*/*z* 1005 $(M + H)^+$.

Preparation of Microtubule Protein. Microtubule protein was prepared from porcine brain as described previously.[17] The protein concentrations were determined by the method of Lowry et al.[18] using bovine serum albumin as a standard. Microtubule assembly assays were carried out in MES buffer containing 100 mM 2-N-morpholino ethanesulfonic acid (MES), 1 mM ethylenebis(oxyethylenenitrilo)tetraacetic acid (EGTA), 0.5 mM $MgCl_2$, 1 mM 2-mercaptoethanol, and 1 mM guanosine 5′′-triphosphate trisodium salt (GTP) (pH 6.5).

Microtubule Assembly Assay. Microtubule assembly was monitored spectroscopically by using a spectrophotometer equipped with a thermostatically regulated liquid circulator. The temperature was held at 37 °C and changes in turbidity were monitored at 400 nm. For the drug−protein studies, 10 μM of drug dissolved in DMSO concentration was less than 1%. The turbidity changes were monitored throughout the incubation time.

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Supporting Information Available: 1D and 2D NMR spectra for compounds **1**−**3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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