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Bioactive *neo*-Clerodane Diterpenoids from the Whole Plants of *Ajuga ciliata* Bunge

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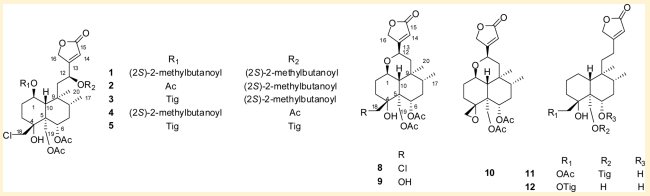
Supporting Information

ABSTRACT: Ten new *neo*-clerodane diterpenes, ajugaciliatins A–J (**1**–**5**, **8**–**12**), along with 17 known analogues (**6**, **7**, **13**–**27**) were isolated from the whole plants of *Ajuga ciliata* Bunge. Their structures were elucidated by spectroscopic data analysis (IR, ESIMS, HRESIMS, 1D and 2D NMR), and the configuration of **1** was confirmed by X-ray crystallography. All of the compounds were assessed for neuroprotective effects against MPP^+-induced neuronal cell death in dopaminergic neuroblastoma SH-SY5Y cells. Compounds **2**, **6**, **7**, **9**, **10**, **15**–**17**, **19**, and **20** exhibited moderate neuroprotective effects.



The genus *Ajuga*, a member of the Labiatae family, is distributed over the temperate parts of Asia and Europe. Several species of this genus have been reported to be rich sources of bioactive metabolites, including diterpenes,[1−19] steroids,[20−26] and iridoids,[27−32] which display insect antifeedant,[4,33] antibacterial,[2,3] antimycobacterial,[34] antiplasmodial,[28] cytotoxic, and vasoconstrictor activities.[35,36] The whole plants of *Ajuga ciliata* Bunge are used in folk medicine in China for the treatment of inflammation. Although many bioactive constituents of the genus *Ajuga* have been reported, phytochemical and pharmacological studies on *A. ciliata* are limited. In the course of our search for bioactive metabolites having neuroprotective effects, 10 new *neo*-clerodane diterpenes, ajugaciliatins A–J (**1**–**5**, **8**–**12**), and 17 known analogues (**6**, **7**, **13**–**27**) have been isolated from the whole plants of *A. ciliata*. In this paper, we report the isolation and structural elucidation of these clerodane diterpenes and their neuroprotective activities against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPP^+)-induced neuronal cell death in human dopaminergic neuroblastoma SH-SY5Y cells.

RESULTS AND DISCUSSION

Compound **1** was obtained as colorless flakes. Its molecular formula was determined as $C_{34}H_{51}ClO_{11}$ by HR-ESIMS (*m*/*z* 693.3021 $[M(^{35}Cl) + Na]^+$, 695.2986 $[M(^{37}Cl) + Na]^+$). The presence of hydroxy (3496 cm^{-1}) and carbonyl groups (1782 and 1729 cm^{-1}) was evident in its IR spectrum. The ^{13}C NMR spectroscopic data of **1** (Table 1) exhibited 20 typical resonances for a *neo*-clerodane diterpene skeleton,[9−11] including two methyl [(δ_C 15.5 (C-17) and 17.7 (C-20)], seven methylene [δ_C 29.5 (C-2), 30.4 (C-3), 32.4 (C-7), 43.0 (C-11), 70.6 (C-16), 49.2 (C-18), and 62.8 (C-19)], six methine [δ_C 70.0 (C-1), 74.3 (C-6), 35.6 (C-8), 47.5 (C-10), 66.3 (C-12), and 115.7 (C-14)], and five quaternary [δ_C 76.2 (C-4), 50.0 (C-5), 39.5 (C-9), 169.0 (C-13), and 172.5 (C-15)] carbons. Corresponding to the above carbon signals, the ^1H NMR spectrum of **1** (Table 2) showed characteristic proton resonances of a *neo*-clerodane diterpene, including one tertiary methyl group at δ_H 0.84 (3H, s, H_3-20), one secondary methyl group at δ_H 0.87 (3H, d, J = 6.2 Hz, H_3-17), three groups of downfield methylene protons at δ_H 4.89 and 4.76 (each, 1H, dd, J = 17.6, 1.6 Hz, H-16a and H-16b), 4.03 and 3.86 (each, 1H, d, J = 11.7 Hz, H-18a and H-18b), and 4.93 and 4.72 (each, 1H, d, J = 13.3 Hz, H-19a and H-19b), three oxygenated methine protons at δ_H 5.73 (1H, td, J = 11.3, 4.5 Hz, H-1), 5.00 (1H, dd, J = 9.8, 5.2 Hz, H-6), and 5.89 (1H, br d, J = 8.0 Hz, H-12), one olefinic proton at δ_H 5.96 (1H, br s, H-14), and partially overlapped resonances due to three methylenes and two methines from δ_H 1.25 to δ_H 2.70. In addition, two sets of acetoxy substituents [δ_H 2.05 (3H, s, H_3-2″); δ_C 169.8 (C-1″) and 21.6 (C-2″) and δ_H 2.19 (3H, s, H_3-2‴′); δ_C 170.2 (C-1‴′) and 21.4 (C-2‴′)] were connected to C-6 and C-19, which was established by the HMBC correlations of H-6/C-1″ and H_2-19/C-1‴′, respectively. Also, the locations of two sets of 2-methylbutanoyloxy units [δ_H 2.31 (1H, qt, J = 6.9, 6.9 Hz, H-2′), 1.55 and 1.73 (each, 1H, m, H-3′a and H-3′b), 0.91 (3H, t, J = 7.4 Hz, H_3-4′), and 1.16 (3H, d, J = 6.9 Hz, H_3-5′); δ_C 175.8 (C-1′), 41.6 (C-2′), 27.0 (C-3′), 11.6 (C-4′), and 16.3 (C-5′) and δ_H 2.45 (1H, qt, J = 6.9, 7.2 Hz, H-2‴), 1.55 and 1.73 (each, 1H, m, H-3‴a and H-3‴b), 0.94 (3H, t, J = 7.4 Hz, H_3-4‴), and 1.20 (3H, d, J = 6.9 Hz, H_3-5‴); δ_C 175.8 (C-1‴), 40.6 (C-2‴), 27.0 (C-3‴), 11.3 (C-4‴), and

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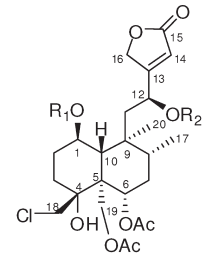
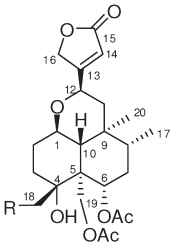
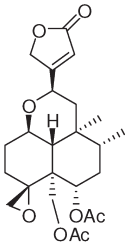


	R_1	R_2
1	(2*S*)-2-methylbutanoyl	(2*S*)-2-methylbutanoyl
2	Ac	(2*S*)-2-methylbutanoyl
3	Tig	(2*S*)-2-methylbutanoyl
4	(2*S*)-2-methylbutanoyl	Ac
5	Tig	Tig
6	Tig	Ac
7	Tig	H



	R
8	Cl
9	OH



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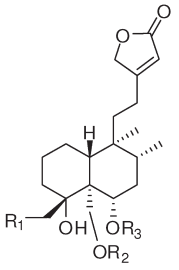
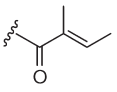
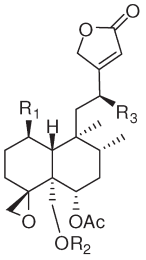


	R_1	R_2	R_3
11	OAc	Tig	H
12	OTig	H	H
13	Cl	Tig	Ac
14	OH	Tig	H



Tig: tigloyl



	R_1	R_2	R_3
15	(2*S*)-2-methylbutanoyloxy	Ac	(2*S*)-2-methylbutanoyloxy
16	(2*S*)-2-methylbutanoyloxy	Ac	OTig
17	OTig	Ac	OTig
18	OTig	Ac	(2*S*)-2-methylbutanoyloxy
19	OTig	Ac	OH
20	OAc	Ac	OTig
21	H	Tig	H
22	(2*S*)-2-methylbutanoyloxy	Ac	OH
23	H	Ac	OH
24	H	Ac	OTig
25	OTig	Ac	OAc
26	OAc	Ac	OAc
27	OAc	Ac	OH

Figure 1. Structures of compounds **1**−**27** from *A. ciliata*.

15.1 (C-5‴)] at C-1 and C-12 were confirmed by the HMBC correlations of H-1/C-1′ and H-12/C-1‴ (Figure 2), respectively. Thus, the planar structure of **1** was established.

The relative configuration of **1** was elucidated by the NOESY spectrum (Figure 3). NOESY correlations observed for H-1/H-2α, H-2β/H_2-18, and H-10/H_2-18 but not for H-1/H-3α and H-3α/H_2-19 in ring A and correlations for H-6/H-8, H-10/H-8, H-6/H-10, H-7α/H_3-20, and H_2-19/H_3-20 in ring B suggested that the two six-membered rings were *trans*-fused and existed in a twist-boat conformation for ring A and a chair conformation for ring B, respectively. H-10 and H_2-18 were β-axially oriented, H_2-19 and H_3-20 were α-axially oriented, and H_3-17 was α-equatorially oriented, respectively. These assignments were consistent with the configuration of reported *neo*-clerodane diterpenes.[1,10,11] All of the known clerodane-type diterpenes from the genus *Ajuga* possess the *neo*-clerodane absolute configuration.[13] The assignment of the 12*S* absolute configuration of **1** was based on comparison of the ^{13}C NMR chemical shifts of C-11 (δ_C 43.0), C-12 (δ_C 66.3), C-13 (δ_C 169.0), C-14 (δ_C 115.7), C-15 (δ_C 172.5), and C-16 (δ_C 70.6) in **1** with those of diacetylajugamarin E1 [C-11 (δ_C 41.6), C-12 (δ_C 66.4), C-13 (δ_C 169.1), C-14 (δ_C 116.0), C-15 (δ_C 172.4), and C-16 (δ_C 70.6)].[9] The 2′*S* and 2‴*S* absolute configuration of the two 2-methylbutanoyloxy groups was inferred from those of the co-occurring diterpenes like **15**, **16**, **18**, and **22** in the *Ajuga* genus.[10,15,16] Ultimately, the absolute configuration of **1** was confirmed by X-ray crystallographic analysis (Figure 4).[37] On the basis of the above analysis, the structure of **1** was determined to be (12*S*,2′*S*,2‴*S*)-6α,19-diacetoxy-18-chloro-4α-hydroxy-1β,12-(di-2-methylbutanoyloxy)-*neo*-clerod-13-en-15,16-olide, which is named ajugaciliatin A.

Analysis of the ^{13}C and ^{1}H NMR spectroscopic data of compounds **2**−**5** (Tables 1 and 2), aided by DEPT, ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY spectral data, revealed that all of the compounds had characteristic *neo*-clerodane skeletons closely resembling those of **1**. The only difference between **2** and **1** was that the 2-methylbutanoyloxy moiety at C-1 in **1** was replaced by an acetoxy moiety [δ_H 2.07 (3H, s, H_3-2′); δ_C 169.4 (C-1′) and 21.8 (C-2′)] in **2**. The difference between **3** and **1** was that the 2-methylbutanoyloxy unit at C-1 in **1** was replaced by a tigloyloxy unit [δ_H 7.02 (1H, qd, *J* = 7.0, 1.1 Hz, H-3′), 1.82

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Table 1. ^{13}C NMR Spectroscopic Data (δ_C) of Compounds 1−5 and 8−12 ($CDCl_3$, 100 MHz)[a]

position		1	2	3	4	5	position		8	9	10	position		11	12
1		70.0	70.2	70.0	70.0	70.0	1		71.1	71.2	70.5	1		22.0	22.5
2		29.5	29.1	29.7	29.5	29.3	2		28.5	28.6	31.4	2		21.0	20.7
3		30.4	30.3	30.4	30.5	30.3	3		29.7	30.7	30.1	3		30.1	31.4
4		76.2	76.3	76.2	76.2	76.3	4		75.3	75.7	64.7	4		77.0	80.0
5		50.0	49.9	50.1	50.0	50.2	5		47.9	46.7	43.4	5		47.1	47.7
6		74.3	74.2	74.3	74.3	73.8	6		73.5	75.9	72.6	6		74.4	76.0
7		32.4	32.5	32.4	32.4	32.6	7		32.6	32.0	32.6	7		35.5	35.5
8		35.6	35.8	35.7	35.6	35.1	8		41.8	41.8	41.4	8		34.9	35.2
9		39.5	39.5	39.5	39.5	39.7	9		35.7	35.7	35.4	9		38.8	38.8
10		47.5	47.2	48.3	47.3	47.9	10		51.5	51.5	53.6	10		44.2	44.0
11		43.0	43.5	42.3	43.0	41.1	11		47.0	47.2	45.9	11		35.2	35.6
12		66.3	66.0	66.6	66.5	66.6	12		69.2	69.1	69.2	12		21.9	22.2
13		169.0	168.9	168.8	168.6	169.0	13		170.5	170.4	170.4	13		170.9	170.3
14		115.7	115.8	115.8	116.0	115.8	14		114.5	114.6	114.5	14		114.9	115.2
15		172.5	172.5	172.4	172.4	172.4	15		173.8	173.7	173.5	15		174.3	174.0
16		70.6	70.6	70.6	70.5	70.7	16		71.2	71.2	71.0	16		73.2	73.1
17		15.5	15.7	15.5	15.5	15.3	17		14.1	14.2	14.0	17		15.4	15.5
18		49.2	49.3	49.2	49.3	49.3	18		49.9	64.5	50.2	18		66.6	66.2
19		62.8	62.8	62.9	62.8	63.1	19		63.3	63.3	63.3	19		64.1	63.1
20		17.7	17.6	17.6	17.7	17.9	20		14.4	14.7	14.3	20		17.8	18.8
1-OR	1′	175.8	169.4	166.7	175.8	166.6	6-OAc	1′	170.3	170.2	169.8	18-OR	1′	171.5	168.3
	2′	41.6	21.8	129.5	41.5	129.4		2′	21.4	21.6	21.0		2′	21.0	128.3
	3′	27.0		138.0	27.1	138.0	19-OAc	1″	170.1	170.2	170.4		3′		138.3
	4′	11.6		14.5	11.6	14.5		2″	21.6	21.6	21.2		4′		14.5
	5′	16.3		12.3	16.3	12.4							5′		12.1
6-OAc	1″	169.8	169.8	169.7	169.8	169.8						19-OTig	1″	168.0	
	2″	21.6	21.6	21.6	21.6	21.5							2″	128.5	
12-OR	1‴	175.8	175.7	175.9	169.8	166.7							3″	137.6	
	2‴	40.6	40.7	40.7	21.1	128.2							4″	14.5	
	3‴	27.0	27.0	27.1		140.8							5″	12.2	
	4‴	11.3	11.5	11.4		14.8									
	5‴	15.1	15.3	15.1		12.2									
19-OAc	1⁗	170.2	170.1	170.2	170.2	170.2									
	2⁗	21.4	21.3	21.4	21.4	21.4									

[a] Assignments of ^{13}C NMR data are based on DEPT, $^1H-^1H$ COSY, HMQC, and HMBC experiments.

(3H, d, J = 7.0 Hz, H_3-4′), and 1.88 (3H, br s, H_3-5′); δ_C 166.7 (C-1′), 129.5 (C-2′), 138.0 (C-3′), 14.5 (C-4′), and 12.3 (C-5′)] in **3**. Compound **4** possessed an acetoxy moiety [δ_H 2.17 (3H, s, H_3-2‴); δ_C 169.8 (C-1‴) and 21.1 (C-2‴)] at C-12 instead of the 2-methylbutanoyloxy moiety in **1**. Compound **5** possessed two tigloyloxy groups [δ_H 6.93 (1H, qd, J = 7.0, 1.1 Hz, H-3′), 1.78 (3H, d, J = 7.0 Hz, H_3-4′), and 1.86 (3H, br s, H_3-5′); δ_C 166.6 (C-1′), 129.4 (C-2′), 138.0 (C-3′), 14.5 (C-4′), and 12.4 (C-5′) and δ_H 7.15 (1H, qd, J = 7.1, 1.1 Hz, H-3‴), 1.91 (3H, d, J = 7.1 Hz, H_3-4‴), and 1.96 (3H, br s, H_3-5‴); δ_C 166.7 (C-1‴), 128.2 (C-2‴), 140.8 (C-3‴), 14.8 (C-4‴), and 12.2 (C-5‴)] at C-1 and C-12 rather than two 2-methylbutanoyloxy groups in **1**. Therefore, **2**−**5** were elucidated as (12*S*,2‴*S*)-1β,6α,19-triacetoxy-18-chloro-4α-hydroxy-12-(2-methylbutanoyloxy)-*neo*-clerod-13-en-15,16-olide (**2**), (12*S*,2‴*S*)-6α,19-diacetoxy-18-chloro-4α-hydroxy-12-(2-methylbutanoyloxy)-1β-tigloyloxy-*neo*-clerod-13-en-15,16-olide (**3**), (12*S*,2′*S*)-6α,12,19-triacetoxy-18-chloro-4α-hydroxy-1β-(2-methylbutanoyloxy)-*neo*-clerod-13-en-15,16-olide (**4**), and (12*S*)-6α,19-diacetoxy-18-chloro-4α-hydroxy-1β,12-ditigloyloxy-*neo*-clerod-13-en-15,16-olide (**5**), which are named ajugaciliatins B−E, respectively.

Compound **8** was obtained as colorless flakes, and the molecular formula was determined to be $C_{24}H_{33}ClO_8$ by HR-ESIMS (m/z 507.1762 [M(^{35}Cl) + Na]$^+$, 509.1764 [M(^{37}Cl) + Na]$^+$). The ^{13}C NMR spectroscopic data of **8** (Table 1) also displayed 20 characteristic carbon resonances for a *neo*-clerodane diterpene skeleton, including two methyl [(δ_C 14.1 (C-17) and 14.4 (C-20)], seven methylene [δ_C 28.5 (C-2), 29.7 (C-3), 32.6 (C-7), 47.0 (C-11), 71.2 (C-16), 49.9 (C-18), and 63.3 (C-19)], six methine [δ_C 71.1 (C-1), 73.5 (C-6), 41.8 (C-8), 51.5 (C-10), 69.2 (C-12), and 114.5 (C-14)], and five quaternary [δ_C 75.3 (C-4), 47.9 (C-5), 35.7 (C-9), 170.5 (C-13), and 173.8 (C-15)] carbons. The ^1H NMR spectrum of **8** (Table 3) showed typical proton signals of the *neo*-clerodane diterpene including one tertiary methyl group at δ_H 1.06 (3H, s, H_3-20), one secondary methyl group at δ_H 0.87 (3H, d, J = 6.7 Hz, H_3-17), three groups of downfield methylene protons at δ_H 4.87 (2H, br s, H_2-16), 4.09 and 4.00 (each, 1H, d, J = 11.3 Hz, H-18a and H-18b), and

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Table 2. ^{1}H NMR Spectroscopic Data (δ_H) of Compounds 1−5 ($CDCl_3$, 400 MHz)[a]

position		1	2	3	4	5
1		5.73 td (11.3, 4.5)	5.71 td (11.3, 4.6)	5.86 m	5.74 td (11.3, 4.4)	5.95 td (11.2, 4.6)
2α		1.93 m	1.92 m	1.91 m	1.93 m	1.90 m
2β		1.25 m	1.23 m	1.23 m	1.23 m	1.20 m
3α		2.23 m	2.18 m	2.21 m	2.21 m	2.12 m
3β		1.97 m	2.00 m	1.97 m	1.99 m	1.89 m
6		5.00 dd (9.8, 5.2)	5.03 dd (9.8, 5.1)	5.00 dd (10.2, 5.2)	5.00 dd (9.7, 5.3)	4.95 dd (11.2, 4.6)
7α		1.70 m	1.68 m	1.70 m	1.69 m	1.65 m
7β		1.75 m	172 m	1.76 m	1.75 m	1.76 m
8		1.79 m	1.73 m	1.81 m	1.76 m	1.90 m
10		2.11 d (11.3)	2.17 m	2.06 m	2.13 d (11.3)	2.16 d (11.2)
11a		2.70 dd (16.1, 8.0)	2.53 dd (16.3, 7.0)	2.72 dd (16.3, 9.1)	2.66 dd (16.1, 7.6)	2.84 dd (16.0, 11.2)
11b		1.64 dd (16.1, 2.0)	1.72 m	1.54 m	1.67 m	1.49 br d (16.0)
12		5.89 br d (8.0)	5.94 br d (7.0)	5.87 m	5.94 br d (7.6)	6.06 br d (11.2)
14		5.96 br s	5.98 br s	5.90 br s	5.97 br s	5.88 br s
16a		4.89 dd (17.6, 1.6)	4.90 dd (17.6, 1.7)	4.84 dd (17.6, 1.6)	4.87 dd (17.6, 1.6)	4.79 dd (17.6, 1.6)
16b		4.76 dd (17.6, 1.6)	4.78 dd (17.6, 1.7)	4.71 dd (17.6, 1.6)	4.77 dd (17.6, 1.6)	4.72 dd (17.6, 1.6)
17		0.87 d (6.2)	0.87 d (5.3)	0.86 d (6.5)	0.87 d (6.1)	0.88 d (6.4)
18a		4.03 d (11.7)	4.13 d (11.7)	3.97 d (11.6)	4.05 d (11.7)	3.84 s
18b		3.86 d (11.7)	3.89 d (11.7)	3.85 d (11.6)	3.87 d (11.7)	
19a		4.93 d (13.3)	4.94 d (13.3)	4.95 d (13.2)	4.93 d (13.3)	4.94 d (13.3)
19b		4.72 d (13.3)	4.71 d (13.3)	4.74 d (13.2)	4.72 d (13.3)	4.71 d (13.3)
20		0.84 s	0.83 s	0.85 s	0.84 s	0.87 s
1-OR	2′	2.31 qt (6.9, 6.9)	2.07 s		2.31 qt (6.9, 6.9)	
	3′a	1.55 m		7.02 qd (7.0, 1.1)	1.55 m	6.93 qd (7.1, 1.1)
	3′b	1.73 m			1.73 m	
	4′	0.91 t (7.4)		1.82 d (7.0)	0.92 t (7.4)	1.78 d (7.1)
	5′	1.16 d (6.9)		1.88 br s	1.15 d (6.9)	1.86 br s
6-OAc	2″	2.05 s	2.05 s	2.06 s	2.06 s	2.02 s
12-OR	2‴	2.45 qt (6.9, 7.2)	2.42 qt (6.9, 7.2)	2.47 qt (6.9, 7.2)	2.17 s	
	3‴a	1.55 m	1.53 m	1.56 m		7.15 qd (7.1, 1.1)
	3‴b	1.73 m	1.78 m	1.77 m		
	4‴	0.94 t (7.4)	0.94 t (7.5)	0.95 t (7.4)		1.91 d (7.1)
	5‴	1.20 d (6.9)	1.19 d (6.9)	1.22 d (6.8)		1.96 br s
19-OAc	2⁗	2.19 s	2.18 s	2.21 s	2.19 s	2.21 s

[a] Proton coupling constants (J) in Hz are given in parentheses. Assignments of ^{1}H NMR data are based on ^{1}H−^{1}H COSY, HMQC, and HMBC experiments.

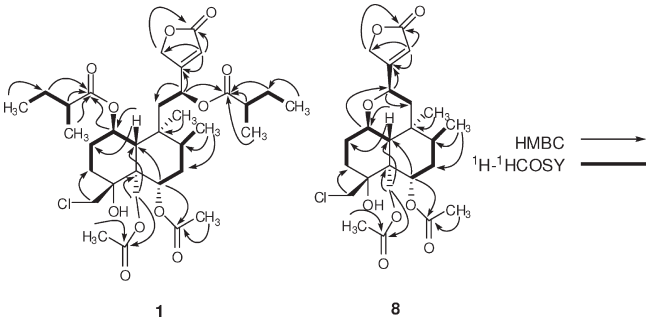

Figure 2. Key HMBC and ^{1}H−^{1}H COSY correlations of **1** and **8**.

4.92 and 4.45 (each, 1H, d, J = 13.2 Hz, H-19a and H-19b), three oxygenated methine protons at δ_H 4.18 (1H, td, J = 10.5, 5.8 Hz, H-1), 5.05 (1H, t, J = 8.0 Hz, H-6), and 4.56 (1H, br d, J = 11.8 Hz, H-12), one olefinic proton at δ_H 5.93 (1H, br s, H-14), and partially overlapped resonances due to three methylenes and two methines from δ_H 1.25 to δ_H 2.05. In addition, two sets of acetoxy groups [δ_H 2.02 (3H, s, H_3-2′); δ_C 170.3 (C-1′) and 21.4 (C-2′) and δ_H 2.16 (3H, s, H_3-2″); δ_C 170.1 (C-1″) and 21.6 (C-2″)] were connected to C-6 and C-19, which was established by the HMBC correlations of H-6/C-1′ and H_2-19/C-1″, respectively. Furthermore, attention should be paid to the correlation from H-1 to C-12 in the HMBC spectrum of **8**, which suggested that C-1 and C-12 were connected to the same oxygen atom to form a pyran ring (Figure 2). Thus, the planar structure of **8** was established.

The NOESY spectrum allowed the stereochemical features of **8** to be assigned (Figure 3). The junctions of rings A and B, rings A and C, and rings B and C were *trans* because the NOESY correlations of H-10/H-1, H-10/H_2-19, and H-10/H_3-20 were not found. Ring A showed a boat conformation because the correlations of H-1/H-3α and H-10/H-2β were not found. The cross-peaks of H-8/H-6, H-10/H-8, H-10/H-11β, H-8/H-11β,

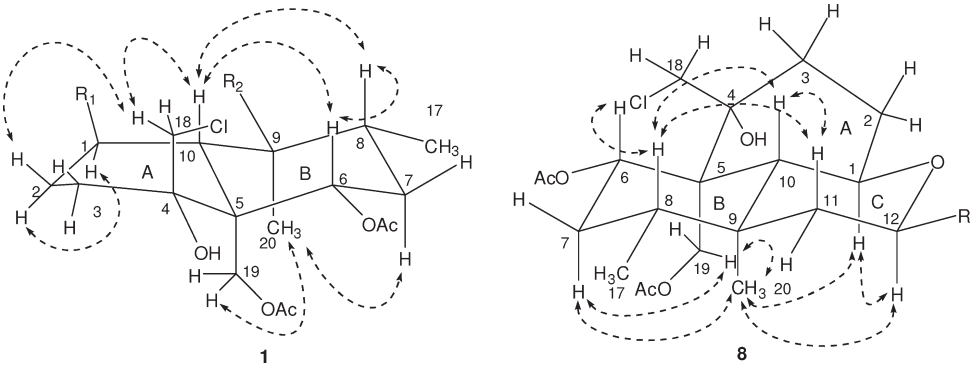

Figure 3. Key NOESY correlations of **1** and **8**.

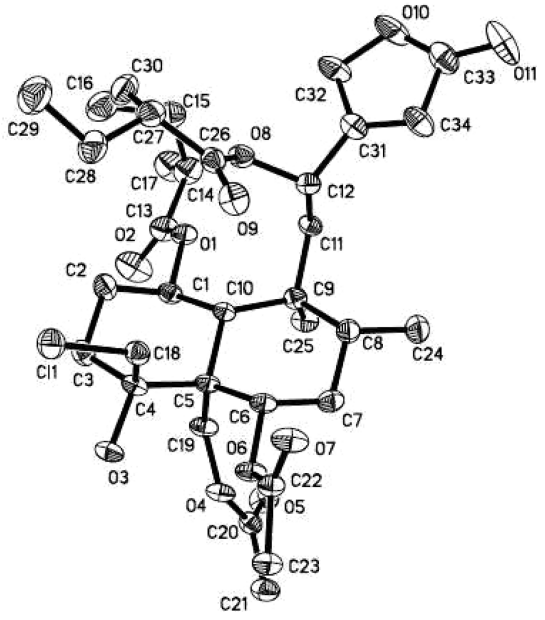

Figure 4. Thermal ellipsoid representation of **1**.

H-1/H_3-20, H-1/H-12, H-7α/H_3-20, and H_2-19/H_3-20 in the NOESY spectrum of **8** indicated that rings B and C possessed chair conformations and that H-6, H-8, and H-10 had axial β-orientations and H-1, H-12, and H_3-20 had axial α-orientations. Therefore, the absolute configuration of C-12 was suggested to be *R*. On the basis of the above analysis, **8** was determined to be (12*R*)-6α,19-diacetoxy-18-chloro-1β,12-epoxy-4α-hydroxy-*neo*-clerod-13-en-15,16-olide, named ajugaciliatin F.

Compounds **9** and **10** exhibited similar NMR spectroscopic features to those of **8** (Tables 1 and 3).

The molecular formula of **9** was inferred as $C_{24}H_{34}O_9$ by HR-ESIMS (*m*/*z* 489.2099 $[M + Na]^+$). Comparison of the NMR data of **9** and **8** indicated that the C-4 chloromethyl [δ_H 4.09 and 4.00 (each, 1H, d, *J* = 11.3 Hz, H-18a and H-18b); δ_C 49.9 (C-18)] group of **8** was replaced by a hydroxymethyl [δ_H 4.02 and 3.71 (each, 1H, d, *J* = 11.1 Hz, H-18a and H-18b); δ_C 64.5 (C-18)] in **9**. Compound **10** gave a molecular formula of $C_{24}H_{32}O_8$ as established by HR-ESIMS (*m*/*z* 471.1988 $[M + Na]^+$). The only difference between **10** and **9** was that the hydroxymethyl and the hydroxyl groups at C-4 of **9** were involved in an intramolecular dehydration reaction to form an epoxide ring [δ_H 3.10 (1H, dd, *J* = 3.7, 2.2 Hz, H-18a) and 2.35 (1H, d, *J* = 3.7 Hz, H-18b); δ_C 50.2 (C-18) and 64.7 (C-4)] in **10**. Unambiguous assignments of all the proton and carbon resonances of **9** and **10** were done by analysis of the HMQC, HMBC, and $^1H-^1H$ COSY spectra of **9** and **10**. Analysis of the NOESY and $[\alpha]_D$ data of **9** and **10** revealed that these compounds have the same configuration as **8**. Hence, the structures of **9** and **10** were elucidated as (12*R*)-6α,19-diacetoxy-1β,12-epoxy-4α,18-dihydroxy-*neo*-clerod-13-en-15,16-olide (**9**) and (12*R*)-6α,19-diacetoxy-1β,12:4α,18-diepoxy-*neo*-clerod-13-en-15,16-olide (**10**), named ajugaciliatins G and H, respectively.

Compound **11** was obtained as colorless needles, and the molecular formula was determined to be $C_{27}H_{40}O_8$ by HR-ESIMS (*m*/*z* 515.2606 $[M + Na]^+$). Analysis of the ^{13}C NMR (Table 1) and ^1H NMR (Table 3) data showed that **11** displayed characteristics of a *neo*-clerodane diterpene nucleus,[11,19] exhibiting two methyl [C-17 (δ_C 15.4) and C-20 (δ_C 17.8)], nine methylene [C-1 (δ_C 22.0), C-2 (δ_C 21.0), C-3 (δ_C 30.1), C-7 (δ_C 35.5), C-11 (δ_C 35.2), C-12 (δ_C 21.9), C-16 (δ_C 73.2), C-18 (δ_C 66.6), and C-19 (δ_C 64.1)], four methine [C-6 (δ_C 74.4), C-8 (δ_C 34.9), C-10 (δ_C 44.2), and C-14 (δ_C 114.9)], and five quaternary [C-4 (δ_C 77.0), C-5 (δ_C 47.1), C-9 (δ_C 38.8), C-13 (δ_C 170.9), and C-15 (δ_C 174.3)] carbons. Compound **11** also possessed an acetoxy group [δ_H 2.13 (3H, s, H_3-2′); δ_C 171.5 (C-1′) and 21.0 (C-2′)] at C-18 and a tigloyloxy unit [δ_H 6.82 (1H, qd, *J* = 7.0, 1.0 Hz, H-3″), 1.82 (3H, d, *J* = 7.0 Hz, H_3-4″), and 1.86 (3H, br s, H_3-5″); δ_C 168.0 (C-1″), 128.5 (C-2″), 137.6 (C-3″), 14.5 (C-4″), and 12.2 (C-5″)] at C-19, which were established by the HMBC correlations of H_2-18/C-1′ and H_2-19/C-1″, respectively. By analyzing the HMQC, HMBC, and $^1H-^1H$ COSY spectra, all the proton and carbon resonances were assigned unambiguously. The NOESY spectroscopic data of **11** revealed that the two six-membered rings have the same configuration as **1**. Thus, the structure of **11** was assigned as 18-acetoxy-4α,6α-dihydroxy-19-tigloyloxy-*neo*-clerod-13-en-15,16-olide, named ajugaciliatin I (**11**).

Compound **12** gave a molecular formula of $C_{25}H_{38}O_7$ on the basis of its HR-ESIMS (*m*/*z* 473.2502 $[M + Na]^+$). The ^1H and ^{13}C NMR data (Tables 3 and 1) closely resembled those of **11**. The differences between **11** and **12** were that **12** possessed a tigloyloxy unit [δ_H 6.95 (1H, qd, *J* = 7.0, 1.1 Hz, H-3′), 1.82 (3H, d, *J* = 7.0 Hz, H_3-4′), and 1.87 (3H, br s, H_3-5′); δ_C 168.3 (C-1′), 128.3 (C-2′), 138.3 (C-3′), 14.5 (C-4′), and 12.1 (C-5′)] rather than an acetoxy group at C-18, and a hydroxy group rather than a tigloyloxy group at C-19. Analysis of the NOESY correlations of **12** revealed the same configuration as that of **11**. Thus,

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Table 3. ^{1}H NMR Spectroscopic Data (δ_H) of Compounds 8−12 ($CDCl_3$, 400 MHz)[a]

position		8	9	10	position		11	12
1		4.18 td (10.5, 5.8)	4.15 td (10.5, 5.7)	4.09 td (10.4, 5.4)	1α		1.77 m	1.77 m
					1β		1.81 m	1.83 m
2α		2.01 m	1.98 m	2.19 m	2α		1.95 m	1.96 m
2β		1.31 m	1.31 m	1.53 m	2β		1.41 m	1.34 m
3α		2.05 m	1.95 m	2.29 m	3α		1.89 m	1.99 m
3β		1.89 m	1.75 m	1.09 m	3β		1.84 m	1.84 m
6		5.05 dd (8.0, 8.0)	4.97 dd (11.2,4.7)	4.76 dd (10.0, 6.2)	6		3.89 dd (10.8, 3.7)	3.96 dd (10.8, 3.6)
7α		1.68 m	1.70 m	1.59 m	7α		1.76 m	1.82 m
7β		1.66 m	1.59 m	1.56 m	7β		1.65 m	1.73 m
8		1.42 m	1.44 m	1.41 m	8		1.64 m	1.58 m
10		1.29 d (10.5)	1.31d (10.5)	1.28 d (10.4)	10		1.39 m	1.30 m
11a		1.81 m	1.79 dd (16.1, 7.4)	1.78 dd (12.9, 1.4)	11a		1.61 m	1.57 m
11b		1.25 m	1.25 m	1.22 m	11b		1.58 m	1.52 m
12		4.56 br d (11.8)	4.54 br d (11.7)	4.58 m	12a		2.33 m	2.26 m
14		5.93 br s	5.91 br s	5.92 br s	12b		2.11 m	2.05 m
16		4.87 br s	4.86 br s	4.86 br s	14		5.84 br s	5.83 br s
17		0.87 d (6.7)	0.86 d (6.6)	0.86 d (6.7)	16		4.78 br s	4.75 br s
18a		4.09 d (11.3)	4.02 d (11.1)	3.10 dd (3.7, 2.2)	17		0.87 d (6.0)	0.87 d (6.7)
18b		4.00 d (11.3)	3.71 d (11.1)	2.35 d (3.7)	18a		4.53 d (11.5)	4.55 d (11.8)
19a		4.92 d (13.2)	4.92 d (13.0)	4.61 d (12.6)	18b		4.40 d (11.5)	4.50 d (11.8)
19b		4.45 d (13.2)	4.43 d (13.0)	4.35 d (12.6)	19a		4.93 d (13.3)	4.45 d (12.6)
20		1.06 s	1.06 s	1.05 s	19b		4.67 d (13.3)	4.37 d (12.6)
6-OAc	2′	2.02 s	2.02 s	1.97 s	20		0.82 s	0.81 s
19-OAc	2″	2.16 s	2.15 s	2.14 s	18-OR	2′	2.13 s	
						3′		6.95 qd (7.0, 1.1)
						4′		1.82 d (7.0)
						5′		1.87 br s
					19-OR	3″	6.82 qd (7.0, 1.0)	
						4″	1.82 d (7.0)	
						5″	1.86 br s	

[a] Proton coupling constants (J) in Hz are given in parentheses. Assignments of ^{1}H NMR data are based on ^{1}H−^{1}H COSY, HMQC, and HMBC experiments.

compound **12** was determined as 4α,6α,19-trihydroxy-18-tigloyloxy-*neo*-clerod-13-en-15,16-olide, named ajugaciliatin J (**12**).

The 17 known *neo*-clerodane diterpenes were identified by comparison of experimental and reported spectroscopic data as ajugamarin A2 chlorohydrin (**6**),[13] ajugamarin A1 chlorohydrin (**7**),[10] ajugacumbin A chlorohydrin (**13**),[14] ajugacumbin F (**14**),[19] ajugatakasin B (**15**),[15] ajugamarin H1 (**16**),[16] ajugatakasin A (**17**),[15] ajugamarin G1 (**18**),[16] ajugamarin A1 (**19**),[10] ajuganipponin A (**20**),[17] ajugacumbin A (**21**),[12] ajugamarin B1 (**22**),[10] ajugalide C (**23**),[18] ajuganipponin B (**24**),[17] ajugamarin A2 (**25**),[16] ajugapantin A (**26**),[10,18] and ajugalide B (**27**).[18]

Compounds **1**−**27** were evaluated for their neuroprotective activities against MPP^+-induced neuronal cell death in dopaminergic neuroblastoma SH-SY5Y cells using an established MTT assay with a slight modification.[38,39] Guanosine was used as a positive control.[40] Compounds **2**, **6**, **7**, **9**, **10**, **15**−**17**, **19**, and **20** exhibited moderate neuroprotective effects (Table 4), and other compounds were inactive. The above active compounds (3−30 μM) neither affected the cell viability nor showed any cytotoxcity (data not shown).

In our search for neuroprotective active substances, we have isolated 27 *neo*-clerodane diterpenes from *A. ciliata* for the first time, including the new (**1**−**5**, **8**−**12**) and 17 known (**6**, **7**, **13**−**27**) structures. Naturally occurring *neo*-clerodane diterpenes containing a pyran moiety formed by C-1 and C-12 connecting to the same oxygen atom[41,42] all possess the 12*S*-configuration except for synthetic products. This is the first report of ajugaciliatins F−H (**8**−**10**) with 12*R*-configuration in this type of naturally occurring *neo*-clerodane diterpenes.

EXPERIMENTAL SECTION

General Experimental Procedures. The optical rotations were measured in MeOH using an Autopal IV automatic polarimeter (Autopal Industries Co. Ltd., India). The IR spectra were recorded on a Bio-Rad FTS 6000 Fourier transform infrared (FTIR) spectrometer with KBr discs (DeFelsko Co. Ltd., America). The ESIMS spectra were obtained on an LCQ-Advantage mass spectrometer (Finnigan Co. Ltd., USA). HR-ESIMS spectra were recorded by an IonSpec 7.0 T FTICR MS (IonSpec Co. Ltd., USA). 1D and 2D NMR data were recorded on a Bruker AV 400 instrument (400 MHz for ^{1}H and 100 MHz for ^{13}C) with TMS as an internal standard (Bruker BioSpin Co. Ltd., Switzerland). HPLC separations were performed on a CXTH system, equipped with a UV3000 detector at 210 nm (Beijing Chuangxintongheng Instruments Co. Ltd., China), and a YMC-pack J'Sphere ODS-M80 (250 × 20 mm) column (YMC Co. Ltd., Japan). X-ray crystallographic analysis was

Table 4. Neuroprotective Effects of Compounds 2, 6, 7, 9, 10, 15−17, 19, and 20 against MPP^+-Induced SH-SY5Y Cell Death

	cell viability (%)[a]		
compd	3 μM	10 μM	30 μM
2	67.4 ± 3.8	80.4 ± 5.3[b]	88.2 ± 6.2[b]
6	71.0 ± 2.5[b]	79.7 ± 3.6[b]	83.7 ± 4.4[b]
7	67.1 ± 7.0	69.2 ± 3.1	73.8 ± 5.0[b]
9	69.1 ± 5.4	73.6 ± 3.6[b]	81.5 ± 6.6[b]
10	65.2 ± 2.7	74.2 ± 4.3[b]	78.2 ± 5.7[b]
15	70.2 ± 3.4[b]	76.5 ± 5.0[b]	84.3 ± 3.1[b]
16	67.3 ± 2.5	70.4 ± 2.8[b]	87.5 ± 2.6[b]
17	66.3 ± 5.7	71.5 ± 5.1[b]	78.2 ± 4.6[b]
19	65.3 ± 4.5	73.4 ± 6.2[b]	81.9 ± 3.4[b]
20	65.4 ± 5.8	69.2 ± 3.5	76.6 ± 4.7[b]
guanosine[c]	70.0 ± 3.1[b]	79.7 ± 2.9[b]	90.1 ± 3.8[b]

[a] Neuroprotective effects of test compounds against MPP^+-induced SH-SY5Y cell death. Cell viability: 100 (control); 61.1 ± 4.3 (treated only with MPP^+). Data are presented as mean ± SEM from triplicate samples. [b] $p < 0.05$, compared with the group treated only with MPP^+. [c] Guanosine was used as a positive control.

carried out on a Rigaku Saturn724 CCD diffractometer equipped with a multilayer-monochromator and Mo Kα radiation (λ = 0.71075 Å) (Rigaku Co. Ltd., Japan). The structure was solved by direct methods (SHELXL-97), expanded using Fourier techniques, and refined with full-matrix least-squares on F^2 (SHELXL-97). Silica gel was used for column chromatography (200−300 mesh, Qingdao Marine Chemical Group Co. Ltd., China). Chemical reagents for isolation were analytical grade and purchased from Tianjin Yuanli Co. Ltd., China. Biological reagents were from Sigma Company. Human dopaminergic SH-SY5Y cells were obtained from the American Type Culture Collection (ATCC).

Plant Material. The whole plants of *A. ciliata* were collected from Zhejiang Province, China, in August 2008. The botanical identification was made by Dr. Yuanqiang Guo (College of Pharmacy, Nankai University, China), and a voucher specimen (No. 20080809) was deposited at the laboratory of the Research Department of Natural Medicine, College of Pharmacy, Nankai University, China.

Extraction and Isolation. The air-dried whole plants of *A. ciliata* (20 kg) were powdered and extracted with MeOH (3 × 40 L) at room temperature for 48 h. The organic solvent was concentrated to afford a crude extract (2.4 kg). The extract was suspended in H_2O (2.5 L) and partitioned successively with petroleum ether, CH_2Cl_2, and *n*-BuOH (3 × 2.5 L). The CH_2Cl_2-soluble part (270.0 g) was subjected to silica gel column chromatography, using a gradient of acetone in petroleum ether, to give 12 fractions (F_1−F_{12}). F_6 (22.7 g) was separated by reversed-phase flash chromatography over C-18 eluting with a step gradient from 20% to 95% MeOH in H_2O to give seven subfractions ($F_{6\text{-}1}$−$F_{6\text{-}7}$) and afforded **21** (356.0 mg). $F_{6\text{-}2}$ was purified by RP HPLC (YMC-pack J'Sphere ODS-M80, 20 × 250 mm, 57% MeOH in H_2O) to afford **10** (16.3 mg). Fractions $F_{6\text{-}3}$−$F_{6\text{-}7}$ were purified using the same protocol, eluting with 63%, 64%, 67%, 70%, and 72% MeOH in H_2O, respectively, to afford **24** (32.4 mg), **6** (11.2 mg), **17** (8.4 mg), **4** (12.4 mg), **13** (8.7 mg), **16** (32.6 mg), **18** (12.5 mg), **5** (15.5 mg), **15** (6.9 mg), **3** (9.4 mg), and **1** (19.8 mg). F_7 (15.7 g), F_8 (11.6 g), and F_9 (6.9 g) were also separated by reversed-phase flash chromatography over C-18, eluting with a step gradient from 20% to 95% MeOH in H_2O, to give fractions $F_{7\text{-}1}$−$F_{7\text{-}5}$, $F_{8\text{-}1}$−$F_{8\text{-}4}$, and $F_{9\text{-}1}$−$F_{9\text{-}4}$. Fractions $F_{7\text{-}4}$, $F_{7\text{-}5}$, $F_{8\text{-}2}$, $F_{8\text{-}3}$, $F_{9\text{-}1}$, and $F_{9\text{-}4}$ were purified using the same protocol as $F_{6\text{-}2}$, eluting with 62.5%, 65.5%, 59%, 63%, 48%, and 60% MeOH in H_2O, respectively, to afford **20** (74.8 mg), **19** (3.2 mg), **8** (25.3 mg), **27** (8.3 mg), **2** (14.4 mg), **23** (10.2 mg), **25** (33.7 mg), **12** (19.2 mg), **22** (26.4 mg), **9** (7.9 mg), **11** (43.8 mg), **7** (21.4 mg), **26** (13.6 mg), and **14** (23.7 mg).

Ajugaciliatin A (***1***): colorless flakes (MeOH); mp 167−169 °C; $[\alpha]^{20}_D$ +11.0 (*c* 0.22, MeOH); IR (KBr) ν_{max} 3496, 2968, 2936, 2876, 1782, 1729, 1461, 1373, 1251, and 1236 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 2; ESIMS *m/z* 693 $[M(^{35}Cl) + Na]^+$, 695 $[M(^{37}Cl) + Na]^+$; HR-ESIMS *m/z* 693.3021 $[M(^{35}Cl) + Na]^+$, 695.2986 $[M(^{37}Cl) + Na]^+$ (calcd. for $C_{34}H_{51}{}^{35}ClO_{11}Na$, 693.3012).

X-ray crystal data of ***1***: $C_{34}H_{51}ClO_{11}$, M_r = 671.20, orthorhombic, space group $P2(1)2(1)2(1)$, a = 8.7420(10) Å, b = 19.5690(18) Å, c = 20.181(2) Å, V = 3452.4(6) $Å^3$, Z = 4, D_{calc} =1.291 g/cm^3, crystal dimensions 0.26 × 0.10 × 0.02 mm were used for measurements on a Rigaku Saturn724 CCD diffractometer with a multilayer monochromator, Mo Kα radiation (λ = 0.71075 Å). The total number of reflections measured was 35 744, of which 8185 were unique and 6855 were observed, $I > 2\sigma(I)$. Final indices: R_1 = 0.0649, wR_2 = 0.1672 for observed reflections, and R_1 = 0.0774, wR_2 = 0.1754 for all reflections.

Ajugaciliatin B (***2***): white powder; $[\alpha]^{20}_D$ +8.7 (*c* 0.31, MeOH); IR (KBr) ν_{max} 3512, 2987, 2955, 2894, 1792, 1744, 1649, 1468, 1376, 1272, and 1243 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 2; ESIMS *m/z* 651 $[M(^{35}Cl) + Na]^+$, 653 $[M(^{37}Cl) + Na]^+$; HR-ESIMS *m/z* 651.2539 $[M(^{35}Cl) + Na]^+$, 653.2538 $[M(^{37}Cl) + Na]^+$ (calcd for $C_{31}H_{45}{}^{35}ClO_{11}Na$, 651.2543).

Ajugaciliatin C (***3***): colorless flakes (MeOH); mp 156−158 °C; $[\alpha]^{20}_D$ +2.1 (*c* 0.24, MeOH); IR (KBr) ν_{max} 3489, 2985, 2954, 2894, 1792, 1745, 1711, 1656, 1468, 1380, 1277, and 1244 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 2; ESIMS *m/z* 691 $[M(^{35}Cl) + Na]^+$, 693 $[M(^{37}Cl) + Na]^+$; HR-ESIMS *m/z* 691.2860 $[M(^{35}Cl) + Na]^+$, 693.2847 $[M(^{37}Cl) + Na]^+$ (calcd for $C_{34}H_{49}{}^{35}ClO_{11}Na$, 691.2856).

Ajugaciliatin D (***4***): white powder; $[\alpha]^{20}_D$ +7.8 (*c* 0.27, MeOH); IR (KBr) ν_{max} 3492, 2984, 2952, 2894, 1792, 1743, 1652, 1468, 1378, 1277, and 1241 cm^{-1}; H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 2; ESIMS *m/z* 651 $[M(^{35}Cl) + Na]^+$, 653 $[M(^{37}Cl) + Na]^+$; HR-ESIMS *m/z* 651.2543 $[M(^{35}Cl) + Na]^+$, 653.2534 $[M(^{37}Cl) + Na]^+$ (calcd for $C_{31}H_{45}{}^{35}ClO_{11}Na$, 651.2543).

Ajugaciliatin E (***5***): white powder; $[\alpha]^{20}_D$ −24.2 (*c* 0.24, MeOH); IR (KBr) ν_{max} 3497, 2995, 2966, 2894, 1800, 1767, 1719, 1664, 1468, 1386, and 1263 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 2; ESIMS *m/z* 689 $[M(^{35}Cl) + Na]^+$, 691 $[M(^{37}Cl) + Na]^+$; HR-ESIMS *m/z* 689.2692 $[M(^{35}Cl) + Na]^+$, 691.2656 $[M(^{37}Cl) + Na]^+$ (calcd for $C_{34}H_{47}{}^{35}ClO_{11}Na$, 689.2699).

Ajugaciliatin F (***8***): colorless flakes (MeOH); mp 236−238 °C; $[\alpha]^{20}_D$ +4.8 (*c* 0.31, MeOH); IR (KBr) ν_{max} 3527, 2973, 2956, 2891, 1788, 1752, 1655, 1466, 1380, and 1249 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 3; ESIMS *m/z* 507 $[M(^{35}Cl) + Na]^+$, 509 $[M(^{37}Cl) + Na]^+$; HR-ESIMS *m/z* 507.1762 $[M(^{35}Cl) + Na]^+$, 509.1764 $[M(^{37}Cl) + Na]^+$ (calcd for $C_{24}H_{33}{}^{35}ClO_8Na$, 507.1756).

Ajugaciliatin G (***9***): colorless flakes (MeOH); mp 202−204 °C; $[\alpha]^{20}_D$ +34.8 (*c* 0.50, MeOH); IR (KBr) ν_{max} 3499, 2979, 2965, 2890, 1787, 1748, 1653, 1462, 1379, 1246, and 1243 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 3; ESIMS *m/z* 489 $[M + Na]^+$; HR-ESIMS *m/z* 489.2099 $[M + Na]^+$ (calcd. for $C_{24}H_{34}O_9Na$, 489.2095).

Ajugaciliatin H (***10***): white powder; $[\alpha]^{20}_D$ +27.4 (*c* 0.31, MeOH); IR (KBr) ν_{max} 2982, 2955, 2893, 1787, 1758, 1652, 1467, 1378, 1272, and 1243 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz,

$CDCl_3$) data, see Tables 1 and 3; ESIMS m/z 471 $[M + Na]^+$; HR-ESIMS m/z 471.1988 $[M + Na]^+$ (calcd for $C_{24}H_{32}O_8Na$, 471.1989).

Ajugaciliatin I (**11**): colorless needles (MeOH); mp 110−112 °C; $[\alpha]^{20}_D$ +5.2 (*c* 0.42, MeOH); IR (KBr) ν_{max} 3475, 2973, 2955, 2893, 1789, 1754, 1647, 1458, 1382, and 1262 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 3; ESIMS m/z 515 $[M + Na]^+$; HR-ESIMS m/z 515.2606 $[M + Na]^+$ (calcd for $C_{27}H_{40}O_8Na$, 515.2615).

Ajugaciliatin J (**12**): white powder; $[\alpha]^{20}_D$ −8.8 (*c* 0.32, MeOH); IR (KBr) ν_{max} 3445, 2973, 2953, 2891, 1789, 1756, 1714, 1646, 1456, 1395, and 1271 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 3; ESIMS m/z 473 $[M + Na]^+$; HR-ESIMS m/z 473.2502 $[M + Na]^+$ (calcd. for $C_{25}H_{38}O_7Na$, 473.2510).

Bioassay Procedure. Human dopaminergic neuroblastoma SH-SY5Y cells were cultured at 37 °C in DMEM supplemented with 10% (v/v) inactivated fetal bovine serum and 100 U/mL penicillin/streptomycin under a water-saturated atmosphere of 95% air and 5% CO_2. The cells were disassociated by incubation with 1 mM ethylene glycol-bis-(2-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid in phosphate-buffered saline for 15 min and then seeded in 96-well culture plates (1×10^4 cells/well). Cells were incubated at 37 °C under a 5% CO_2 humidified air incubator for 24 h. Cells were pretreated for 2 h with various concentrations (3, 10, 30 μM) of compounds before incubation in medium containing MPP^+. MTT dissolved in phosphate-buffered saline was added at the end of incubation to a final concentration of 0.5 mg/mL. After incubation for 4 h at 37 °C and 5% CO_2, the supernatants were removed and the formed formazan crystals in the viable cells were measured at 550 nm using a microplate reader (Molecular Devices, USA). Experiments were carried out in triplicate. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by *post hoc* multiple comparisons using the Student−Newman−Keuls method. The data are expressed as mean ± SEM of three assays.

■ ASSOCIATED CONTENT

Supporting Information. MS and 1D, 2D NMR spectra of compounds **1**−**5** and **8**−**12** and a cif file of X-ray data. This material is available free of charge via the Internet at http://pubs.acs.org.

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