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Cytotoxic and Anti-inflammatory Triterpenoids from *Toona ciliata*

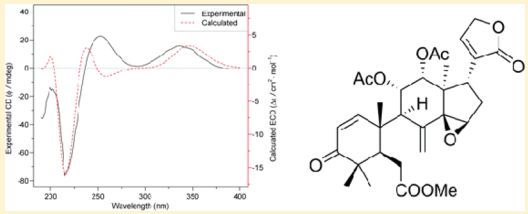
Feng Zhang,[†] Jun-Song Wang,[†] Yu-Cheng Gu,[‡] and Ling-Yi Kong*,[†]

[†]State Key Laboratory of Natural Medicines, Department of Natural Medicinal Chemistry, China Pharmaceutical University, 24 Tong Jia Xiang, Nanjing 210009, People's Republic of China

[‡]Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire RG42 6EY, United Kingdom

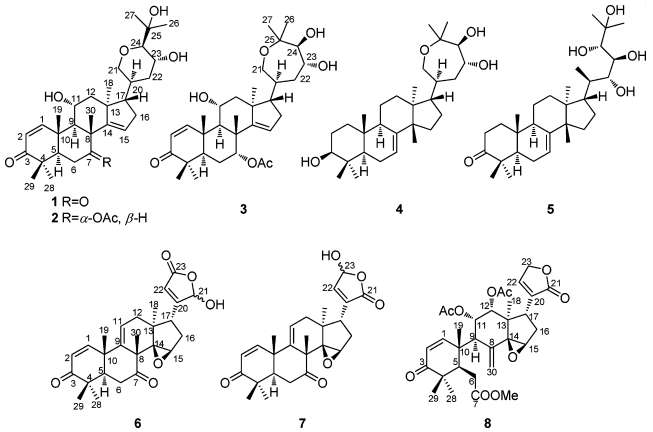
Supporting Information

ABSTRACT: Toonaciliatavarins A–H (**1**–**8**), including three new protolimonoids (**1**–**3**), two new tirucallane-type triterpenoids (**4** and **5**), and three new tetranortriterpenoids (**6**–**8**), and 10 known compounds were isolated from the stem barks of *Toona ciliata Roem.* var. *henryi*. Their structures were identified on the basis of spectroscopic analysis. The absolute configurations of **2** and **8** were determined by ECD calculation. The new isolates were evaluated for their cytotoxicities using six human cancer cell lines and also for their inhibitory effects on lipopolysaccharide-induced nitric oxide production in RAW264.7 cells. Compounds **4** and **5** showed moderate cytotoxicities, and the protolimonoids (**1**–**3**) exhibited marked inhibitory effects on LPS-stimulated NO production.



Limonoids, distributed mainly in the plant family Meliaceae, have been a focus in natural products research for their structural diversity and potential biological significance.[1,2] Plants of the genus *Toona* are well-known for their application to treat dysentery, fever, and menstrual disorders in Chinese folk medicine.[3] Previous chemical investigations on this genus have led to the isolation of a series of limonoids, norlimonoids, and tirucallane-type triterpenoids.[4]

Toona ciliata Roem. var. *henryi* (C. DC.) C. Y. Wu, a timber tree, is a variety of *T. ciliata* that grows mainly in tropical areas of southern mainland China.[5] As part of our ongoing research program[6] on the Meliaceae family, three new protolimonoids (**1**–**3**), two new tirucallane-type triterpenoids (**4** and **5**), and three new tetranortriterpenoids (**6**–**8**), along with 10 known compounds, were isolated from the stem barks of *T. ciliata Roem.* var. *henryi*. Their structures were elucidated by spectroscopic methods. The absolute configurations of compounds **2** and **8** were determined by the ECD calculation method. In this report, we describe the isolation and structural elucidation of these triterpenoids as well as their inhibitory effects on the growth of six human cancer cells and on lipopolysaccharide (LPS)-induced NO production in RAW264.7 cells.



RESULTS AND DISCUSSION

Toonaciliatavarin A (**1**) was obtained as a white, amorphous powder. The molecular formula was determined as $C_{30}H_{44}O_6$ from the HR-ESIMS ion at m/z 523.3051 $[M + Na]^+$ (calcd 523.3030). Its IR spectrum showed the presence of hydroxy (3451 cm^{-1}), carbonyl (1730 cm^{-1}), and olefinic (1640 cm^{-1}) groups. The ^1H NMR spectrum (Table 1) showed seven tertiary methyl singlets (δ_H 1.02, 1.13, 1.13, 1.24, 1.24, 1.48, and 1.54), three olefinic protons (δ_H 8.35, d, 10.0; 5.76, d, 10.0; and 5.76, d, 2.0), and five hydroxy protons (δ_H 2.92, 3.48, 3.84, 4.05, and 4.41). The ^{13}C NMR spectrum (Table 1) showed the presence of seven methyls, five methylenes (one oxygenated), 10 methines (three oxygenated, three olefinic), and eight quaternary (two ketocarbonyls and one oxygenated) carbons. These data indicated that **1** was an *apo*-tirucallane protolimonoid with a modified eight-carbon side chain,[7] as determined by the HMBC experiment (Figure 1a). In the HMBC spectrum, the correlations arising from the tertiary methyl protons to their neighboring carbons enabled the assignment of the backbone. The HMBC correlations originating from oxymethylene protons [δ_H 3.48, 4.05 (H_2-21)] to an oxygenated carbon [δ_C 88.0 (C-24)] revealed the existence of an ether bridge between C-21 and C-24 of the side chain, forming a six-membered pyran ring. The cross-peaks from Me-26 (δ_H 1.24, s, 3H) and Me-27 (δ_H 1.24, s, 3H) to the oxygenated carbon (C-24) also confirmed the connection of a hydroxyisopropyl group at C-24. In combination with the chemical shift, the HMBC correlations from H-23 (δ_H 3.84, td,

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Table 1. ^{1}H and ^{13}C NMR Data of Compounds 1−3 in Methanol-d_4[a]

	1		2		3	
position	δ_H, mult. (J in Hz)	δ_C	δ_H, mult. (J in Hz)	δ_C	δ_H, mult. (J in Hz)	δ_C
1	8.35, d (10.0)	163.7	8.30, d (10.5)	163.5	8.30, d (10.5)	163.9
2	5.76, d (10.0)	124.2	5.76, d (10.5)	122.2	5.75, d (10.5)	123.6
3		206.1		205.6		207.1
4		46.3		44.0		45.4
5	1.95[b]	53.9	2.32[b]	46.5	2.31[b]	48.0
6α	2.33, dd (14.0, 2.5)	37.0	1.84, dt (15.0, 2.5)	23.2	1.83, br d (14.5)	24.6
6β	3.12, t (14.0)		2.11, dd (15.0, 2.0)		2.09[b]	
7		211.6	5.23, br t (2.5)	74.7	5.23, br s	76.0
8		54.4		42.3		43.7
9	2.15[b]	54.3	2.07, s	47.2	2.03, m	48.2
10		42.1		40.6		42.1
11	4.41, ddd (9.6, 6.5, 4.0)	68.0	4.41, ddd (8.0, 6.0, 2.0)	66.2	4.41, m	67.6
12α	1.93, dd (14.0, 4.0)	50.2	2.10[b]	46.0	2.10[b]	47.3
12β	2.14, dd (14.0, 8.0)		2.28, dd (14.0, 2.0)		2.30[b]	
13		48.0		45.9		47.3
14		154.2		160.0		161.5
15	5.76, d (2.0)	127.4	5.33, d (3.0)	118.0	5.30, br s	119.1
16α	2.12[b]	36.3	2.08[b]	34.2	2.11[b]	35.8
16β	2.36, dd (8.5, 5.0)		2.31[b]		2.29[b]	
17	2.19, d (9.5)	53.5	2.09[b]	52.3	1.94[b]	55.6
18	1.02, s (3H)	21.8	1.12, s (3H)	18.7	1.13, s (3H)	20.0
19	1.54, s (3H)	20.4	1.36, s (3H)	19.3	1.36, s (3H)	20.7
20	1.94[b]	37.4	2.09[b]	35.8	1.94[b]	37.8
21α	3.48, dd (11.5, 2.5)	71.2	3.51, dd (11.5, 2.2)	69.7	3.61, d (13.5)	65.0
21β	4.05, d (11.5)		4.11, d (11.5)		3.72, d (13.5)	
22α	1.58, ddd, (14.5, 10.0, 4.5)	37.4	1.59, ddd (13.0, 11.0, 4.0)	35.9	1.62, t (9.0)	38.9
22β	2.01, m		1.99[b]		1.95[b]	
23	3.84, td (9.0, 5.0)	65.7	3.86, ddd (11.0, 9.0, 4.0)	64.1	3.76, d (8.5)	68.9
24	2.92, d (9.0)	88.0	2.92, d (9.0)	86.4	3.40, d (8.5)	81.3
25		74.5		72.9		77.7
26	1.24, s (3H)	28.1	1.26, s (3H)	28.4	1.15, s (3H)	23.3
27	1.24, s (3H)	24.9	1.26, s (3H)	23.3	1.30, s (3H)	26.4
28	1.13, s (3H)	21.6	1.10, s (3H)	26.5	1.10, s (3H)	21.9
29	1.13, s (3H)	27.2	1.11, s (3H)	20.4	1.11, s (3H)	27.3
30	1.48, s (3H)	29.7	1.25, s (3H)	28.4	1.25, s (3H)	29.8
7-OAc				170.4		171.9
			1.97, s (3H)	19.7	1.98, s (3H)	21.3

[a]Recorded at 500 MHz (^{1}H) and 125 MHz (^{13}C). [b]Signal pattern unclear due to overlapping.

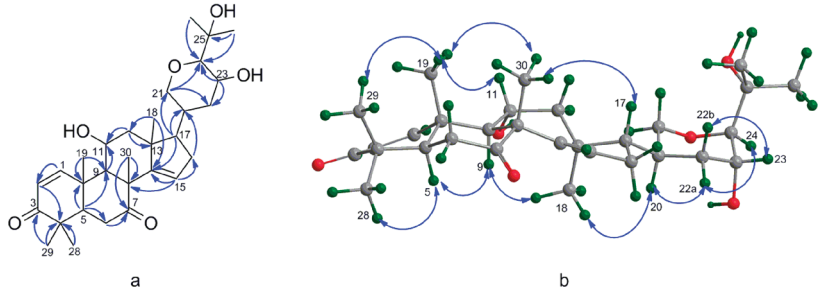

Figure 1. Selected HMBC (H → C) (a) and NOESY (←→) correlations (b) of 1.

10.2, 4.7) to C-24 and C-22 (δ_C 37.4) placed a hydroxy group at C-23. The existence of an α,β-unsaturated carbonyl moiety in ring A was revealed by the HMBC correlations from two *gem*-dimethyl protons (Me-28 and Me-29) to the carbonyl and the correlation from the olefinic proton to C-4. The second carbonyl group was placed at C-7 on the basis of the observed HMBC cross-peaks from Me-30 (δ_H 1.48, s, 3H) and H_2-6 (δ_H 2.33, dd, 14.0, 2.5; 3.12, t, 14.0) to the carbonyl at δ_C 211.6. The HMBC correlations from H-9 and H_2-12 to the oxygenated carbon allowed the assignment of the remaining hydroxy group to C-11. Analysis of its HMBC spectrum further confirmed the planar structure.

The NOESY experiment of **1** (Figure 1b) established the relative configuration of the tetracyclic core, as shown in Figure

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1b. Thus, the NOESY correlations of H-5/Me-28, H-5/H-9, H-9/Me-18, and Me-18/H-20 indicated that H-5, Me-28, H-9, and Me-18 were cofacial, and these were randomly assigned as being α-oriented. In contrast, the NOESY correlations of Me-29/Me-19, Me-19/Me-30, Me-19/H-11, and Me-30/H-17 suggested that they were cofacial and thus β-oriented. The large coupling constant between H-23 and H-24 (J = 9.0 Hz) indicated that the tetrahydropyran ring of the side chain was in a chair conformation. Other correlations of H-20/H-22a, H-22a/H-24, and H-22b/H-23 indicated that the isopropyl group at C-24 was in a β-orientation and the hydroxy group at C-23 in an α-orientation, and distinguished the two C-22 protons. Thus, the structure of **1** was assigned as depicted.

Toonaciliatavarin B (**2**), a white, amorphous powder, exhibited a molecular formula of $C_{32}H_{48}O_7$, as indicated by the observed ion at m/z 567.3306 $[M + Na]^+$ (calcd 567.3292) in the HR-ESIMS. The IR and NMR spectra of **2** were similar to those of toonaciliatavarin A (**1**). The major difference was that C-7 of **2** was an oxymethine (δ_C 74.7) instead of a ketocarbonyl (δ_C 211.6) in **1**, which was confirmed by the HMBC correlations from H_2-6 (δ_H 1.84, 2.11) and the protons of a methyl group (δ_H 1.25, s, 3H) to C-7 (δ_C 74.7). Additionally, an HMBC correlation between H-7 (δ_H 5.23, br t, 2.5) and an acetoxy carbonyl carbon (δ_C 170.4) indicated that OH-7 is acetylated. The broad H-7 singlet suggested that it was in an *equatorial* position and β-orientated. A NOESY experiment allowed the establishment of the relative configuration of **2** as the same as **1**, except for the α-orientation of AcO-7, which was confirmed according to the NOESY correlation between H-7 and Me-30. Therefore, the structure of toonaciliatavarin B was established as **2**.

To determine its absolute configuration, the ECD spectrum of **2** was measured in CH_3CN, which revealed a positive Cotton effect at 231 nm ($\Delta\varepsilon$ +3.32) and a negative Cotton effect at 207 nm ($\Delta\varepsilon$ −1.31). However, lack of proper model compounds for reference and the absence of applicable exciton coupling in the ECD spectrum of **2** made the assignment of its absolute configuration unreliable by direct analysis of the CD curve.[8] Therefore, we calculated the ECD spectrum by time-dependent density functional theory (TDDFT)[9,10] and compared the result with the experimental ECD data of **2**. The conformational analysis was performed by means of the semiempirical PM3 method, as implemented in the program package Gaussian 09, starting from preoptimized geometries generated by the MM2 force field in Chem 3D software overlaid with key correlations observed in the NOESY spectrum. The corresponding minimum geometries were further optimized by DFT calculations at the B3LYP/6-31G(d) level. The calculated ECD of **2** (Figure 2) matches the experimental result very well, allowing the assignment of the absolute configuration of **2** as depicted.

Toonaciliatavarin C (**3**) gave a molecular formula of $C_{32}H_{48}O_7$, as determined from the HR-ESIMS ion at m/z 567.3289 $[M + Na]^+$ (calcd 567.3292). Signals for five oxygenated protons and eight methyl singlets (one acetyl) in the ^{1}H NMR spectrum and 32 carbon signals in the ^{13}C NMR spectrum were observed. Comparison of the NMR data of **3** with those of **2** revealed the carbon framework of the two compounds to be similar, with the only differences occurring in the side chain. The HMBC correlations (Figure 3a) originated from oxymethylene protons [δ_H 3.61, 3.72 (H_2-21)] to an oxygenated quaternary carbon [δ_C 77.7 (C-25)], revealed the existence of an ether bridge between C-21 and C-25 of the side

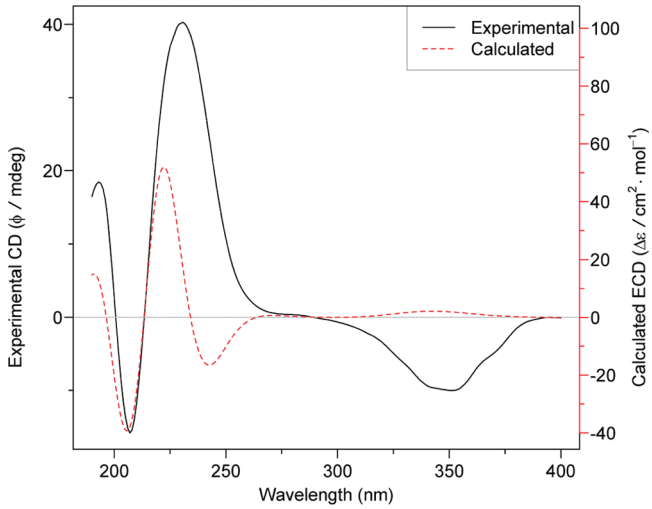

Figure 2. ECD spectra of **2**.

chain, forming a seven-membered cyclic ether ring. The two hydroxy groups were placed at C-23 and C-24 according to the HMBC correlation from H-24 to C-25 and from H-23 to C-22. In the ^{1}H NMR spectrum of **3**, the broad doublet of one proton of the methylene at C-6 and the broad singlet of H-7 suggested that the two protons were nearly perpendicular to each other and that H-7 was in an *equatorial* position and β-orientated. The NOESY correlations (Figure 3b) of H-5/Me-28 and Me-19/H-29 distinguished the two *gem*-methyls at C-4. Other NOESY correlations of H-5/H-9, H-9/Me-18, H-5/Me-18, and Me-18/H-20 determined these protons to be α-oriented; those of Me-19/Me-30, Me-30/H-17, and Me-30/H-7 elucidated them as β-oriented. Further NOESY cross-peaks of H-20/H-22α, H-22α/H-24, H-22β/H-23, and H-17/H-23 allowed the establishment of the relative configuration in the ring attached to C-17. The structure of **3** was therefore established as depicted.

Toonaciliatavarin D (**4**) was obtained as a white, amorphous powder. The HR-ESIMS data displayed a pseudomolecular ion at m/z 497.3598 $[M + Na]^+$ (calcd for $C_{30}H_{50}O_4Na$, 497.3601), consistent with a molecular formula of $C_{30}H_{50}O_4$. IR absorption bands revealed the presence of a hydroxy (3423 cm^{-1}) group and an olefinic bond (1641 cm^{-1}). The ^{1}H NMR data (Table 2) indicated the presence of seven tertiary methyls at δ_H 0.80, 0.86, 0.88, 0.98, 1.05, 1.15, and 1.28 (each 3H, s) and one olefinic proton at δ_H 5.31 (1H, q, 3.3). The ^{13}C NMR spectrum showed 30 carbon resonances, which were classified from the ^{1}H NMR and HSQC spectra as two olefinic carbons (δ_C 118.0 and 145.5), seven methyls, nine sp^3 methylenes, seven sp^3 methines, and five sp^3 quaternary carbons. A comparison of the NMR data of **4** with those of hispidone,[11] previously isolated from the same genus, revealed that the structures of the two compounds are closely related, with the main differences occurring at the A-ring in **4**. The HMBC cross-peaks from C-2 and C-4 to the oxygenated methine proton at δ_H 3.21 (1H, dd, 10.0, 5.5) and from C-3 (δ_C 78.5) to the *gem*-methyls (Me-28 and Me-29) were used to place the hydroxy group at C-3, adopting an α-orientation. Compound **4** shares the same relative configuration as those of other tirucallane-type triterpenoids. The NOESY correlations of H-5/Me-28, H-5/H-9, H-9/Me-18, Me-18/H-20, and H-20/H-24 suggested that these hydrogens and methyl groups were α-oriented. Subsequently, the NOESY correlations of Me-29/Me-19, Me-

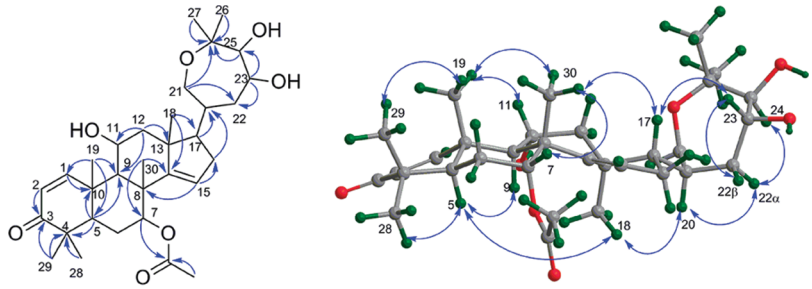

Figure 3. Selected HMBC (H → C) (a) and NOESY (←→) correlations (b) of **3**.

19/Me-30, Me-30/H-17, H-17/H-22b, and H-22b/H-23 indicated that these groups were cofacial and were randomly assigned to be *β*-oriented. The structure of **4** was thus defined as shown.

Table 2. ^{1}H and ^{13}C NMR Data of Compounds 4 and 5 in Methanol-d_4[a]

	4		5	
position	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)	δ_C
1*α*	1.18, dd (15.0, 6.0)	37.2	1.47, td (14.0, 4.0)	39.7
2*β*	1.72, dt (15.0, 3.0)		2.05[b]	
2*α*	1.64[b]	27.0	2.19, dt (14.5, 2.5)	35.8
2*β*			2.84, td (14.5, 5.5)	
3	3.21, dd (10.0, 5.5)	78.5		219.2
4		38.6		49.7
5	1.33, dd (12.0, 5.5)	50.8	1.74, dd (11.0, 6.0)	53.8
6*α*	2.04, m	23.7	2.13, m	25.4
6*β*	2.17, m			
7	5.31, q (3.3)	118.0	5.34, q (3.0)	119.0
8		145.5		147.3
9	2.25, m	49.0	2.35, m	49.9
10		34.6		36.1
11*α*	1.56[b]	17.7	1.62[b]	19.2
11*β*	1.57[b]		1.65[b]	
12*α*	1.94[b]	32.7	1.68, dd (15.0, 9.0)	35.1
12*β*	1.95[b]		1.87, m	
13		43.1		44.6
14		51.0		52.5
15*α*	1.40, m	33.6	1.55, dd (12.0, 7.0)	35.2
15*β*	1.53, m		1.64[b]	
16*α*	1.39, m	27.7	1.37, m	28.4
16*β*	2.04[b]		2.04[b]	
17	1.95[b]	47.7	1.95, dd (10.5, 8.2)	49.7
18	0.86, s (3H)	21.5	0.88, s (3H)	22.5
19	0.80, s (3H)	12.2	1.05, s (3H)	13.2
20	1.81, m	38.4	1.91 t (6.0)	37.6
21*α*	3.47, dd, (13.0, 6.0)	63.9	0.91, d (6.0, 3H)	12.3
21*β*	3.68, dd, (13.0, 4.0)			
22*α*	1.63[b]	36.9	3.73, d (8.5)	77.0
22*β*	1.94[b]			
23	3.78, td (8.5, 3.0)	68.1	3.61, dd (8.5, 7.5)	71.9
24	3.37, d (8.5)	79.8	3.42, d (7.5)	80.9
25		76.4		74.6
26	1.15, s (3H)	21.6	1.26, s (3H)	24.6
27	1.28, s (3H)	25.0	1.24, s (3H)	27.6
28	0.88, s (3H)	14.0	1.03, s (3H)	25.1
29	0.98, s (3H)	26.9	1.13, s (3H)	22.0
30	1.05, s (3H)	26.4	1.09, s (3H)	28.1

[a]Recorded at 500 MHz (^{1}H) and 125 MHz (^{13}C). [b]Signal pattern unclear due to overlapping.

Toonaciliatavarin E (**5**) gave a molecular formula of $C_{30}H_{50}O_5$, as deduced from the HR-ESIMS at *m/z* 513.3569 $[M + Na]^+$ (calcd for $C_{30}H_{50}O_5Na$, 513.3550). The IR absorption bands implied the presence of hydroxy (3443 cm^{-1}), carbonyl (1700 cm^{-1}), and double-bond (1640 cm^{-1}) functionalities. The ^{1}H NMR spectrum showed the presence of seven tertiary methyls at δ_H 0.88, 1.03, 1.05, 1.09, 1.13, 1.24, and 1.26 (each 3H, s), one methyl doublet at δ_H 0.91 (3H, d, 6.0), and one olefinic proton at δ_H 5.34 (1H, q, 3.0). The ^{13}C NMR spectrum showed 30 carbon resonances, which were classified by the HSQC data as eight methyls, seven sp^3 methylenes, seven sp^3 methines, five sp^3 quaternary carbons, one ketocarbonyl (δ_C 219.2), and two olefinic carbons (δ_C 119.0, 147.3). Comparison of the NMR data of **5** with those of hispidone[11] revealed a similar carbon framework for the two compounds, with the only differences being the C-17 side chain. In the HMBC experiment (Figure 4a), the C-21 methyl group and the C-22 hydroxy were assigned by the HMBC correlations of Me-21/C-17, C-20, and C-22, which also indicated the linkages between C-17 and C-20. The HMBC correlations from two methyls (Me-26 and Me-27) to an oxygenated quaternary carbon (δ_C 74.6) were used to assign a hydroxy group at C-25. Meanwhile, the HMBC correlations from an oxygenated methine proton (δ_H 3.42, d, 7.5) to C-25, C-26, and C-27 showed the presence of another hydroxy group at C-24 (δ_C 80.9). The HMBC correlations from H-22 and H-24 to another oxygenated carbon assigned the C-23 (δ_C 71.9). The relative configuration of **5** was established by the NOESY spectrum, which showed that the side chain adopted a preferred conformation (Figure 4b). The usual correlations of Me-18/H-20 revealed a typical tirucallane stereochemistry, while the cross-peaks of H-17/Me-21, H-17/H-22, Me-21/H-22, and H-22/H-24 indicated the configurations of C-22*S**, C-23*S**, and C-24*R**, consistent with the stereochemistry of the side chain of tirucallanes.[7,12−14]

Toonaciliatavarin F (**6**) was determined to have a molecular formula of $C_{26}H_{30}O_6$ according to the pseudomolecular ion at *m/z* 461.1913 $[M + Na]^+$ (calcd for $C_{26}H_{30}O_6Na$, 461.1935) in the HR-ESIMS. The IR absorption bands implied the presence of hydroxy (3526 cm^{-1}), carbonyl (1735, 1710 cm^{-1}), and olefinic (1640 cm^{-1}) functionalities. The ^{1}H NMR spectrum showed signals for five tertiary methyl groups at δ_H 0.58, 1.12, 1.14, 1.26, and 1.69, two oxygenated methine protons at δ_H 3.89 (1H, s) and 6.11 (1H, br s), two mutually coupled olefinic protons (δ_H 7.52, d, 10.5; 5.95, d, 10.5), and two additional olefinic protons at δ_H 5.85 (1H, d, 7.5) and 5.99 (1H, s). The ^{13}C NMR spectrum showed 26 carbon resonances, which were

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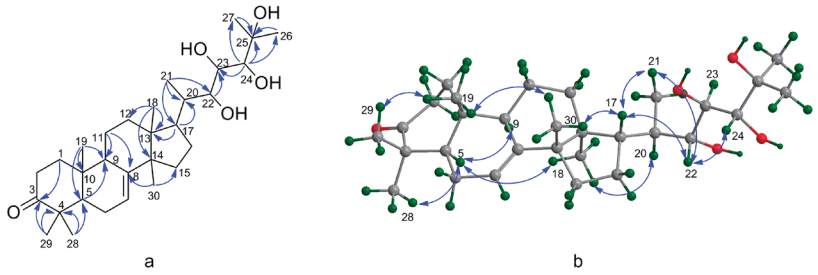

Figure 4. Selected HMBC (H → C) (a) and NOESY (←→) correlations (b) of **5**.

Table 3. ^{1}H and ^{13}C NMR Data of Compounds 6–8 in Methanol-d_4[a]

	6		7		8	
position	δ_H, mult. (J in Hz)	δ_C	δ_H, mult. (J in Hz)	δ_C	δ_H, mult. (J in Hz)	δ_C
1	7.52, d (10.5)	157.2	7.54, d (10.5)	157.3	7.32, d (10.5)	152.7
2	5.95, d (10.5)	123.2	5.94, d (10.5)	123.2	6.15, d, (10.5)	126.1
3		203.8		203.8		203.7
4		44.1		44.1		45.8
5	2.28[b]	50.3	2.28, dd (16.0, 3.0)	50.4	2.60[b]	44.6
6α	2.51, dd (16.0, 3.0)	35.6	2.49, dd (16.0, 3.0)	35.7	2.08, dd (13.5, 11.0)	31.1
6β	3.19, t (16.0)		3.19, t (16.0)		2.58[b]	
7		208		208.1		174.5
8		53.7		53.7		135.4
9		144.6		144.2	2.68, s	57.2
10		43.2		43.1		41.8
11	5.85, d (7.5)	121.9	5.85, d (7.5)	122.0	5.49, d (4.5)	69.8
12α	2.17, d (16.5)	34.2	2.19, d (16.5)	34.2	5.14, d (4.5)	75.1
12β	2.37, d (16.5)		2.39, d (16.5)			
13		41.8		41.8		44.1
14		69.8		70.0		71.9
15	3.89, s	58.0	3.87, s	58.0	4.10, s	60.1
16α	2.15, d (16.0)	30.0	2.10, m	30.0	2.19, dd (14.0, 7.0)	31.0
16β	2.26[b]		2.24, m		2.58[b]	
17	2.64, dd (10.5, 6.5)	41.8	2.60, dd (8.5, 4.0)	41.8	2.76, dd (11.0, 7.0)	37.8
18	0.58, s (3H)	16.5	0.54, s (3H)	16.7	1.00, s (3H)	13.1
19	1.69, s (3H)	22.2	1.68, s (3H)	22.3	1.05, s (3H)	19.0
20		169.5		136.5		131.0
21	6.11, br s	99.2		181.1		175.0
22	5.99, s	119.2	5.99, s	149.1	7.49, s	149.3
23		171.7	6.21, br s	98.1	4.88[b]	70.6
28	1.12, s (3H)	19.7	1.12, s (3H)	19.7	1.06, s (3H)	21.9
29	1.14, s (3H)	23.8	1.13, s (3H)	23.9	1.12, s (3H)	21.8
30	1.26, s (3H)	22.6	1.25, s (3H)	22.6	5.52, s; 5.38, s	121.0
11-OAc						169.9
					2.01, s (3H)	19.5
12-OAc						170.2
					1.78, s (3H)	19.4
OMe					3.67, s (3H)	51.0

[a]Recorded at 500 MHz (^{1}H) and 125 MHz (^{13}C). [b]Signal pattern unclear due to overlapping.

classified by HSQC experiments as five methyls, three sp^3 methylenes, four sp^3 methines (one oxygenated and one hemiacetal), five sp^3 quaternary carbons (one oxygenated), three carbonyl carbons, and six olefinic carbons (δ_C 157.2, 123.2; 144.6, 121.9; 169.5, 119.2). The aforementioned data indicated that compound **6** featured a tetranortriterpenoid skeleton sharing the A–D ring system of 21-hydroxycedrelonelide.[15] Olefinic protons centered at δ_H 7.52 (J = 10.5 Hz) and δ_H 5.95 (J = 10.5 Hz) comprise an AX system corresponding to the protons in an α,β-unsaturated carbonyl moiety, which was indicated by the carbon resonances at δ_C 157.2, 123.2, and 203.8, the locations of which were determined by the HMBC correlations from Me-28 and Me-29 to the C-3 carbonyl and from Me-19 to the C-1 olefinic carbon. The second carbonyl group was located at C-7 on the basis of its HMBC correlations with Me-30 and H_2-6. A double bond was assigned at C-9 and C-11 according to the HMBC correlations from Me-19 and Me-30 to the olefinic quaternary carbon at δ_C 144.6. The HMBC correlations from Me-18, Me-30, and H-15 (δ_H 3.89, s) to an oxygenated quaternary carbon (δ_C 69.8) and the HMBC correlations from H_2-16 to C-15 indicated that a three-membered oxygenated ring was at C-14 and C-15, thus

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establishing the tetracyclic core of **6**. Using an HMBC experiment, the remaining carbons at δ_C 171.7, 169.5, 119.2, and 99.2 could be attributed to C-23, C-20, C-22, and C-21, comprising a rare γ-hydroxybutyrolactone moiety[16] with the corresponding ^{1}H NMR resonances at δ_H 5.99 and 6.11, assigned to H-22 and H-21, respectively.[17]

The relative configuration of **6** was established via NOESY data. The NOESY correlations of Me-29/Me-19, Me-19/Me-30, and Me-30/H-17 indicated that Me-29, M-19, Me-30, and H-17 were cofacial and were randomly assigned to be β-oriented. Likewise, the NOESY correlations of M-28/H-5, H-5/H-18, and H-15/Me-18 indicated that they were cofacial and α-oriented. Compound **6** was thus elucidated as depicted, featuring a 21-hydroxy-20(22)-ene-21,23-γ-lactone ring in the side chain.

Toonaciliatavarin G (**7**), showing an ion at m/z 461.1937 (calcd 461.1935) in the HR-ESIMS, has the same molecular formula of $C_{26}H_{30}O_6$ as that of **6**. Inspection of the 1D and 2D NMR data (Table 3) of **6** and **7** revealed differences as far as the C-17 substituent was concerned. The presence of a γ-hydroxybutyrolactone unit[18] in the side chain in **7** was evidenced by the signals at δ_H 5.99 (H-22) and 6.21 (H-23) in the ^{1}H NMR spectrum as well as the hemiacetalic carbon at δ_C 98.1 (C-23) and an α,β-unsaturated-γ-lactone moiety[18] [δ_C 136.5 (C-20), 181.1 (C-21), and 149.1 (C-22)] in the ^{13}C NMR spectrum, which was confirmed by the NOESY spectrum. Thus, toonaciliatavarin G (**7**) is a regioisomer of compound **6**.

Toonaciliatavarin H (**8**) was obtained as a white, amorphous solid. The HR-ESIMS exhibited a molecular ion at m/z 593.2343 (calcd 593.2357), corresponding to a molecular formula of $C_{31}H_{38}O_{10}$ and indicating 13 degrees of unsaturation. The IR spectrum exhibited absorption bands for carbonyl (1744, 1710 cm^{-1}) and olefinic (1636 cm^{-1}) functionalities. The NMR data featured a *seco* B-ring limonoid. Two broad singlets at δ_H 5.52 and 5.38 and the corresponding carbon signals at δ_C 135.4 and 121.0 were characteristic of a terminal double bond at C-8. A carbomethoxy group was evidenced by the carboxy carbon signal at δ_C 174.5, which showed an HMBC correlation with the methoxy protons at δ_H 3.67 (3H, s). The location of this group was determined by the HMBC correlation between H_2-6 and the carboxy carbon at C-7, the same as in *seco* B-ring limonoids.[19] An α,β-unsaturated carbonyl moiety in A ring was indicated by a pair of doublets at δ_H 7.32 (J = 10.5 Hz, H-1) and 6.15 (J = 10.5 Hz, H-2) and carbon resonances at δ_C 152.7, 126.1, and 203.7 assigned to C-1, C-2, and C-3, respectively. The common $\Delta^{14(15)}$-double bond was replaced by a 14,15-oxirane moiety, as indicated by resonances at δ_C 71.9 and 60.1 and a resonance ascribed to H-15 at δ_H 4.10. Proton resonances at δ_H 2.01 (3H, s) and 1.78 (3H, s) were ascribable to two acetoxy groups, which were placed at C-11 and C-12 according to their HMBC correlations with H-11 and H-12, respectively. The remaining four carbons at δ_C 131.0, 175.0, 149.3, and 70.6 comprised an α,β-unsaturated lactone ring in the side chain, which was inferred by the strong HMBC correlations from the broad oxymethylene singlet at δ_H 4.88 to C-22 as well as the HMBC correlation from H-17 to the ester carbonyl carbon.

The 14,15-oxirane function was established to be β-orientated according to a NOESY correlation between H-15 and H-30. The singlet H-9 resonance and small coupling constant of $J_{11,12}$ (4.5 Hz) indicated that the acetate groups at C-11 and C-12 were both α-oriented,[19] which was also confirmed by NOESY correlations from H-11 to Me-19 and from H-12 to H-11. Thus structure **8** is proposed for toonaciliatavarin H.

The absolute configuration of **8** was determined using the ECD method as for compound **2**. The calculated ECD spectrum of **8** (Figure 7) matches the experimental result very well, allowing the assignment of the absolute configuration of **8** as depicted.

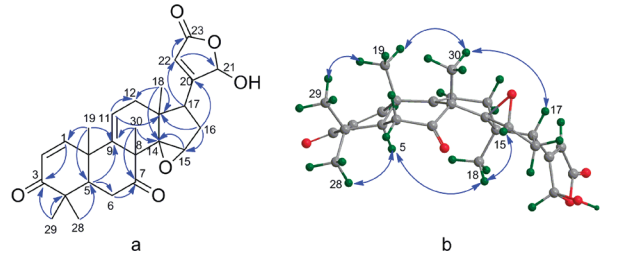

Figure 5. Selected HMBC (H → C) (a) and NOESY (←→) correlations (b) of **6**.

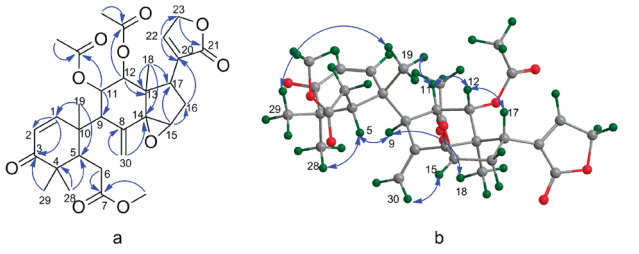

Figure 6. Selected HMBC (H → C) (a) and NOESY (←→) correlations (b) of **8**.

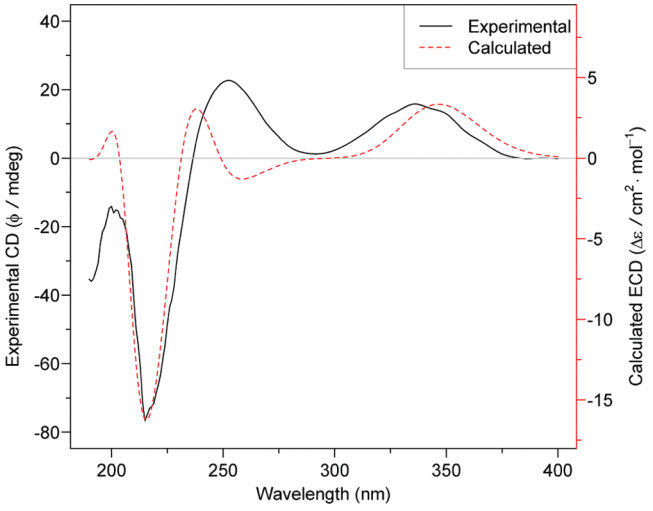

Figure 7. ECD spectra of **8**.

The known compounds hispidone,[11] bourjotinolone A,[20] 3-episapeline A,[20] 23-hydroxytoonacilide,[19] 21-hydroxytoonacilide,[19] bourjotinolone B,[7] piscidinol B,[14] piscidinol A,[21] aglaiodiol,[22] and odoratol[23] were identified by comparison of their spectroscopic data with those reported. Compounds **1**–**5** are precursors of tetranortriterpenoids, e.g., **6**–**8**. It would be interesting to determine the amount of these isolates during different life stages of the title plant.

Compounds **1**–**8** were evaluated for their cytotoxicity using K562 (leukemia), SMMC-7721 (hepatocellular carcinoma),

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Table 4. Cytotoxicity of Isolates against Six Cancer Cell Lines[a,b]

compound	MCF-7	MCF-7/ADR	KB	KB/VCR	SMMC-7721	K562
4	>50	>50	39.5 ± 2.0	>50	31.4 ± 2.4	43.1 ± 2.1
5	17.1 ± 1.4	22.6 ± 3.8	10.2 ± 1.1	32.1 ± 2.3	19.4 ± 1.9	11.2 ± 0.9
5-fluorouracil[c]	>100	>100	6.8 ± 1.2	33.4 ± 2.5	>100	2.1 ± 0.3
doxorubicin[c]	0.54 ± 0.07	>50	0.012 ± 0.005	0.45 ± 0.03	0.37 ± 0.06	0.17 ± 0.02

[a]Results are expressed as IC_{50} values in μM. [b]Compounds **1**–**3** and **6**–**8** were inactive for all cell lines (IC_{50} > 50 μM). [c]Positive controls.

MCF-7 (breast cancer), and KB (oral epithelial cancer) human cell lines, as well as multidrug-resistant cell lines MCF-7/ADM and KB/VCR (Table 4). Compounds **4** and **5** were found to show moderate cytotoxicities.

Nine isolates were further tested for their inhibitory effect on LPS-stimulated NO production in RAW 264.7 cells (Table 5).

Table 5. Effects of Compounds on NO in LPS-Stimulated RAW 264.7 Cells (n = 4)

	IC_{50} (μM)		IC_{50} (μM)
compound	NO	compound	NO
1	9.4 ± 0.2	**7**	15.2 ± 0.9
2	7.9 ± 0.4	**8**	20.9 ± 1.4
3	11.0 ± 0.7	23-hydroxytoonacilide	41.4 ± 2.2
4	33.4 ± 1.1	21-hydroxytoonacilide	38.9 ± 1.5
6	28.8 ± 2.7	dexamethasone[a]	0.98 ± 0.06

[a]Positive control.

The protolimonoids **1**–**3** exhibited moderate inhibitory abilities, with IC_{50} values lower than 12 μM, as compared with tetranortriterpenoids **6**–**8**, 23-hydroxytoonacilide, and 21-hydroxytoonacilide, showing weak inhibitory effects, which suggested a superiority of protolimonoids to tetranortriterpenoids in this regard.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with a JASCO P-1020 polarimeter. IR spectra (KBr disks) were recorded on a Bruker Tensor 27 spectrometer. ECD spectra were obtained on a JASCO 810 spectropolarimeter. NMR spectra were recorded on a Bruker ACF-500 NMR instrument (^{1}H: 500 MHz, ^{13}C: 125 MHz), with TMS as internal standard. Mass spectra were obtained on an MS Agilent 1100 Series LC/MSD ion-trap mass spectrometer (ESIMS) and a Mariner ESITOF spectrometer (HR-ESIMS). All solvents used were of analytical grade (Jiangsu Hanbang Science and Technology. Co., Ltd.). Silica gel (Qingdao Haiyang Chemical Co., Ltd.), Sephadex LH-20 (Pharmacia), and RP-C_{18} (40–63 μm, FuJi) were used for column chromatography. Preparative HPLC was carried out using an Agilent 1100 Series instrument with a Shim-pack RP-C_{18} column (20 × 200 mm) and a 1100 Series multiple wavelength detector.

Plant Material. The air-dried stem barks of *Toona ciliate Roem.* var. *henryi* (C. DC.) C. Y. Wu were collected from Xishuangbanna, Yunnan Province, People's Republic of China, in May 2009, and were authenticated by Professor Jing-Yun Cui, Xishuangbanna Botanical Garden, Chinese Academy of Sciences, People's Republic of China. A voucher specimen has been deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University (accession number TC200905).

Extraction and Isolation. The air-dried stem barks (10 kg) were extracted with 95% aqueous EtOH (3 × 20 L) under reflux. The EtOH extract was concentrated under reduced pressure (2000 g) and extracted with $CHCl_3$ to give 135 g of material. The oily chloroform extract was dissolved in 2 L of 50% aqueous MeOH and extracted with petroleum ether. After removal of the fatty components, 65 g of the extract was obtained, which was subjected to passage over a silica gel column eluted with $CHCl_3$–MeOH in a gradient (1:0 to 1:2), to afford four fractions (A–D), monitored by TLC. Fraction B (4 g) was chromatographed on a column of silica gel, eluted successively with a gradient of petroleum ether–EtOAc (10:1 to 1:1), to give three subfractions (B1–B3). Subfraction B2 (230 mg) was chromatographed on a column of reversed-phase C_{18} silica gel, eluted with MeOH–H_2O (5:5 to 9:1), to give three subfractions (B2a–B2c). Subfraction B2a (80 mg) was separated over ODS, using MeOH–H_2O (75:25) as the mobile phase, to give bourjotinolone B (8 mg), piscidinol B (6 mg), and piscidinol A (30 mg). Subfraction B3 (180 mg) was chromatographed on a column of reversed-phase C_{18} silica gel, eluted with MeOH–H_2O (5:5 to 9:1), to give four subfractions (B3a–B3d). Of these, subfraction B3b (70 mg) was separated by preparative HPLC, using MeOH–H_2O (70:25, 10 mL/min) as the mobile phase, to give **4** (11 mg), aglaiodiol (20 mg), and odoratol (5 mg). Fraction C (12 g) was chromatographed on a column of silica gel, eluted successively with a gradient of petroleum ether–EtOAc (4:1 to 1:2), to give three subfractions (C1–C3). Subfraction C1 (1 g) was chromatographed on a column of reversed-phase C_{18} silica gel, eluted with MeOH–H_2O (5:5 to 7:3), to give four subfractions (C1a–C1d). Subfraction C1b (100 mg) was separated by preparative HPLC, using MeOH–H_2O (70:30, 10 mL/min) as the mobile phase, to give **5** (6 mg) and bourjotinolone A (13 mg). Subfraction C1d (300 mg) was separated by preparative HPLC using MeOH–H_2O (64:35, 10 mL/min) as the mobile phase to give **1** (9 mg), **2** (11 mg), hispidone (20 mg), and 3-episapeline A (5 mg). Subfraction C3 (800 mg) was chromatographed on a column of reversed-phase C_{18} silica gel, eluted with MeOH–H_2O (5:5 to 7:3), to give four subfractions (C3a–C3d). Subfraction C3b (120 mg) was separated by preparative HPLC, using MeOH–H_2O (65:35, 10 mL/min) as the mobile phase, to give **3** (6 mg), **6** (4 mg), and 21-hydroxytoonacilide (12 mg). Subfraction C3a (90 mg) was separated by preparative HPLC using MeOH–H_2O (65:35, 10 mL/min) as the mobile phase to give **7** (4 mg), **8** (10 mg), and 23-hydroxytoonacilide (15 mg).

*Toonaciliatavarin A (**1**):* white, amorphous powder; $[\alpha]^{25}_D$ −11 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3451, 2947, 1730, 1640, 1379, 1249, 1038 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; negative ESIMS *m/z* 535.4 $[M + Cl]^-$ (100); negative ESIMS *m/z* 535.4 $[M + Cl]^-$ (100); positive ESIMS *m/z* 501.3 $[M + H]^+$ (100); HR-ESIMS *m/z* 523.3051 $[M + Na]^+$ (calcd for $C_{30}H_{44}O_6Na$, 523.3030).

*Toonaciliatavarin B (**2**):* white, amorphous powder; $[\alpha]^{25}_D$ +15 (*c* 0.1, MeOH); UV (CH_3CN) λ_{max} (log ε): 208 (3.91) nm; ECD (CH_3CN): 207 nm (Δε −1.31), 231 nm (Δε +3.32), 184 nm (Δε +1.61); IR (KBr); ν_{max} 3450, 2942, 1711, 1640, 1377, 1249, 1030 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; negative ESIMS *m/z* 579.5 $[M + Cl]^-$ (100); positive ESIMS *m/z* 545.4 $[M + H]^+$ (100); HR-ESIMS *m/z* 567.3306 $[M + Na]^+$ (calcd for $C_{32}H_{48}O_7Na$, 567.3292).

*Toonaciliatavarin C (**3**):* white, amorphous powder; $[\alpha]^{25}_D$ −13 (*c* 0.2, MeOH); IR (KBr) ν_{max} 3455, 2927, 1733, 1641, 1384, 1350 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; negative ESIMS *m/z* 543.4 $[M - H]^-$ (100); positive ESIMS *m/z* 545.4 $[M + H]^+$ (100); HR-ESIMS *m/z* 567.3289 $[M + Na]^+$ (calcd for $C_{32}H_{48}O_7Na$, 567.3292).

*Toonaciliatavarin D (**4**):* white, amorphous powder; $[\alpha]^{25}_D$ −46 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3423, 2933, 1641, 1463, 1384, 1060, 1034 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 2; negative ESIMS *m/z* 473.2 $[M - H]^-$ (100); positive ESIMS *m/z* 475.2 $[M + H]^+$ (100); HR-ESIMS *m/z* 497.3598 $[M + Na]^+$ (calcd for $C_{30}H_{50}O_4$, 497.3601).

*Toonaciliatavarin E (**5**):* white, amorphous powder; $[\alpha]^{25}_D$ −68 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3443, 2972, 2948, 1696, 1642, 1386, 1167, 1030, 983, 652 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 2; negative ESIMS

m/z 489.5 $[M - H]^-$ (100); HR-ESIMS *m/z* 513.3569 $[M + Na]^+$ (calcd for $C_{30}H_{50}O_5Na$, 513.3550).

*Toonaciliatavarin F (**6**):* white, amorphous powder; $[\alpha]^{25}_D$ −57 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3526, 2983, 1735, 1710, 1664, 1385, 1273, 1137 cm^{-1}; ^1H and ^{13}C NMR, see Table 3; negative ESIMS *m/z* 473.2 $[M + Cl]^-$ (100); positive ESIMS *m/z* 456.2 $[M + NH_4]^+$ (100); HR-ESIMS *m/z* 461.1913 $[M + Na]^+$ (calcd for $C_{26}H_{30}O_6Na$, 461.1935).

*Toonaciliatavarin G (**7**):* white amorphous powder; $[\alpha]^{25}_D$ +39 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3525, 2983, 1735, 1710, 1664, 1446, 1273, 1137 cm^{-1}; ^1H and ^{13}C NMR, see Table 3; negative ESIMS *m/z* 473.2 $[M + Cl]^-$ (100); positive ESIMS *m/z* 456.2 $[M + NH_4]^+$ (100); HR-ESIMS *m/z* 461.1937 $[M + Na]^+$ (calcd for $C_{26}H_{30}O_6Na$, 461.1935).

*Toonaciliatavarin H (**8**):* white, amorphous powder; $[\alpha]^{25}_D$ +40 (*c* 0.1, MeOH); UV (CH_3CN) λ_{max} (log ε) 202 (4.88) nm; ECD (CH_3CN) 200 nm ($\Delta\varepsilon$ −1.22), 215 nm ($\Delta\varepsilon$ −6.62), 272 nm ($\Delta\varepsilon$ +1.82); IR (KBr) ν_{max} 3446, 2974, 1744, 1636, 1384, 1243, 1043 cm^{-1}; ^1H and ^{13}C NMR, see Table 3; negative ESIMS *m/z* 605.5 $[M + Cl]^-$ (100); positive ESIMS *m/z* 588.3 $[M + NH_4]^+$ (100); HR-ESIMS *m/z* 593.2343 $[M + Na]^+$ (calcd for $C_{31}H_{38}O_{10}Na$, 593.2357).

Determination of Cytotoxic Activities. The following human tumor cell lines were used: K562 (leukemia), SMMC-7721 (hepatocellular carcinoma), MCF-7 (breast cancer), KB (oral epithelial cancer), and multidrug-resistant cells of MCF-7/ADM and KB/VCR. All cells were cultured in RPMI-1640 or DMEM medium (Hyclone, Logan, UT, USA), supplemented with 10% fetal bovine serum (Hyclone) in 5% CO_2 at 37 °C. The cytotoxicity assay was performed according to the MTT method in 96-well microplates.[23] Briefly, 180 μL of the cell suspension was seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before test compound addition, while suspended cells were seeded just before test compound addition with an initial density of 1×10^5 cells/mL. Each tumor cell line was exposed to each test compound at concentrations of 0.1, 1, 10, 100, and 500 μM in triplicate for 48 h, with 5-fluorouracil and doxorubicin (Sigma, St. Louis, MO, USA) used as positive controls. After treatment, cell viability was detected and IC_{50} values were calculated by the Reed and Muench method.[25]

Determination of Nitrite Activity Assay. Mouse macrophage cell line (RAW 264.7) was obtained from the Chinese Academy of Science Cell Bank (Shanghai, China). The cells were cultured in RPMI 1640 medium (Gibco) supplemented with 10% heated-deactivated fetal bovine serum (FBS), penicillin (100 U/mL), and streptomycin (15 μM) at 37 °C atmosphere and 5% CO_2.

RAW 264.7 cells were placed in 24-well cell culture plates at a density of 1×10^5 with 500 μL of culture medium and incubated for 24 h. The cells were pretreated with different compounds (40, 20, 10, 5, 2.5 μM) for 2 h, which were solubilized with DMSO diluted with RPMI 1640 medium and then stimulated with lipopolysaccharide (Sigma) (2 μg/mL) for 18 h. The final concentration of DMSO did not exceed 0.1% in the culture medium. After incubation, the supernatants (100 μL) were added to a solution of 100 μL of Griess reagent (1% sulfanilamide and 0.1% naphthyl ethylene diamine dihydrochloride in 5% H_3PO_4). Using $NaNO_2$ to generate a standard curve, nitrite production was measured by a microplate reader (IQuantTM, BIO-TEK Instrument Inc., Winooski, VT, USA) at 540 nm.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

HRESIMS, ^1H and ^{13}C NMR, and 2D NMR spectra of toonaciliatavarins A−H (**1**−**8**). This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +86-25-83271405. Fax: +86-25-85301528. E-mail: cpu_lykong@126.com.

Notes

The authors declare no competing financial interest.

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