Cytotoxic Withanolides from the Flowers of *Datura metel*

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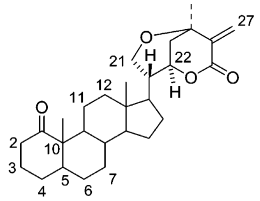
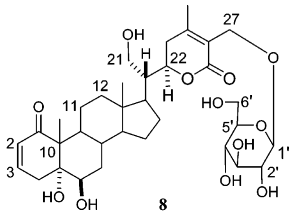
Chemical investigation of a methanol extract of the flowers of *Datura metel* has led to the isolation of 10 new withanolides, withametelins I−P (**1**−**8**), 1,10-*seco*-withametelin B (**9**), and 12β-hydroxy-1,10-*seco*-withametelin B (**10**), together with seven known withanolides. The structures of **1**−**10** were elucidated by means of spectroscopic methods, and the absolute stereochemistry of **1** was confirmed by single-crystal X-ray analysis. Compounds **1**, **3**, **4**, and **6** exhibited cytotoxic activities against A549 (lung), BGC-823 (gastric), and K562 (leukemia) cancer cell lines, with IC_{50} values ranging from 0.05 to 3.5 μM.

The withanolides are a group of naturally occurring steroidal lactones that have been isolated from the genera *Acnistus*, *Datura*, *Dunalia*, *Jaborosa*, *Lycium*, *Physalis*, and *Withania* of the family Solanaceae. Many of these compounds exhibit a variety of biological activities, including anti-inflammatory, antioxidant, antitumor, and immunosuppressive properties.[1] Withanolides can inhibit tumor cell proliferation[2] and angiogenesis[3] and induce the phase II enzyme quinone reductase.[4]

The flowers of *Datura metel* L. (Solanaceae), known as "Yangjinhua", have been used in traditional Chinese medicine for the treatment of asthma, convulsions, pain, and rheumatism for centuries.[5] In the present investigation, bioassay-guided fractionation of a methanol extract of the flowers of *D. metel* has led to the purification of 10 withanolides, withametelins I−P (**1**−**8**), 1,10-*seco*-withametelin B (**9**), and 12β-hydroxy-1,10-*seco*-withametelin B (**10**), together with seven known withanolides. We describe herein the isolation and structure determination of these 10 new withanolides (**1**−**10**). Their cytotoxic activities against A549 (lung), BGC-823 (gastric), and K562 (leukemia) cancer cells are also reported herein.



1 Δ^2, 5β, 6β-epoxy, OH-12β
2 Δ^2, 5α-OH, OH-6β, OH-12β
3 $\Delta^{2,4}$, OH-6β, OH-12β
4 $\Delta^{2,5}$, OH-12β
5 $\Delta^{3,5}$, OH-12β
6 OH-3α, 5β, 6β-epoxy
7 Δ^2, OH-5β, OH-6α



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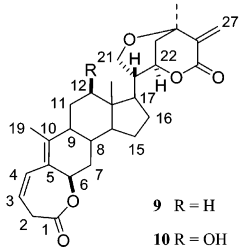

9 R = H
10 R = OH

Results and Discussion

The ethyl acetate-soluble fraction obtained from a methanol extract of the flowers of *D. metel* showed significant cytotoxicity against selected human cancer cell lines. Further investigation of this fraction by repeated chromatography over silica gel and RP-18 resulted in the isolation of 10 new compounds (**1**−**10**). The ^{1}H NMR spectroscopic data of **1**−**10** are shown in Tables 1 and 2, with their ^{13}C NMR spectroscopic data in Table 3.

Compound **1** was obtained as colorless crystals with a molecular formula of $C_{28}H_{36}O_6$ as determined from its HRESIMS and NMR data. The UV absorption maximum at 216 nm and strong IR absorptions at 3430, 1726, and 1663 cm^{-1} indicated the presence of an α,β-unsaturated enone, an α,β-unsaturated δ-lactone, and a hydroxyl group, respectively. The ^{1}H and ^{13}C NMR spectra of **1** showed the presence of three tertiary methyls at δ_H 0.68, 1.24, and 1.42 (Me-18, Me-19, and Me-28, respectively) and two singlet olefinic protons at δ_H 6.00 and 6.75 (each 1H, s, H_2-27), assigned to a terminal methylene conjugated with the lactone carbonyl. Also observed were two remaining olefinic protons at δ_H 6.85 (1H, ddd, J = 10.0, 6.0, 2.4 Hz, H-3) and 6.02 (1H, dd, J = 10.0, 2.5 Hz, H-2), associated with a 2-en-1-one system, and two oxygenated carbons with chemical shift values at δ_C 61.8 and 63.4 (5β,6β-epoxy), almost identical to those of withametelin F.[6] Compound **1** differed from the latter compound by its increase in molecular mass of 16 amu and by the presence of an additional oxygenated methine carbon at δ_C 76.9 that correlated with the proton resonating at δ_H 3.52 (1H, dd, J = 11.1, 4.2 Hz) in the HSQC spectrum, disclosing an additional hydroxyl group in **1**. The ^{1}H−^{1}H COSY couplings between the above-mentioned methine and two methylene protons observed at δ_H 1.50 (1H, m, H-11α) and 2.40 (1H, dt, J = 12.9, 4.2 Hz, H-11β) revealed the hydroxyl group to be located at C-12, which was confirmed by the long-range correlations between C-12 and H-11 and H-18, C-11 and H-12, and C-18 and H-12 (Figure 1). The relative configuration at C-12 was determined as β on the basis of the analysis of the coupling constant of 11.1 Hz between H-11β and H-12, which was in good agreement with the upfield shift of δ_C 7.5 for the C-18 methyl signal due to a γ-gauche effect induced by a 12β-hydroxyl group.[7] The absolute configuration of **1** was established by means of X-ray crystallographic diffraction analysis. A perspective view of the structure is shown in Figure 2. This analysis revealed that the A/B rings of **1** are *cis*-fused with

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Table 1. ^{1}H NMR Data of Compounds **1**−**5**

proton	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[b]
2a	6.02 (dd;10,2.5)	5.87 (dd;10.2,2.4)	5.96 (dd;9.6,0.9)	5.85 (dd;9.9,2.1)	3.28 (d;20.4)
2b					2.75 (dd;20.4,4.5)
3	6.85 (ddd;10,6,2.4)	6.59 (ddd;10.2,4.8,2.4)	6.99 (dd;9.6,6)	6.79 (ddd;9.9,5.1,2.7)	5.60 (dt;9.6,4.5)
4a	3.01 (dd;21.6,2.4)	3.34 (dt;19.8,2.7)		3.27 (dd;21.3,2.1)	
4b	1.90 m	2.07 m	6.15 (d;6)	2.85 (dd;21.3,4.8)	6.05 (dd;9.6,2.1)
6	3.13 (d;2.4)	3.65 (br, s)[c]	4.40 (br, s)	5.55 (d;6)	5.64 (d;5.1)
7a	2.10 (dd;14.4,3)	1.58−1.68 m	2.01 m	1.98 m	2.18 (dt;17.7,5.1)
7b	1.18 m		1.18 m	1.51 m	1.52 m
8	1.48 m	1.66 m	1.98 m	1.36 m	1.38 m
9	1.18 m	1.90 m	1.90 m	1.74 m	1.89 m
11a	2.20 (dt;12.9,4.2)	2.43 (dt;12.3,3.3)	2.02 m	2.40 (dt;12.6,4.2)	1.98 m
11b	1.45 m	1.34 m	1.55 m	1.50 m	1.35 m
12	3.52 (dd;11.1,4.2)	3.65[c]	3.36 (dd;11,4.8)	3.71[c]	3.71[c]
14	0.98 m	1.24 m	1.15 m	1.13 m	1.13 m
15	1.65 m	1.24−1.70 m	1.70 m	1.28−1.70 m	1.70 m
16	1.78 m	1.75 m	1.70 m	1.46−1.78 m	1.42 m
17	1.80 m	1.88 m	1.95 m	1.88 m	1.86 m
18	0.70 s	0.70 s	0.74 s	0.70 s	0.72 s
19	1.24 s	1.30 s	1.45 s	1.21 s	1.35 s
20	1.85 m	1.90 m	1.85 m	1.88 m	1.85 m
21a	4.54 (d;13.5)	4.54 (d;13.5)	4.56 (d;13.5)	4.58 (d;13.5)	4.56 (d;13.5)
21b	3.70 (dd;13.5,3.3)	3.68 (dd;13.5,2.7)[c]	3.62 (dd;13.5,3)	3.68 (dd;13.5,3)[c]	3.68 (dd;13.5,3)[c]
22	4.66 (br, s)	4.69 (br, s)	4.69 (br, s)	4.69 (br, s)	4.69 (br, s)
23	1.85−1.95 m	1.58−1.68 m	1.98 m	1.92−2.04 m	1.91−1.98 m
27a	6.75 s	6.75 s	6.96 s	6.75 s	6.76 s
27b	5.99 s	6.00 s	6.01 s	6.00 s	6.01 s
28	1.42 s	1.42 s	1.42 s	1.43 s	1.43 s

[a] Spectra recorded at 300 MHz in $CDCl_3$ at 25 °C. [b]Spectra recorded at 300 MHz in $CDCl_3$ with a small amount of CD_3OD added at 25 °C. The values are ppm downfield from TMS, and assignments were made by COSY, HMQC, and HMBC experiments. [c]Signals partly overlapped.

Table 2. ^{1}H NMR Data of Compounds **6**−**10**

proton	**6**[a]	**7**[a]	**8**[b]	**9**[a]	**10**[a]
2a	2.78 (dd;14.1,6.3)	6.02 (dd;9.9,2.4)	5.77 (dd;9.9,2.4)	3.79 (br,d;17.4)	3.82 (br,d;17.4)
2b	2.64 (dd;14.1,5.4)			3.18 (dd;17.4,8.7)	3.18 (dd;17.4,8.7)
3	4.32 (br, m)	6.75 (ddd;9.9,5.4,2.4)	6.64 (ddd;9.9,5.1,2.1)	5.50 (dd;11.4,8.7)	5.50 (dd;11.4,8.7)
4a	2.33 (dd;14.7,4.5)	2.80 (dt;20.7,2.7)	3.25 (dt;19.8,2.7)	6.55 (dd;11.4,3.3)	6.55 (dd;11.4,3.3)
4b	1.45 m	2.50 (dd;20.7,5.1)	2.05 (dd;19.8,5.1)		
6	3.24 (d; 1.5)	3.88 (dd;11.7,5.1)	3.52 (br, s)	5.1 (br, d; 3.3)	5.1 (br, d; 3.3)
7a	2.12 (dt;14.1,2.7)	1.98 m	1.70 m	2.01 m	2.12 (dd;12.3,4.2)
7b	1.44 m		1.50 m		1.34 (dd;12.3,3.6)
8	1.48 m	1.20 m	1.78 m	1.69 m	1.40 m
9	1.20 m	1.30 m	1.80 m	1.69 m	1.69 m
11a	1.35−1.45 m	1.36 m	2.22 m	1.25 m	2.05 m
11b				1.65 m	1.38 m
12a	1.40 m	1.80−1.85 m	2.05 m	2.05 m	3.60 (dd;10.8,4.5)
12b		1.25−1.30 m	1.45 m		
14	0.98 m	1.04 m	1.24 m	1.25 m	1.16 m
15a	1.65 m	1.70 m	1.70 m	1.90 m	1.78 m
15b			1.24 m		
16	1.70 m	1.73 m	1.82 m	1.80 m	1.80 m
17	1.78 m	1.68−1.80 m	1.78 m	1.80 m	1.90 m
18	0.62 s	0.65 s	0.80 s	0.71 s	0.72 s
19	1.14 s	1.15 s	1.30 s	1.79 s	1.79 s
20	1.80 m	1.75−1.85 m	1.90 m	1.90 m	1.90 m
21a	3.83 (d;13.2)	3.82 (d;13.2)	3.78 (dd;11.4,4.2)	3.88 (d;13.5)	4.50 (d;13.5)
21b	3.67 (dd;13.2,3.3)	3.67 (dd;13.2,2.7)	3.92 (dd;11.4,2.1)	3.74 (dd;13.5,2.7)	3.68 (d;13.5)
22	4.61 (br, s)	4.61 (br, s)	4.56 (dt;13.2,3.3)	4.66 (br, s)	4.68 (br, s)
23a	1.80−1.97 m	1.85−2.00 m	2.97 (d;18.6)	1.85−2.0 m	1.85−2.05 m
23b			2.32 (dd;18.6,2.7)		
27a	6.71 s	6.73 s	4.62 (d;11.4)	6.76 s	6.75 s
27b	5.98 s	5.99 s	4.47 (d;11.4)	6.01 s	6.01 s
28	1.37 s	1.39 s	1.42 s	1.43 s	1.42 s
1′			4.32 (d;7.8)		
2′			3.16 (dd;8.7,7.8)		
3′			3.26 (t;8.7)		
4′			3.28 (t;8.7)		
5′			3.32 (dt;9.5,2.4)		
6′a			3.86 (d;12)		
6′b			3.66 (dd;12,4.8)		

[a] Spectra recorded at 300 MHz in $CDCl_3$ at 25 °C. [b]Spectra recorded at 300 MHz in CD_3OD at 25 °C. The values are ppm downfield from TMS, and assignments were made by COSY, HMQC, and HMBC experiments.

the C-10 methyl and the epoxide oxygen lying along the β-face, and the hydroxyl group at C-12 and the methyl at C-13 are also positioned in the same orientation, with the C-17 side chain exhibiting β-orientation. The stereochemistry at C-20 of **1** was determined as *R* on the basis of biogenetic arguments because all the reported withanolides unsubstituted at C-20 have the same configuration. Accordingly, the structure of **1** was proposed as (20*R*,22*R*,24*R*)-5β,6β-epoxy-21,24-epoxy-12β-hydroxy-1-oxowitha-2,25(27)-dienolide, and this compound has been named withametelin I.

Compound **2** was obtained as colorless crystals with a molecular formula of $C_{28}H_{38}O_7$, as determined by HRESIMS and from its NMR data. The ^{1}H and ^{13}C NMR spectra of **2** were closely related to those of **1**. The main difference was the chemical shifts of two

Table 3. ^{13}C NMR Data of Compounds **1**−**10**

C	1[a]	2[a]	3[a]	4[a]	5[b]	6[a]	7[a]	8[c]	9[a]	10[a]
1	203.2	204.3	207.5	204.0	210.4	210.9	201.3	208.1	172.6	172.5
2	129.1	128.7	118.7	127.8	39.6	45.2	127.8	129.5	35.6	35.5
3	144.7	141.3	142.3	145.4	126.7	64.1	143.5	144.4	117.3	117.6
4	32.8	35.7	126.7	33.3	129.2	39.7	30.3	37.1	129.4	129.1
5	61.8	77.2	159.6	135.8	140.8	62.2	78.9	78.8	124.9	125.8
6	63.4	74.7	73.9	124.5	121.7	61.6	72.0	75.7	73.1	73.0
7	30.8	33.4	40.7	30.3	30.6	31.3	35.9	34.6[d]	33.1	35.5
8	29.0	29.3	30.8	32.2	30.8	29.1	33.6	32.0	32.7	31.9
9	43.3	39.8	50.3	41.8	40.0	42.6	45.5	43.1	47.6	46.0
10	47.9	51.9	54.7	50.2	51.9	51.8	55.0	53.5	142.0	140.8
11	33.9	33.7	32.2	34.1	32.9	22.2	22.9	25.0	23.1	32.7
12	76.9	76.9	76.3	76.5	77.2	39.1	39.2	41.0	40.1	77.7
13	47.5	47.7	48.6	47.6	48.0	42.6	43.0	44.6	43.4	48.5
14	53.8	53.4	54.0	54.1	54.2	55.6	55.5	57.5	54.3	52.5
15	23.5	23.4	24.5	23.6	23.6	23.9	23.8	25.8	25.6	22.7
16	26.3	25.7	26.5	26.4	26.8	26.4	26.4	28.6	26.6	26.7
17	49.3	49.3	48.8	49.3	49.3	47.4	47.3	50.4	47.6	49.2
18	7.3	7.6	8.2	7.4	7.4	12.4	12.7	13.6	12.9	7.5
19	15.1	15.5	19.6	18.9	20.2	14.0	9.2	16.8	15.7	15.6
20	38.1	37.5	38.3	38.2	38.7	39.6	39.6	47.4	39.6	38.3
21	61.8	62.0	62.9	61.8	61.7	60.4	60.4	60.6	60.4	61.7
22	75.6	75.7	75.7	75.7	75.7	75.4	75.4	79.9	75.3	73.0
23	34.0	33.9	34.7	33.9	33.9	33.2	33.2	34.5[d]	33.3	33.9
24	69.1	69.2	69.9	69.1	69.2	69.3	69.3	161.4	69.4	69.1
25	138.9	138.8	139.6	138.9	138.9	138.8	138.8	124.0	138.8	138.8
26	165.3	165.3	167.1	165.3	165.3	165.3	165.2	169.1	165.2	165.2
27	130.0	130.0	131.2	130.0	130.0	130.0	130.1	64.1	130.1	130.1
28	26.3	25.8	26.2	25.7	25.7	25.5	25.6	21.2	25.7	25.7
1′								104.4		
2′								75.5		
3′								78.5		
4′								72.1		
5′								78.5		
6′								63.3		

[a] Spectra recorded at 75 MHz in $CDCl_3$ at 25 °C. [b]Spectra recorded at 75 MHz in $CDCl_3$ with a small amount of CD_3OD added at 25 °C. [c]Spectra recorded at 75 MHz in CD_3OD at 25 °C. The values are ppm downfield from TMS, and assignments were made by COSY, DEPT, HMQC, and HMBC experiments. [d]The values may be interchanged.

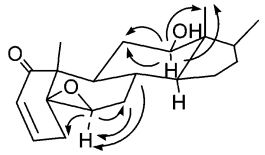

Figure 1. Key HMBC correlations in the B/C rings of **1**.

carbons bearing oxygen. The ^{13}C NMR and DEPT spectra exhibited signals corresponding to oxygenated carbons at δ_C 76.6 and 74.6, which were assigned to C-5 and C-6, and their downfield chemical shifts suggested the presence of a 5,6-diol group in the A/B rings. Furthermore, the ^1H and ^{13}C NMR data (Tables 1 and 3, respectively) of **2** were almost identical with those of withametelin G[6] except for the presence of an additional β-hydroxyl group at δ_H 3.65 and δ_C 76.9 at C-12, suggesting the 5α, 6β orientation of two of the hydroxyl groups. Therefore, the structure of **2** was elucidated as (20*R*,22*R*,24*R*)-21,24-epoxy-5α,6β,12β-trihydroxy-1-oxowitha-2,25(27)-dienolide and was named withametelin J.

Compound **3** was obtained as light yellow crystals from EtOAc with a molecular formula of $C_{28}H_{36}O_6$ as determined by HRESIMS. This compound was recognized as an analogue of withametelin J from the observation of characteristic signals in the ^1H NMR spectrum (Table 1). The only difference between these substances came from the signals of ring A assigned to three contiguous olefinic protons at δ_H 5.96 (1H, dd, J = 9.6, 0.9 Hz, H-2), 6.99 (1H, dd, J = 9.6, 6.0 Hz, H-3), and 6.15 (1H, d, J = 6.0 Hz, H-4) in **3**, which was consistent with its UV absorption maxima at 210 and 312 nm. Thus, the presence of a 2,4-dien-1-one moiety was revealed, and the chemical shift and splitting pattern of H-6 at δ_H 4.40 (1H, brs) indicated a β-oriented hydroxyl group at C-6, as in withametelin B.[8] Consequently, the structure of **3** was established as (20*R*,22*R*,24*R*)-21,24-epoxy-6β,12β-dihydroxy-1-oxowitha-2,4,-25(27)-trienolide and was named withametelin K.

Compound **4** was obtained as colorless crystals with a molecular formula of $C_{28}H_{36}O_5$, as determined by its HRESIMS and NMR data. The spectroscopic data of **4** were also very similar to those of **1**, except for NMR signals due to the substitution pattern at C-5 and C-6, as shown in Tables 1 and 3. In the ^1H NMR spectrum of **4**, a signal for an oxygenated proton at δ_H 3.13 (1H, d, J = 2.4 Hz) assigned to H-6 in **1** was absent, with instead a signal at δ_H 5.55 (d, 1H, J = 6.0 Hz) being observed. In the ^{13}C NMR and DEPT spectra the signals in **1** of the oxygenated sp^3 methine at δ_C 63.4 and the oxygenated sp^3 quaternary carbon at δ_C 61.8 were replaced in **4** by two sp^2 carbons at δ_C 135.8 and 124.5, respectively. These data were consistent with the presence of a ring A/B 2,5-dien-1-one moiety, which has been found in withametelin, the first withanolide isolated from *D. metel*.[9] Therefore, the structure of **4** was elucidated as (20*R*,22*R*,24*R*)-21,24-epoxy-12β-hydroxy-1-oxowitha-2,5,25(27)-trienolide and was named withametelin L.

Withametelin M (**5**) gave the same formula, $C_{28}H_{36}O_5$, as compound **4**, as determined from the HRESIMS and NMR data, indicating they are a pair of isomers. The ^1H NMR spectrum of **5** also showed signals for five olefinic protons, of which two, at δ_H 6.01 and 6.76 (each 1H, s), were assigned to two exomethylene protons conjugated with the lactone carbonyl group. The signal at δ_H 5.64 (1H, d, J = 5.1 Hz) was ascribed to H-6, as for **4**, and other signals were observed at δ_H 5.60 (1H, dt, J = 9.6, 4.5 Hz) and 6.05 (1H, dd, J = 9.6, 2.1 Hz), with a different splitting pattern and coupling constants. Furthermore, the carbonyl carbon of ring A gave a downfield shift at δ_C 210.4, consistent with a 3,5-dien-1-one system, as described for isowithametelin.[10] This was confirmed by the analysis of the HMBC spectrum, in which long-range correlations were observed between C-1 and H-2b and H-19, H-2b

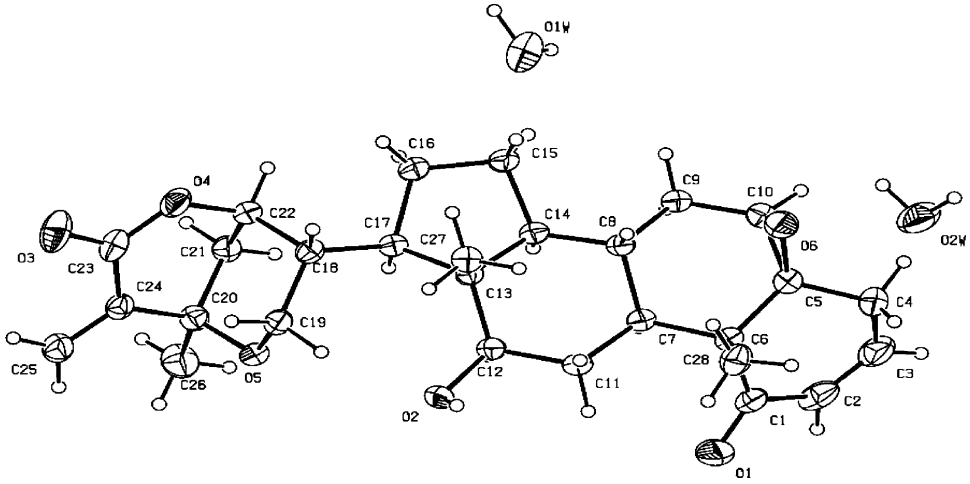

Figure 2. X-ray crystallographic displacement ellipsoid diagram of **1**.

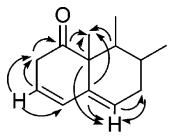

Figure 3. Key HMBC correlations in the A/B rings of **5**.

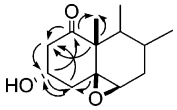

Figure 4. Key HMBC correlations in the ring A of **6**.

and C-1 and C-3, and H-6 and C-5, C-7, and C-10, as shown in Figure 3. Therefore, the structure of **5** (withametelin M) was established as (20*R*,22*R*,24*R*)-21,24-epoxy-12β-hydroxy-1-oxowitha-3,5,25(27)-trienolide.

Compound **6** was obtained as colorless crystals, and its molecular formula was determined from its HRESIMS and NMR data as $C_{28}H_{38}O_6$. On comparison with analogous data of compound **1**, the NMR spectra of **6** lacked any signals for a C-2/C-3 double bond, whereas an oxygenated methine signal was observed at δ_H 4.32 (1H, m), and this could be correlated with a tertiary carbon at δ_C 64.1 in the HSQC spectrum, which was assigned to C-3. Furthermore, the downfield signal of C-1, appearing at δ_C 210.9, and the splitting pattern and coupling constants of H-2 (δ_H 2.64, dd, J = 14.1, 5.4 Hz; 2.78, dd, J = 14.1, 6.3 Hz, each 1H) were consistent with a 2,3-dihydro-3-hydroxy system in ring A. This was confirmed by HMBC correlations, as shown in Figure 4. Comparison of the chemical shift value and the coupling pattern of H-3 with those of the compounds previously isolated from a soft coral led to the determination of an α-configuration hydroxyl group at C-3.[11] Thus the structure of **6** (withametelin N) was established as (20*R*,22*R*,24*R*)-5β,6β-epoxy-21,24-epoxy-2,3-dihydro-3α-hydroxy-1-oxowitha-25(27)-enolide.

Compound **7** exhibited the same molecular formula, $C_{28}H_{38}O_6$, as withametelin G, showing that they are a pair of isomers. The ^{1}H and ^{13}C NMR spectra of these compounds closely resembled one another except for the upfield shifts of C-19 and C-4 that appeared at δ_C 9.17 and 30.3 in the ^{13}C NMR spectrum of **7**, while in withametelin G these signals occur at δ_C 15.6 and 35.7, respectively. These significant upfield shifts could be interpreted by the γ-gauche effect induced by a 5β-hydroxyl group. Its ^{1}H NMR spectrum exhibited a doublet of doublets at δ_H 3.88 (1H, dd, J = 11.7, 5.1 Hz), instead of the broad doublet at δ_H 3.65 (1H, J = 2.7 Hz) in withametelin G, indicating an axial proton at C-6. Since a positive Cotton effect around 250 nm indicated a 22*R* configuration in withametelin,[10] the CD spectrum of **7** showed a positive Cotton effect at 259 nm, disclosing a 22*R* configuration. Thus a 5β,6α-diol substitution was assigned in the A/B rings, and on the basis of the above analysis, the structure of **7** (withmetelin O) was established as (20*R*,22*R*,24*R*)-21,24-epoxy-5β,6α-dihydroxy-1-oxowitha-2,25(27)-dienolide.

Compound **8** was obtained as a white powder with a molecular formula of $C_{34}H_{50}O_{12}$, as determined from its HRESIMS and NMR data. The acid hydrolysis of **8** (MeOH$-$H$_2$O, 1:1) with 1 M HCl provided withametelin G and withametelin H. The chemical shifts and coupling constants in the ^{1}H NMR spectrum as well as the ^{13}C NMR spectroscopic data (Tables 2 and 3) revealed that rings A$-$D of **8** were consistent with those of withametelin G[6] and indicated a β-D-glucosyl unit (δ_H 4.32 d, J = 7.8 Hz, H-1′; δ_C 104.4, C-1′). The ^{1}H NMR spectrum of **8** also showed signals for three methyls, two angular methyls (δ_H 0.80, 1.30 each 3H, s) at H-18 and H-19, respectively, and one vinylic methyl group (δ_H 2.12, 3H, s H-28). Moreover, the diagnostic double triplets for H-22 (δ_H 4.56, 1H, dt, J = 13.2, 3.3 Hz) indicated unambiguously that the C-17 side chain of **8** is not bicyclic like in **1**. Further analysis of the ^{1}H NMR spectrum showed that the signals of two terminal methylene protons had disappeared but that an additional oxygenated methylene group at δ_H 4.47 (1H, d, J = 11.4 Hz, H-27a) and 4.62 (1H, d, J = 11.4 Hz, H-27b) had appeared. In the HMBC spectrum, a long-range correlation was observed from H-1′ to C-27 as well as from H-27 to C-1′ and indicated the β-D-glucosyl unit is attached to C-27. Therefore, the structure of compound **8** was determined as (20*R*,22*R*)-5α,6β,21-trihydroxy-1-oxowitha-2,24-dienolide-27-*O*-β-D-glucopyranoside, and this compound was named withmetelin P.

Compound **9** was obtained as colorless crystals with a molecular formula of $C_{28}H_{36}O_5$, as determined by its HRESIMS and NMR data. The NMR spectra also showed it to be an analogue of withametelin with the main differences being in the A/B rings. In the ^{1}H NMR spectrum, a signal at δ_H 5.50 (1H, J = 11.4, 8.7 Hz), resonating as a broad double doublet, was assigned to H-3, and a downfield signal at δ_H 1.79 (3H, s) indicated an allylic methyl at H-19. In addition, a signal at δ_H 5.10 (1H, br, d, J = 3.3 Hz) together with the signal in the ^{13}C NMR spectrum of a keto-carbonyl of C-1 upfield of δ_C 172.5 due to an ester group of CO$-$O$-$CH indicated that **9** possesses a skeleton of 1,10-*seco*-withanolide. Further support of this assumption was obtained from the HMBC spectrum, in which long-range correlations were observed between C-1 (δ_C 172.5) and H-2α, H-2β, H-3, and H-6, between H-4 (δ_H 6.55, 1H, dd, J = 11.4, 3.3 Hz) and C-2 (CH2), C-5 (C), C-6 (CH), and C-10 (C), and between C-5 and H-19, as shown in Figure 5. Thus, a seven-membered β,γ-unsaturated lactonic moiety was determined in ring A.

The stereochemistry of C-6 in **9** was determined through a NOE NMR experiment. Irradiation of the signal of H-6 (δ_H 5.10) resulted in the enhancement of H-2α, H-7α, and H-7β, disclosing that H-6

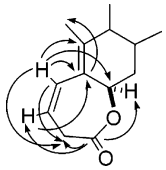

Figure 5. Key HMBC correlations in the A/B rings of **9**.

Table 4. Cytotoxicity of Compounds **1**−**10** against Three Human Cancer Cell Lines

	cell lines (IC_{50} μM)[a]		
compound	A549	BGC-823	K562
1	1.2	1.3	0.05
2	>10	5.2	2.5
3	3.5	1.9	0.12
4	2.0	1.6	0.55
6	1.7	1.0	0.46
adriamycin	0.75	0.35	0.58

[a] IC_{50} is the concentration of agent that reduced cell growth by 50% under the experimental conditions. [b]Compounds **5**, **7**, **8**, **9**, and **10** exhibit no cytotoxicity (IC_{50} > 10 μM).

has the same configuration as H-2α. Since a small coupling constant between H-2α and H-3 in the ^{1}H NMR spectrum indicated the dihedral angle between them to be about 90°, a β-configuration of the lactone bond at C-6 was inferred, which is in agreement with that of stoloniolide I.[12] Accordingly, **9** was elucidated to be 1,10-*seco*-withametelin B.

Compound **10** was obtained as colorless crystals with a molecular formula of $C_{28}H_{36}O_6$, as determined by its HRESIMS and NMR data. The ^{1}H and ^{13}C NMR data of **10** were closely related to those of **9** except for the presence of an oxygenated methine carbon signal at δ_C 77.7, which correlated with the proton at δ_H 3.60 (1H, dd, J = 10.8, 4.5 Hz). As described for compound **1**, compound **10** was found to be the 12β-hydroxyl derivative of **9** and has been named 12β-hydroxy-1,10-*seco*-withametelin B.

Compounds **9** and **10** are novel withanolides obtained from a plant in the genus *Datura* with an unusual seven-membered β,γ-unsaturated lactone of ring A. They may be related biogenetically to withametelin B, as formulated in previous literature.[12]

The cytotoxicity of the 10 new withanolides was tested against selected human cancer cell lines, namely, A549 (lung), BGC-823 (gastric), and K562 (leukemia). Compounds **1**, **3**, **4**, and **6** exhibited cytotoxicity against all three cell lines with IC_{50} values of 0.05−3.5 μM (Table 4). Compound **2** showed moderate cytotoxic activity against BGC-823 and K562 but less cytotoxicity against A549.

Experimental Section

General Experimental Procedures. Melting points were measured on a WRR micromelting point apparatus and are uncorrected. Optical rotations were measured on a JASCO PE-341 digital polarimeter. UV spectra were obtained on a TV-1901 spectrophotometer. IR spectra were recorded on a Nicolet 5700 FT-IR spectrometer. CD spectra were carried out on a JASCO J-810 CD spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity 300 spectrometer in $CDCl_3$ and CD_3OD, with tetramethylsilane (TMS) as an internal standard. HRESIMS were acquired on a Micromass LCT mass spectrometer. Column chromatography was performed on silica gel H (300−400 mesh; Qingdao Marine Chemical Ltd.), Sephadex LH-20 (25−100 μm; Pharmacia), and RP-18 (20−45 μm; Fuji Silysia Chemical Ltd.). Precoated silica gel GF_{254} plates for analytical TLC were obtained from Yantai Huiyou Chemical Ltd. All solvents used were of analytical grade (Shanghai Chemical Plant).

Plant Material. The flowers of *Datura metel* were collected from Yuxi Town, Fuqing City, Fujian Province, People's Republic of China, in September 2005. The plant material was identified by Dr. Keli Chen, and a voucher specimen is on deposit in Sundia Meditech Company, Ltd., Shanghai, People's Republic of China (voucher no. H20051120-3).

Extraction and Isolation. The dried, powdered flowers of *D. metel* (10 kg) were extracted exhaustively with MeOH at room temperature. The extract was concentrated to a thick syrup (1 L) under vacuum and diluted with four times its volume with water, and the aqueous suspension was sequentially extracted with *n*-hexane and EtOAc. The EtOAc-soluble fraction was subjected to silica gel column chromatography, first eluting with petroleum ether (60−90 °C)−acetone gradients and then with CH_2Cl_2−MeOH (4:1 to 0:1). The fraction that eluted with petroleum ether−acetone (9:1) was subjected to column chromatography over silica gel with CH_2Cl_2−EtOAc gradients to afford compounds **1** (120 mg), **9** (48 mg), and a mixture of **4** and **5**. This mixture was separated by RP-HPLC (Gilson 215) on a C_{18} column (Phenomenex, 5 μm, 250 mm × 21.4 mm), eluting with MeCN−H_2O (from 30:70 to 60:40, flow rate 25 mL/min), to afford pure **4** (18 mg) and **5** (8 mg). The fraction eluted by petroleum ether−acetone (5:1) was further chromatographed over silica gel, eluting with CH_2Cl_2−EtOAc gradients, and Sephadex LH-20, eluting with MeOH, to afford pure compounds **2** (24 mg), **3** (64 mg), **6** (55 mg), **7** (12 mg), and **10** (38 mg). The fraction eluted by CH_2Cl_2−MeOH (4:1) was first chromatographed over a Sephadex LH-20 column (MeOH) and then fractionated over RP-18 (MeOH−H_2O, 35:65) to afford impure **8**, which was further purified by RP-HPLC on the same column as above with gradient elution (MeCN−H_2O, 20:80 for 10 min; then from 20:80 to 90:10 for 15 min, flow rate 25 mL/min) to give pure **8** (15 mg).

Withametelin I (1): colorless crystals (EtOAc); mp 185−187 °C; $[\alpha]^{20}_D$ −59 (*c* 0.20, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 216 (4.13) nm; IR ν_{max} (KBr) 3453, 2939, 1720, 1672, 1053, 968 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3, respectively; HRESIMS *m*/*z* 491.2387 $[M + Na]^+$ (calcd for $C_{28}H_{36}NaO_6$, 491.2410).

Withametelin J (2): colorless crystals (EtOAc); mp 203−205 °C; $[\alpha]^{20}_D$ −35 (*c* 0.09, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 210 (4.00) nm; IR ν_{max} (KBr) 3433, 1721, 1683 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3, respectively; HRESIMS *m*/*z* 509.2508 $[M + Na]^+$ (calcd for $C_{28}H_{38}NaO_7$, 509.2515).

Withametelin K (3): light yellow crystals (EtOAc); mp 244−247 °C; $[\alpha]^{20}_D$ −110 (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 226 (4.05), 312 (3.70) nm; IR ν_{max} (KBr) 3450, 1701, 1680 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3, respectively; HRESIMS *m*/*z* 491.2391 $[M + Na]^+$ (calcd for $C_{28}H_{36}NaO_6$, 491.2410).

Withametelin L (4): colorless crystals (EtOAc); mp 219−222 °C; $[\alpha]^{20}_D$ −122 (*c* 0.20, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 215 (4.16) nm; IR ν_{max} (KBr) 3500 (br), 2981, 1716, 1679, 1051, 810 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3, respectively; HRESIMS *m*/*z* 475.2451 $[M + Na]^+$ (calcd for $C_{28}H_{36}NaO_5$, 475.2460).

Withametelin M (5): colorless solid; mp 145−148 °C; $[\alpha]^{20}_D$ −70 (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 216 (4.05) nm; IR ν_{max} (KBr) 3483, 1715, 1678 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3, respectively; HRESIMS *m*/*z* 475.2453 $[M + Na]^+$ (calcd for $C_{28}H_{36}NaO_5$, 475.2460).

Withametelin N (6): colorless crystals (EtOAc); mp 236−239 °C; $[\alpha]^{20}_D$ −130 (*c* 0.09, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 215 (4.15) nm; IR ν_{max} (KBr) 3485, 2921, 1716, 1668 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3, respectively; HRESIMS *m*/*z* 493.2562 $[M + Na]^+$ (calcd for $C_{28}H_{38}NaO_6$, 493.2566).

Withametelin O (7): colorless solid; mp 161−164 °C; $[\alpha]^{20}_D$ −50 (*c* 0.12, $CHCl_3$); CD (nm) $\Delta\epsilon$ + 6.1 (226, *c* 0.0005, MeOH); UV (MeOH) λ_{max} (log ϵ) 210 (4.15) nm; IR ν_{max} (KBr) 3534, 2921, 1702, 1679 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3, respectively; HRESIMS *m*/*z* 493.2562 $[M + Na]^+$ (calcd for $C_{28}H_{38}NaO_6$, 493.2566).

Withametelin P (8): white solid; $[\alpha]^{20}_D$ +14 (*c* 0.10, CH_3OH); IR ν_{max} (KBr) 3423 (br), 2921, 1679, 1398, 1043, 968 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3, respectively; HRESIMS *m*/*z* 673.3181 $[M + Na]^+$ (calcd for $C_{40}H_{50}NaO_{12}$, 673.3200), 689.2971 $[M + K]^+$ (calcd for $C_{34}H_{50}KO_{12}$, 689.2939).

1,10-*seco*-Withametelin B (9): colorless crystals (EtOAc); mp 232−234 °C; $[\alpha]^{20}_D$ −39 (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 240 (4.26) nm; IR ν_{max} (KBr) 2921, 1727, 1629 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3, respectively; HRESIMS *m*/*z* 453.2639 $[M + H]^+$ (calcd for $C_{28}H_{37}O_5$, 453.2641).

12β-Hydroxy-1,10-*seco*-withametelin B (10): colorless crystals (EtOAc); mp 157−159 °C; $[\alpha]^{20}_D$ −60 (*c* 0.20, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 243 (4.29) nm; IR ν_{max} (KBr) 3494, 2921, 1716, 1625 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3, respectively; HRESIMS *m*/*z* 469.2576 $[M + H]^+$ (calcd for $C_{28}H_{37}O_6$, 469.2590).

Acid Hydrolysis of 8. A MeOH−H_2O (1:1) solution of **8** (8 mg) with a few drops of 1 M HCl was stirred at 60 °C for 2 h in a water bath. The mixture was neutralized, filtered, and extracted with CH_2Cl_2. The CH_2Cl_2 layer was chromatographed over silica gel eluting with CH_2Cl_2−EtOAc (4:1) to afford two compounds, which were identified as withametelin G and withafastuosin H by HPLC and comparison of the ^1H NMR and ^{13}C NMR data in the literature.[6,13] The aqueous layer was dried and dissolved in anhydrous pyridine under Ar gas. L-Leucine methyl ester hydrochloride (2 mg) was added, and the mixture was warmed at 60 °C for 1 h. Then, $NaBH_4$ (2 mg) was added, and the mixture was stirred for 1 h at room temperature. Me_3SiCl (0.2 mL) was added, and heating was conducted at 60 °C. The silylated Leu derivatives were analyzed by GC (Shimadzu GC-17A; CP-Sil 5CB column; column temperature 200 °C, injection temperature 250 °C, carrier gas N_2, flow rate 32.2 mL/min), which led to the identification of D-glucose by comparison of retention times with an authentic sample of this sugar.

Single-Crystal X-ray Structure Determination of 1. Single-crystal X-ray measurements were performed on a Bruker SMART CCD diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.71073). Lattice determination and data collection were performed using SMART. The structures were solved by direct methods with SHELXS-97 and refined by full matrix least-squares in F^2 using SHELXL-97. The hydrogen atoms were treated by a mixture of independent and constrained refinement. Pertinent results are given in the figures and below; full details are deposited in the Cambridge Crystallographic Data Centre with deposition number CCDC 638783.[14]

Crystallographic data and data collection parameters: colorless $C_{28}H_{40}O_8$, MW 504.60 ($C_{28}H_{36}O_6 \cdot 2H_2O$), orthorhombic, $P2_12_12_1$, cell constants a = 10.6156(7) Å, b = 11.2307(7) Å, c = 21.7108(14) Å, V = 2588.4(3) Å^3, D_{calc} = 1.295 g cm^{-3}, Z = 4, $F_{(000)}$ = 1088, $\mu_{(Mo\ K\alpha)}$ = 0.094 mm^{-1}, crystal dimensions 0.46 × 0.37 × 0.19 mm. A total of 5908 reflections were collected with 5202 unique reflections (R_{int} = 0.033).

Cytotoxicity Assays. Three selected human cancer cell lines were purchased from Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Science. Cytotoxicity assays of the test compounds **1**−**10** were performed using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma Chemical Co., Ltd.] colorimetric method. All the cell lines were grown in RPMI-1640 (Gibco) medium, supplemented with 10% fetal bovine serum, at 37 °C under 5% CO_2 in a humidified incubator. For the assay, 6 × 10^4 cells/mL, with 100 μL/well of the cell suspensions were seeded in 96-well microtiter plates and incubated to allow cell attachment. After 24 h, 100 μL solutions of test samples at the desired dilutions and the positive controls were added to each well and incubated for 48 h. MTT (0.5 mg/mL) was added to each of the 96 wells and incubated for 3 h. The plates were then shaken, and optical density (OD) was recorded using a microplate reader (Multiskan MK3, Thermo) at 550 nm. The IC_{50} values for **1**−**10** and the positive control, adriamycin, are listed in Table 4.

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Supporting Information Available: NMR spectra for compounds **1**−**10** and the seven known withanolides isolated in this investigation and a discussion of compound **8**. This information is available free of charge via the Internet at http://pubs.acs.org.

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(14) Crystallographic data for the structure reported in this paper have been deposited at the Cambridge Crystallographic Data Centre with the deposition number CCDC 638783. Copies of the data can be obtained free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

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