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Cytotoxic Withanolides from *Tubocapsicum anomalum*

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Fifteen new withanolides (**1**−**8**, **11**−**17**) and two known withanolides, withanolide D (**9**) and 17α-hydroxywithanolide D (**10**), were isolated from the stems, roots, and leaves of *Tubocapsicum anomalum* using bioassay-directed fractionation. The structures were determined by spectroscopic and chemical methods, and the absolute configurations were established by CD analysis and by the Mosher ester method. The structure of **1** and **3** were further confirmed by X-ray crystallographic analysis. Compounds **1**, **4**−**6**, **8**−**10**, and **13** showed significant cytotoxic activity against Hep G2, Hep 3B, A-549, MDA-MB-231, MCF-7, and MRC-5 cell lines.

The family Solanaceae includes over 95 genera, which are mainly distributed in tropical and subtropical regions. Ten genera, *Brugmansia*, *Capsicum*, *Datura*, *Lycianthes*, *Lycium*, *Lycopersicon*, *Physaliasrtum*, *Physalis*, *Solanum*, and *Tubocapsicum*, grow in Taiwan.[1] However, only *Tubocapsicum anomalum* (Franch. & Sav.) Makino is native to Taiwan.[1] Seven withanolide derivatives have been isolated from this genus.[2,3] Many withanolides and their derivatives have been isolated from solanaceous plants. Some of them possess significant pharmacological effects, including antitumor,[4] cytotoxic,[5,6] immunosuppressive,[6] anti-inflammatory,[7,8] and antifeedant activities.[9,10] It has been reported that withaferin A disrupts F-actin organization via an interaction with annexin II and consequently causes concentration-dependent cytotoxicity and marked anti-invasive activity in tumor cells.[11] These results suggest a useful, previously unexploited target for therapeutic intervention and support the further investigation and development of withanolides as potential anticancer agents.

As part of our research on solanaceous plants, crude extracts of *T. anomalum* showed cytotoxic activity against Hep G2, Hep 3B, A-549, MDA-MB-231, and MCF-7 cancer cell lines. Using bioassay-directed fractionation, 17 withanolides (**1**−**17**) and 10 apparent or presumed artifacts of isolation (compounds **18**−**27**; see Supporting Information for structures and experimental details) were isolated. Withanolide D (**8**)[12] and 17α-hydroxywithanolide D (**9**)[13] are known compounds. In this paper we report the structures of these new withanolides.

Results and Discussion

Compounds **1**−**17** were isolated from methanolic extracts of *T. anomalum* (**1**−**4**, **7**, **11**−**14**, **16**, **17** from stems and leaves and **5**, **6**, **8**−**10**, **15** from roots). The molecular formula of **1** ($C_{28}H_{36}O_6$) was determined by HRFABMS. The ^{13}C NMR spectrum (Table 1) exhibited 28 signals (5 methyl, 5 methylene, 9 methine, and 9 quaternary carbons), and the ^1H NMR spectrum (see Experimental Section) showed typical steroidal signals. The 2D (COSY, HMQC, HMBC) NMR spectra suggested that **1** contained a 4-hydroxy-α,β-unsaturated ketone, an α,β-unsaturated δ-lactone, and a tetrasubstituted olefin: features of a $\Delta^{13,14}$-16-hydroxywithanolide skeleton.[3] Compound **1** also contained an epoxy group linked at C-5/6, on the basis of NMR signals at δ_H 3.37 (br s, H-6), δ_C 64.8 (s, C-5) and 60.1 (d, C-6), and confirmed by HMBC analysis. Evidence for an OH group at C-16 was suggested by O-linked signals at δ_H 4.45 (t, H-16) and δ_C 81.6 (d, C-16) and further supported by COSY and HMBC analysis. The structure and relative configuration of **1** were confirmed by X-ray crystallographic analysis (Figure 1). Compound **1** was established as 4β,16α-dihydroxy-5β,6β-epoxy-1-oxowitha-2,13,24-trienolide and was named tubocapsenolide A.

The molecular formulas of **2** ($C_{28}H_{38}O_7$) and **3** ($C_{28}H_{37}ClO_6$) were established by HRESIMS data. The $^1H/^{13}C$ NMR, COSY, HMQC, and HMBC spectra of **1**, **2**, and **3** were similar and contained 28 carbon signals including those of an α,β-unsaturated δ-lactone. The signals at δ_H 4.05 (d, H-4), 3.37 (br s, H-6) and δ_C 64.8 (s, C-5) in **1** were shifted to δ_H 5.37 (br s, H-4), 3.81 (dd, H-6) and δ_C 80.5 (s, C-5) in **2**, and the NOESY spectrum showed correlations between H-4/H-9, H-8/H-6, H-19/H-6, and H-8/H-19, suggesting that the 5β,6β-epoxide in **1** was replaced by a 5β,6α-diol in **2**. The NMR data clearly showed that compounds **2** and **1** differed only by the substituents at C-5 and C-6. In addition, the proton signals at δ_H 3.81 (dd, H-6) and 6.91 (dd, H-3) in **2** were shifted to δ_H 4.45 (dd, H-6) and 6.47 (dd, H-3) in **3** and the carbon signals at δ_C 61.8 (d) and 147.8 (d) to δ_C 66.8 (d) and 143.2 (d), respectively. The NMR data and molecular formula of **3** suggested that one hydroxy group at C-4−6 in **2** was replaced by a chlorine in **3**.[14] To determine the exact position of the chlorine substituent, an acetylation experiment was performed. In the resulting ester, the NMR signal at δ_H 5.04 (t, H-4) is shifted to 6.44 (t, H-4), but the NMR signal of H-6 remains unchanged. Thus, the chlorine atom was assigned at C-6. The structure of **3** was further confirmed by X-ray crystallographic analysis (Figure 1). Thus, the structures of **2** and **3** were established as shown and were named tubocapsenolide F and tubocapsenolide G.

Compounds **4**−**6** both had NMR signals characteristic of a 4-hydroxy-α,β-unsaturated ketone and an α,β-unsaturated δ-lactone but, unlike **1**, did not show ^{13}C NMR signals for a tetrasubstituted olefin. In addition, the ^1H and ^{13}C methyl group signals at δ_H 1.21 (3H, s, H-18)/δ_C 25.3 (q, C-18) in **1** were shifted upfield to δ_H 0.89 (3H, s, H-18)/δ_C 15.8 (q, C-18) in **4**. These facts together with HMBC and COSY assignments indicated that the C-18 methyl group in **4** was located at C-13.[2,3] $^1H/^{13}C$ NMR signals at δ_H 3.59 (br s, H-16)/δ_C 61.2 (d, C-16) and δ_C 70.9 (s, C-17) together with similar signals at δ_H 3.20 (br s, H-6)/δ_C 59.8 (d, C-6) and δ_C 64.5

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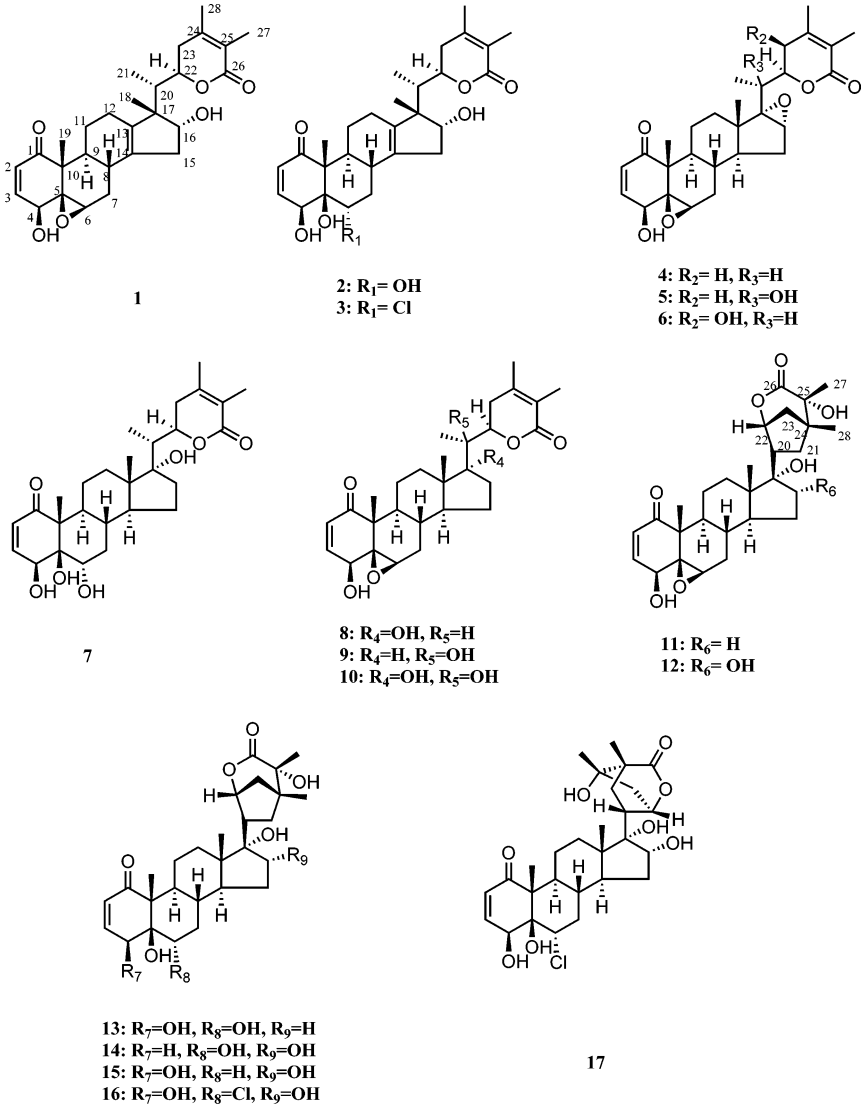
Chart 1



(s, C-5) indicated the presence of a second epoxy group, the first at C-16/17 and the latter at C-5/6. HMBC analysis confirmed these assignments. The molecular formula of **4** was defined as $C_{28}H_{36}O_6$ from HRFABMS data. The structure and relative stereochemistry of **4** were established by NOESY data as 5β,6β:16α,17α-diepoxy-4β-hydroxy-1-oxo-witha-2,24-dienolide, and **4** was named tubocapsanolide A.

The molecular formulas for **5** and **6** were both defined as $C_{28}H_{36}O_7$ (**5**: $[M + Na]^+$ *m*/*z* 507.2353, **6**: $[M + Na]^+$ *m*/*z* 507.2361, calcd 507.2359). The NMR signals at δ_C 34.4 (d, C-20) and 33.6 (t, C-23) in **4** were shifted downfield to δ_C 73.3 (s, C-20) in **5** and δ_C 66.6 (d, C-23) in **6**, respectively. Thus, together with COSY and HMBC analysis, OH groups were placed at C-20 (**5**) and C-23 (**6**), respectively. The structures and relative stereochemistry were established by NOESY data as 5β,6β:16α,17α-diepoxy-4β,20-dihydroxy-1-oxo-witha-2,24-dienolide (**5**) and 5β,6β:16α,-17α-diepoxy-4β,23-dihydroxy-1-oxo-witha-2,24-dienolide (**6**). Hence, **5** and **6** are hydroxy derivatives of **4**, and they were named 20-hydroxytubocapsanolide A (**5**) and 23-hydroxytubocapsanolide A (**6**).

Compound **7** showed a pseudomolecular FABMS ion peak at *m*/*z* 489 $[M + H]^+$, and the molecular formula was established by HRESIMS. The ^{1}H and the ^{13}C NMR spectra were similar to the spectra of the above-mentioned withanolides. The H-4 and H-6 signals and the C-5 carbon signal of **7** were downfield relative to those of **4** and are similar to those found in compound **2**. Thus, compound **7** had a 5β,6α-diol rather than the epoxide found in **4**. Furthermore, the ^{1}H signals for the C-16/17 epoxy group in **4** were replaced by signals consistent with a methylene at C-16 and a quaternary carbon bearing an OH group at C-17. Thus, the structure and relative stereochemistry of **7** were established as 4β,5β,6α,-17α-tetrahydroxy-1-oxo-witha-2,24-dienolide, and **7** was named tubocapsanolide D.

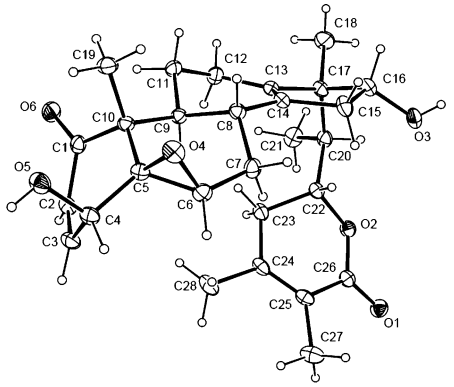
The NMR spectra and therefore the structures of **7** and **8** were very similar, except that **8** had a 5,6-epoxide rather than a 5β,6α-diol. This was supported by COSY and HMBC experimental results and was consistent with the molecular formula ($C_{28}H_{38}O_6$). Thus, **8** was established as 5β,6β-epoxy-4β,17α-dihydroxy-1-oxo-witha-2,24-dienolide and named tubocapsanolide F.

Comparison of NMR signals of compounds **1**−**10** and reported withanolides including an α,β-unsaturated δ-lactone side chain at C-17[15,16] demonstrated that compounds **11**−**16** did not have this structural feature. Compounds **11**−**16** had instead a 4-hydroxy-4,5-dimethyl-2-oxa-bicyclo[3.2.1]octan-3-one ring on C-17, similar to acnistin-type withanolides.[17] Characteristic signals in the ^{1}H/^{13}C NMR spectra of compound **11** were assigned to an epoxy group at C-5/6 and OH groups at C-4 and C-17. The ^{13}C NMR spectra of **11** and of acnistin E[18] were similar. Differences in the chemical shifts of carbon atoms near the linkage between the gonane skeleton and the bicyclic side chain [δ_C **11**: 52.9 (d, C-20), 34.3 (t, C-21); acnistin E: 51.4 (d, C-20), 37.1 (t, C-21)] were similar to those of 17-epiacnistin-A[16] and acnistin A,[18] indicating that the 4-hydroxy-

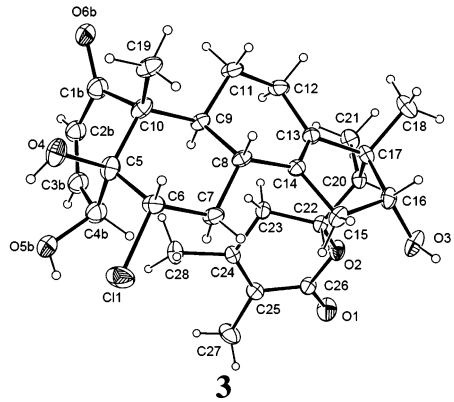
Table 1. ^{13}C NMR Data (100 MHz) of **1**−**8** in C_5D_5N and $CDCl_3$

position	**1**[a]	**2**[a]	**3**[b]	**4**[a]	**5**[a]	**6**[a]	**7**[b]	**8**[a]
1	202.4 (s)	201.5 (s)	200.0 (s)	202.4 (s)	202.6(s)	202.4 (s)	200.0 (s)	202.5 (s)
2	132.2 (d)	127.2 (d)	127.7 (d)	132.3 (d)	132.9 (d)	132.2 (d)	127.9 (d)	132.3 (d)
3	(145.5 d)	147.8 (d)	143.2 (d)	145.0 (d)	145.3 d)	145.0 (d)	142.9 (d)	144.9 (d)
4	70.1 (d)	65.8 (d)	66.1 (d)	70.3 (d)	70.8 (d)	70.2 (d)	66.1 (d)	70.3 (d)
5	64.8 (s)	80.5 (s)	78.2 (s)	64.5 (s)	65.0 (s)	64.4 (s)	78.1 (s)	64.4 (s)
6	60.1 (d)	61.8 (d)	66.8 (d)	59.8 (d)	60.2 (d)	59.7 (d)	66.6 (d)	59.9 (d)
7	30.9 (t)	36.1 (t)	38.1 (t)	31.3 (t)	31.8 (t)	31.2 (t)	39.2 (t)	31.9 (t)
8	31.1 (d)	37.4 (d)	36.0 (d)	28.7 (d)	28.6 (d)	28.6 (d)	36.1 (d)	30.5 (d)
9	44.1 (d)	43.3 (d)	43.3 (d)	44.9 (d)	45.0 (d)	44.8 (d)	45.1 (d)	44.3 (d)
10	48.4 (s)	57.7 (s)	57.1 (s)	48.6 (s)	49.0 (s)	48.5 (s)	57.0 (s)	48.5 (s)
11	23.2 (t)	24.8 (t)	24.0 (t)	21.5 (t)	21.7 (t)	21.3 (t)	22.5 (t)	21.5 (t)
12	24.4 (t)	24.8 (t)	24.0 (t)	33.1 (t)	34.1 (t)	32.5 (t)	31.7 (t)	24.0 (t)
13	140.3 (s)	139.3 (s)	139.2 (s)	42.3 (s)	43.0 (s)	42.4 (s)	47.9 (s)	48.0 (s)
14	134.8 (s)	134.7 (s)	133.4 (s)	45.5 (d)	47.3 (d)	45.3 (d)	49.5 (d)	50.5 (d)
15	41.6 (t)	41.1 (t)	40.8 (t)	27.7 (t)	27.6 (t)	27.6 (t)	22.7 (t)	32.2 (t)
16	81.6 (d)	81.4 (d)	81.7 (d)	61.2 (d)	58.1 (d)	61.7 (d)	35.2 (t)	37.3 (t)
17	53.0 (s)	52.9 (s)	53.5 (s)	70.9 (s)	73.0 (s)	71.2 (s)	85.2 (s)	84.3 (s)
18	25.3 (q)	25.5 (q)	24.9(q)	15.8 (q)	17.3 (q)	15.6 (q)	14.4 (q)	14.6 (q)
19	16.2 (q)	10.6 (q)	10.4 (q)	17.0 (q)	17.1 (q)	16.9 (q)	9.9 (q)	17.1 (q)
20	42.2 (d)	42.1 (d)	40.2 (d)	34.3 (d)	73.3 (s)	32.8 (d)	40.2 (d)	43.6 (d)
21	11.8 (q)	11.9 (q)	12.9 (q)	12.6 (q)	24.2 (q)	14.3 (q)	13.0 (q)	9.9 (q)
22	78.7 (d)	78.5 (d)	78.6 (d)	76.2 (d)	80.0 (d)	82.9 (d)	78.3 (d)	79.2 (d)
23	32.8 (t)	32.7 (t)	34.5 (t)	33.6 (t)	30.7 (t)	66.6 (d)	33.1 (t)	33.1 (t)
24	149.9 (s)	149.4(s)	149.9 (s)	149.1 (s)	150.6 (s)	149.8 (s)	150.9 (s)	150.5 (s)
25	121.9 (s)	121.8 (s)	121.8 (s)	121.9 (s)	121.8 (s)	123.4 (s)	121.1(s)	121.4 (s)
26	166.9 (s)	166.7 (s)	166.4 (s)	165.9 (s)	166.2 (s)	164.3 (s)	167.5 (s)	166.8 (s)
27	12.1 (q)	12.5 (q)	12.3 (q)	12.7 (q)	13.0 (q)	12.8 (q)	12.3 (q)	12.5 (q)
28	20.2 (q)	20.2 (q)	ep (q)	20.0 (q)	20.6 (q)	17.7 (q)	20.5 (q)	20.0 (q)

[a] Measured in C_5D_5N. [b]Measured in $CDCl_3$; all assignments were confirmed by DEPT, COSY, and HMQC.



1



3

Figure 1. Computer-generated ORTEP diagrams of compounds **1** and **3**.

4,5-dimethyl-2-oxa-bicyclo[3.2.1]octan-3-one substituent was β configured and that the OH group was α. These assignments were further established by COSY, HMBC, and NOESY data. Therefore, compound **11** is the 17-epimer of acnistin E and was named anomanolide A.

The molecular formula of **12** was established by HRESIMS as $C_{28}H_{38}O_8$. As observed for **11**, the $^1H/^{13}C$ NMR spectra of **12** had signals for C-5/C-6 epoxy and α-C-17 OH groups.[15,16] However, additional signals at δ_H 4.51 (dd, H-16)/δ_C 75.5 (d, C-16) suggested the presence of an OH substituent at C-16. Therefore, the structure of **12** was established as shown, and it was named anomanolide C.

The NMR spectra of **13** and **11** were similar; however the signals of H-4 and H-6 in **13** were shifted to δ_H 5.03 (br s, H-4) and δ_H 4.42 (dd, 4.0 Hz, H-6). Additionally, δ_C 63.8 (s, C-5) was shifted to δ_C 78.1 (s). These characteristics were also found in compounds **1** and **2** and indicated that the epoxy group of **11** was replaced with a diol group in **13**. Therefore, the structure of **13** was established as shown, and it was named anomanolide B.

The molecular formulas of **14** and **15** were both established as $C_{28}H_{40}O_8$. The $^1H/^{13}C$ NMR signals were similar to those of **12**, including a C-16 OH group and the 5β,6α-diol. Compound **14** had NMR signals at δ_H 3.73 (ddd, H4a), 2.39 (d, H4b)/δ_C 36.7 (t, C-4), proving the absence of an OH group at C-4. Therefore, **14** is the 16-hydroxy 4-dehydroxy derivative of **13**, and it was named anomanolide E. Compound **15** was the 6-hydroxy derivative of **13** and was named anomanolide F.

The molecular formula of **16**, $C_{28}H_{39}ClO_8$, was established by HRESIMS. The $^1H/^{13}C$ NMR signals were similar to those of **12**, including a C-16 hydroxyl group. However, the NMR for H-4 was shifted downfield to δ_H 6.59, similar to that of **3**. Additionally, acetylation of **16** was performed to ascertain the position of the chlorine substituent.[14] Thus, the chlorine substituent was assigned at C-6, and **16** was named anomanolide D.

The HRESIMS spectrum of **17** contained a pseudomolecular ion at *m*/*z* 561.2232 $[M + Na]^+$ (calcd 561.2226), corresponding to $C_{28}H_{39}ClO_8$. Analysis of the $^1H/^{13}C$ NMR, COSY, HMQC, and HMBC spectra suggested a 4-hydroxy-α,β-unsaturated ketone and a δ-lactone ring in the C_{28} skeleton. The NMR signals of **17** were similar to those in **16**, except that the signals characteristic of a 4-hydroxy-4,5-dimethyl-2-oxa-bicyclo[3.2.1]octan-3-one substituent at C-17 were absent in **17** and replaced with those for a 2-oxa-bicyclo[2.2.2]octan-3-one, typical of withajardin-type withanolides.[17] The structure was further established by analysis of COSY, HMBC, and NOESY experiments and by comparison with reported compounds.[14,17,19] Therefore, the structure of **17** was determined to be as shown, and it was named tubonolide A.

Table 2. ^{13}C NMR Data (100 MHz) of **11**−**17** in C_5D_5N or $CDCl_3$

position	**11**[b]	**12**[a]	**13**[b]	**14**[a]	**15**[a]	**16**[a]	**17**[a]
1	202.3 (s)	202.5 (s)	200.0 (s)	204.9 (s)	202.0 (s)	201.6 (s)	202.0 (s)
2	132.1 (d)	132.3 (d)	127.7 (d)	129.1 (d)	127.2 (d)	126.1 (d)	126.7 (d)
3	142.0 (d)	144.9 (d)	142.8 (d)	142.1 (d)	146.8 (d)	146.9 (d)	147.4 (d)
4	69.8 (d)	70.4 (d)	66.2 (d)	36.7 (t)	67.7 (d)	64.6 (d)	65.2 (d)
5	63.8 (s)	64.4 (s)	78.1 (s)	77.6 (s)	79.7 (s)	79.5 (s)	80.0 (s)
6	62.6 (d)	59.9 (d)	66.6 (d)	74.7 (d)	38.2 (t)	65.8 (d)	66.3 (d)
7	31.1 (t)	31.7 (t)	39.4 (t)	34.4 (t)	24.0 (t)	39.8 (t)	40.3 (t)
8	29.9 (d)	29.9 (d)	35.2 (d)	30.7 (d)	34.3 (d)	35.0 (d)	35.7 (d)
9	43.7 (d)	44.2 (d)	45.5 (d)	41.8 (d)	45.1 (d)	46.2 (d)	46.7 (d)
10	47.6 (s)	48.4 (s)	57.2 (s)	52.5 (s)	56.7 (s)	58.1 (s)	58.6 (s)
11	21.8 (t)	20.9 (t)	22.3 (t)	23.3 (t)	23.2 (t)	22.6 (t)	23.1 (t)
12	31.7 (t)	32.5 (t)	31.6 (t)	33.8 (t)	32.1 (t)	32.5 (t)	32.4 (t)
13	47.1 (s)	47.7 (s)	47.7 (s)	48.4 (s)	47.6 (s)	48.2 (s)	49.5 (s)
14	50.3 (d)	48.6 (d)	49.8 (d)	48.5 (d)	50.1 (d)	47.8 (d)	48.1 (d)
15	23.7 (t)	36.7 (t)	23.4 (t)	36.8 (t)	38.1 (t)	36.3 (t)	36.2 (t)
16	37.6 (t)	75.5 (d)	37.9 (t)	75.6 (d)	74.3 (d)	75.2 (d)	74.0 (d)
17	84.3 (s)	82.1 (s)	84.1 (s)	82.3 (s)	83.4 (s)	82.0 (s)	83.3 (s)
18	15.6 (q)	15.4 (q)	15.7 (q)	16.3 (q)	15.7 (q)	15.6 (q)	16.4 (q)
19	17.5 (q)	17.1 (q)	9.9 (q)	16.1 (q)	10.7 (q)	10.4 (q)	10.9 (q)
20	52.9 (d)	53.8 (d)	52.9 (d)	54.0 (d)	54.4 (d)	53.8 (d)	42.2 (d)
21	34.3 (t)	35.1 (t)	34.3 (t)	35.2 (t)	39.8 (t)	35.0 (t)	27.7 (t)
22	85.7 (d)	83.8 (d)	85.5 (d)	84.0 (d)	85.0 (d)	83.7 (d)	77.9 (d)
23	41.1 (t)	41.4 (t)	41.2 (t)	41.4 (t)	35.6 (t)	41.4 (t)	43.1 (t)
24	46.7 (s)	47.7 (s)	46.8 (s)	47.7 (s)	47.9 (s)	47.7 (s)	70.9 (s)
25	76.6 (s)	77.2 (s)	77.2 (s)	77.2 (s)	76.9 (s)	77.1 (s)	48.5 (s)
26	178.8 (s)	178.8 (s)	178.7 (s)	178.8 (s)	176.1 (s)	178.7 (s)	178.5 (s)
27	25.5 (q)	25.5 (q)	25.5 (q)	25.5 (q)	20.4 (q)	25.4 (q)	15.7 (q)
28	19.8 (q)	20.3 (q)	19.9 (q)	20.4 (q)	19.9 (q)	20.3 (q)	28.6 (q)

[a] Measured in C_5D_5N. [b]Measured in $CDCl_3$; all assignments were confirmed by DEPT, COSY, and HMQC

The conformations of 6β-oxygenated withanolides were established from analyses of the NOESY spectra and the $^1H-^1H$ coupling constants. Since NOESY correlations were present between H-6 and H-4, but not between H-6 and H-19, the A/B ring junction could be either *cis* or *trans*. These two conformations can be discriminated by the coupling constants between H-6 and H-7. From literature reports, a *cis*-junction shows two similar coupling constant values for H-6 (0−2 Hz); however, a *trans*-junction shows two different coupling constant values (0−2 Hz and 4−6 Hz).[20,21] Each of the H-6 proton signals appeared as a broad singlet; therefore, the A/B ring conformations were *cis*. Additionally, in the NOESY spectrum of the isolates, the proton signals for CH_3-19 and H-9 showed correlations to H-8 and H-2a, respectively, indicating that the B/C ring conformations were *trans*.

The absolute configurations of these compounds were determined by preparing Mosher esters[22,23] of **1**, **4**, and **12**. The C-4 OH groups were converted to (*S*)-(−)- and (*R*)-(+)-*R*-methoxy-*R*-(trifluoromethyl)phenylacetyl (MTPA) ester derivatives. Distribution of the positive and negative δ values of the MTPA esters established the C-4 chiral center as having an *S*-configuration (Table S1, Supporting Information). The C-16 OH groups of **1** and **12** were assigned as *R* by using the same method.

CD spectroscopic analysis was also used to establish the absolute stereochemistry of the new withanolides. According to literature reports,[24] withanolides with *R*-configuration at C-22 show a positive Cotton effect at ca. 250 nm in the CD spectrum of the α,β-unsaturated δ-lactone. Compounds **1**−**8** and **10** each showed a positive Cotton effect at ca. 250 nm. Therefore their C-22 chiral centers were defined as *R*.

Compounds **1**−**17** were evaluated against five human cancer cell lines (hepatocellular carcinoma Hep G2 and Hep 3B, breast carcinoma MCF-7 and MDA-MB-231, lung carcinoma A-549) and against the embryonic lung cell line MRC-5. Withaferin A and doxorubicin were used as positive controls. The results are summarized in Table 3. Compounds **1**, **4**−**6**, **8**−**10**, and **13** showed significant cytotoxic activity against all six cell lines (Table 3). Note that the cytotoxic effects of chlorinated withanolides, **3** and **16**, were less than their corresponding hydroxy derivatives **2** and **13**.

Table 3. Cytotoxicities of Withanolides **1**−**17**

	cell lines (IC_{50}, μg/mL)					
	Hep G2	Hep 3B	MCF-7	A-549	MDA-MB-231	MRC-5
1	0.44	0.26	0.97	0.15	0.13	0.20
2	>20	15.07	14.71	6.04	8.41	
3	19.12	>20	>20	19.94	>20	
4	0.86	0.42	1.47	0.47	0.22	0.73
5	0.73	0.99	1.77	1.42	0.99	1.36
6	0.44	0.49	2.05	0.79	1.19	0.90
7	>20	7.19	>20	8.01	13.49	
8	0.64	0.80	1.98	0.88	0.99	0.81
9	0.21	0.47	0.37	0.33	0.28	0.80
10	0.49	0.85	1.45	0.72	0.71	1.89
11	3.11	3.63	2.31	1.01	1.37	1.91
12	4.41	1.85	8.01	2.80	1.58	
13	0.97	3.17	4.84	1.49	0.70	0.20
14	>20	>20	>20	>20	>20	
15	>20	>20	>20	>20	>20	
16	>20	19.03	>20	>20	>20	
17	5.09	6.54	12.03	5.91	>20	
WA	0.06	0.06	0.05	0.02	0.02	0.07
doxo	0.46	0.45	0.34	0.42	0.19	0.77

Ten presumed or suspected artifacts of the isolation procedures (compounds **18**−**27**; see Supporting Information for structures and experimental details) were also isolated during this study. These compounds were likely produced by Michael-type additions of solvents and epoxide ring-opening reactions. In addition, three chlorinated withanolides (compounds **3**, **16**, and **17**) were isolated in the present study. The origin of the chlorine atom in these compounds had been assigned to the mineral NaCl, which is present in sizable amounts in this plant.[14] The structures of **18**−**27** were determined by spectroscopic and chemical methods. In addition, the absolute configurations were established by CD analysis (see Tables S2−S7, Figures S1, S2, and Additional Experimental Details).

Experimental Section

General Experimental Procedures. Melting points were determined on a Melt-Temp II apparatus and are uncorrected, optical rotations were measured with a JASCO P-1020 digital polarimeter, UV spectra were

obtained on a Hitachi 200-20 spectrophotometer, and IR spectra were measured on a Hitachi 260-30 spectrophotometer. CD spectra were measured on a Jasco J-810 spectrometer. 1D (^{1}H, ^{13}C, DEPT) and 2D (COSY, TOCSY, HSQC, HMBC, NOESY) NMR spectra using C_5D_5N and $CDCl_3$ as solvents were obtained on a Varian NMR spectrometer (Unity Plus 400 and Unity INOVA-500) or a Bruker AMX-400 NMR spectrometer. Chemical shifts were internally referenced to the solvent signals in C_5D_5N (^{1}H, δ 7.21; ^{13}C, δ 123.5), $CDCl_3$ (^{1}H, δ 7.26; ^{13}C, δ 77.0), or CD_3OD (^{1}H, δ 3.31, ^{13}C, δ 49.0). Low-resolution ESIMS spectra were obtained on an API 3000 (Applied Biosystems); high-resolution ESIMS spectra, on a Bruker Daltonics APEX II 30e spectrometer. Low-resolution EIMS were recorded on a Quattro GC/MS spectrometer having a direct inlet system. Low-resolution FABMS spectra were recorded on a VG Biotech Quattro 5022 spectrometer; high-resolution FABMS, on a Finnigan/Thermo Quest MAT 95XL spectrometer. Shimadzu LC-10AT pumps, a SPD-10A UV−vis detector, and Hypersil ODS 5 μm (250 × 4.6 mm i.d.) and preparative ODS 5 μm (250 × 21.2 mm i.d.) columns were employed for HPLC with detection at 230 nm. Diffraction intensity data were acquired with a Rigaku AFC7S single-crystal X-ray diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å).

Plant Material. The initial collection of *T. anomalum* (Solanaceae) was made on July 2003 near Nan Tao and identified by one of the authors (H.-F.Y.). A larger amount of the same plant was re-collected at the Da-Han Mountain, Kaohsiung, in October 2004, and identified by another author (M.-H.Y.). The samples were authenticated and deposited in the Graduate Institute of Natural Products, Kaohsiung Medical University, Taiwan (anom001, anonm002).

Extraction and Isolation. The air-dried stems and leaves (2.5 kg, part A) and roots (1.2 kg, part B) of *T. anomalum* were extracted separately with MeOH at rt. The MeOH extract of part A was partitioned between EtOAc/H_2O to yield EtOAc and H_2O extracts. The H_2O extracts were further partitioned with *n*-BuOH to give *n*-BuOH and H_2O extracts. The residue from the EtOAc extract was separated on a silica gel column (230−400 mesh, 5 × 20 cm) eluting with a gradient of *n*-hexane/$CHCl_3$/MeOH to give 16 fractions (A1−A16). Fraction A8 (529.8 mg) was chromatographed on a silica gel column using *n*-hexane/$CHCl_3$ (2:1) and $CHCl_3$ as eluents and recrystallized from MeOH to give **4** (42.5 mg). Fraction A11 (165.2 mg) was recrystallized from MeOH to afford **1** (101.1 mg). Fraction A12 (120.1 mg) was further purified on Si gel (*n*-hexane/$CHCl_3$ (2:1) and $CHCl_3$) and recrystallized from MeOH to yield **12** (65.3 mg). The mother liquors of fractions A8, A11, and A12 were combined with fractions A9 and A10 and then partitioned with H_2O/MeOH/$CHCl_3$ (1:4:5) to yield fractions TAEWM and TAEWC. Fraction TAEWM was separated by repeated preparative HPLC (ODS, MeOH/H_2O, 65:35, MeOH/H_2O, 1:1, and MeOH/H_2O, 1:1, respectively) to afford **14** (3.5 mg) and **11** (14.0 mg).

The *n*-BuOH extract was further partitioned between $CHCl_3$ and H_2O and then evaporated to give a dark green, viscous residue. The $CHCl_3$ extract was separated on a Sephadex LH-20 column eluting with MeOH to give seven fractions (B1−B7). Fraction B4 (276 mg) was separated on a silica gel column eluting with $CHCl_3$ to yield 21 subfractions (B4A−B4U). Subfractions B4Q (11.1 mg) yielded **2 (**5.1 mg). Fraction B5 (278 mg) was further separated on a Si gel column (230−400 mesh, 2.5 × 27 cm) eluting with $CHCl_3$/MeOH (150:1) to yield 22 subfractions (B5A−B5V). Subfraction B5C (2.5 mg) gave **7** (1.2 mg). Subfraction B5G (39.38 mg) was purified by preparative reversed-phase HPLC and gave **13** (8.3 mg). Subfraction B5K (19.66 mg) was purified by preparative reversed-phase HPLC to yield **3** (11.53 mg). Subfraction B6 was separated on a Si gel column to afford **16** (33.5 mg) and **17** (1.15 mg).

Using similar methods, the MeOH extract of part B was partitioned between EtOAc/H_2O to yield EtOAc and H_2O extracts. The H_2O extract was partitioned with *n*-BuOH to give *n*-BuOH and H_2O extracts. The EtOAc extract was partitioned with *n*-hexane and MeOH to give *n*-hexane and MeOH extracts. The MeOH extract was separated on a Sephadex LH-20 column (MeOH) to give five fractions (C1−C5). The C3 fraction (807 mg) was further separated on Si gel eluting with $CHCl_3$/MeOH to give 28 fractions (C3-1−28). Repeated separations of the various fractions using similar methods yielded **9** (22.1 mg), **5** (1.19 mg), **8** (2.1 mg), **10** (14.2 mg), **6** (4.7 mg), and **15** (2.8 mg).

Tubocapsenolide A (1): colorless prisms (MeOH); mp 223−225 °C; $[\alpha]_D^{24.3}$ −0.57 (*c* 0.1, MeOH); UV (MeOH) λ_{max} 212, 228 nm; CD [θ] +10 560 (255 nm); IR (neat) ν_{max} 3380, 2921, 1677, 1380, 1130 cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MHz) δ 6.47 (1H, d, *J* = 10.0 Hz, H-2), 7.27 (1H, dd, *J* = 10.0, 6.4 Hz, H-3), 4.05 (1H, d, *J* = 6.4 Hz, H-4), 3.37 (1H, br s, H-6), 1.39 (1H, m, H-7a), 2.33 (1H, m, H-7b), 2.35 (1H, m, H-8), 1.37 (1H, m, H-9), 1.26 (1H, ddd, *J* = 12.4, 4.8, 4.0 Hz, H-11a), 2.04 (1H, m, H-11b), 1.77 (1H, m, H-12a), 2.04 (1H, m, H-12b), 2.48 (2H, m, H-15), 4.45 (1H, t, *J* = 8.0 Hz, H-16), 1.21 (3H, s, H-18), 1.81 (3H, s, H-19), 2.58 (1H, qd, *J* = 7.2, 3.6 Hz, H-20), 1.17 (3H, d, *J* = 7.2 Hz, H-21), 5.17 (1H, ddd, *J* = 12.8, 3.6, 3.2 Hz, H-22), 2.47 (1H, m, H-23a), 2.30 (1H, m, H-23b), 1.87 (3H, s, H-27), 1.77 (3H, s, H-28); ^{13}C NMR data, see Table 1; HRFABMS *m*/*z* 469.2594 [M + H]$^+$ (calcd for $C_{28}H_{36}O_6$, 469.2585).

Tubocapsenolide F (2): white powder; mp 214−216 °C; $[\alpha]_D^{24.4}$ +178.9 (*c* 0.1, MeOH); UV (MeOH) λ_{max} 212, 228 nm; CD [θ] +19 000 (255 nm); IR (neat) ν_{max} 3424, 2927, 1679, 1380, 1132 cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MHz) δ 6.28 (1H, dd, *J* = 10.0, 2.0 Hz, H-2), 6.91 (1H, dd, *J* = 10.0, 2.0 Hz, H-3), 5.37 (1H, br s, H-4), 3.81 (1H, dd, *J* = 12.8, 4.0 Hz, H-6), 2.01 (1H, m, H-7a), 2.99 (1H, dt, *J* = 13.2, 4.0 Hz, H-7b), 2.30 (1H, m, H-8), 1.94 (1H, m, H-9), 1.26 (1H, m, H-11a), 2.01 (1H, m, H-11b), 1.66 (1H, m, H-12a), 1.85 (1H, m, H-12b), 2.38 (2H, m, H-15), 4.37 (1H, dd, *J* = 8.0, 7.6 Hz, H-16), 1.18 (3H, s, H-18), 1.73 (3H, s, H-19), 2.55 (1H, qd, *J* = 7.6, 3.2 Hz, H-20), 1.12 (3H, d, *J* = 7.6 Hz, H-21), 5.17 (1H, m, H-22), 2.45 (1H, m, H-23a), 2.30 (1H, dd, *J* = 14.4, 2.4 Hz, H-23b), 1.78 (3H, s, H-27), 1.66 (3H, s, H-28); ^{13}C NMR data, see Table 1; HRESIMS *m*/*z* 509.2520 [M + Na]$^+$ (calcd for $C_{28}H_{38}O_7Na$, 509.2510).

Tubocapsenolide G (3): white powder; mp 264−266 °C; $[\alpha[_D^{24.4}$ +32.5 (*c* 0.1, MeOH); UV (MeOH) λ_{max} 216 nm; CD [θ] +17 700 (256 nm); IR (neat) ν_{max} 3478, 2947, 1723, 1676, 1379, 1126 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.01 (1H, dd, *J* = 10.4, 2.0 Hz, H-2), 6.47 (1H, dd, *J* = 10.4, 2.0 Hz, H-3), 5.04 (1H, t, *J* = 2.0 Hz, H-4), 4.45 (1H, dd, *J* = 10.4, 3.6 Hz, H-6), 1.85 (1H, *J* = 10.4, 10.4 Hz, H-7a), 2.51 (1H, ddd, *J* = 10.4, 3.6, 3.6 Hz, H-7b), 2.33 (1H, m, H-8), 1.50 (1H, td, *J* = 9.6, 1.6 Hz, H-9), 1.28 (1H, m, H-11a), 2.01 (1H, m, H-11b), 1.63 (1H, m, H-12a), 1.85 (1H, m, H-12b), 2.40 (1H, m, H-15a), 2.55 (1H, m, H-15b), 4.09 (1H, dd, *J* = 7.6, 6.0 Hz, H-16), 1.06 (3H, s, H-18), 1.20 (3H, s, H-19), 2.15 (1H, qd, *J* = 7.2, 7.2 Hz, H-20), 0.96 (3H, d, *J* = 7.2 Hz, H-21), 4.37 (1H, ddd, *J* = 12.8, 7.2, 2.4 Hz, H-22), 2.42 (1H, m, H-23a), 2.22 (1H, dd, *J* = 18.0, 2.4 Hz, H-23b), 1.86 (3H, s, H-27), 1.92 (3H, s, H-28); ^{13}C NMR data, see Table 1; HRESIMS *m*/*z* 527.2177 [M + Na]$^+$ (calcd for $C_{28}H_{37}ClO_6Na$, 527.2171).

Tubocapsanolide A (4): white powder; mp 233−235 °C; $[\alpha]_D^{24.4}$ +22.3 (*c* 0.1, MeOH); UV (MeOH) λ_{max} 218 nm; CD [θ] +14 000 (256 nm); IR (neat) ν_{max} 3403, 2918, 1688, 1679, 1380, 1132 cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MHz) δ 6.43 (1H, d, *J* = 9.6 Hz, H-2), 7.23 (1H, dd, *J* = 9.6, 6.0 Hz, H-3), 4.01 (1H, dd, *J* = 6.0, 4.6 Hz, H-4), 3.20 (1H, br s, H-6), 1.17 (1H, ddd, *J* = 14.8, 11.2, 1.2 Hz, H-7a), 2.01 (1H, m, H-7b), 1.63 (1H, ddd, *J* = 11.2, 11.2, 4.0 Hz, H-8), 0.98 (1H, ddd, *J* = 11.6, 11.2, 4.0 Hz, H-9), 1.57 (1H, m, H-11a), 2.01 (1H, m, H-11b), 1.40 (1H, m, H-12a), 1.60 (1H, m, H-12b), 1.24 (1H, ddd, *J* = 12.0, 11.6, 6.4 Hz, H-14), 1.12 (1H, dd, *J* = 12.4, 12.0 Hz, H-15a), 1.86 (1H, dd, *J* = 12.4, 6.4 Hz, H-15b), 3.59 (1H, br s, H-16), 0.89 (3H, s, H-18), 1.81 (3H, s, H-19), 2.45 (1H, qd, *J* = 8.8, 6.8 Hz, H-20), 1.02 (3H, d, *J* = 6.8 Hz, H-21), 3.91 (1H, ddd, *J* = 12.8, 8.8, 3.2 Hz, H-22), 2.22 (1H, dd, *J* = 17.6, 12.4 Hz, H-23a), 2.08 (1H, dd, *J* = 17.6, 3.2 Hz, H-23b), 1.92 (3H, s, H-27), 1.73 (3H, s, H-28); ^{13}C NMR data, see Table 1; HRFABMS *m*/*z* 469.2594 [M + Na]$^+$ (calcd for $C_{28}H_{36}O_6Na$, 469.2585).

20-Hydroxytubocapsanolide A (5): white powder; mp 245−247 °C; $[\alpha]_D^{26.5}$ +14.4 (*c* 0.12, MeOH); UV (MeOH) λ_{max} 214 nm; CD [θ] +7603 (250 nm), +2675 (290 nm), +1512 (340 nm); IR (neat) ν_{max} 3439, 2922, 2856, 1705, 1380, 1236, 1026, 750 cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MHz) δ 6.46 (1H, d, *J* = 9.5 Hz, H-2), 7.22 (1H, dd, *J* = 6.0 Hz, H-3), 4.00 (1H, d, *J* = 6.0 Hz, H-4), 3.21 (1H, br s, H-6), 1.16 (1H, m, H-7a), 2.00 (1H, qd, *J* = 15.0, 2.0 Hz , H-7b), 1.66 (1H, m, H-8), 0.87 (1H, m, H-9), 1.60 (1H, m, H-11a), 1.96 (1H, m, H-11b), 1.85 (1H, m, H-12a), 1.93 (1H, m, H-12b), 1.37 (1H, td, *J* = 6.0 Hz, H-14), 1.10 (1H, td, *J* = 13.0, 12.0 Hz , H-15a), 1.77 (1H, m, H-15b), 3.57 (1H, s, H-16), 1.06 (3H, s, H-18), 1.81 (3H, s, H-19), 1.45 (3H, s, H-21), 4.48 (1H, dd, *J* = 12.5, 3.5 Hz, H-22), 2.17 (1H, dd, *J* = 18.0, 3.5 H-23a), 2.83 (1H, t, *J* = 18.0 Hz, H-23b), 1.91 (3H, s, H-27), 1.74

(3H, s, H-28); ^{13}C NMR data, see Table 1; HRESIMS *m/z* 507.2361 $[M + Na]^+$ (calcd for $C_{28}H_{36}O_7Na$, 507.2359).

23-Hydroxytubocapsanolide A (6): white powder; mp 223−225 °C; $[\alpha]_D^{25.2}$ −34.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} 216 nm; CD [θ] +3953 (250 nm), +1973 (290 nm), +4433 (340 nm); IR (neat) ν_{max} 3409, 2922, 2848, 1690, 1380, 753 cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MHz) δ 6.42 (1H, d, *J* = 9.6 Hz, H-2), 7.21 (1H, dd, *J* = 9.6, 6.4 Hz, H-3), 3.99 (1H, d, *J* = 6.4 Hz, H-4), 3.18 (1H, br s, H-6), 1.16 (1H, m, H-7a), 1.98 (1H, m, H-7b), 1.63 (1H, m, H-8), 0.92 (1H, m, H-9), 2.01 (2H, m, H-11), 1.38 (1H, m, H-12a), 1.56 (1H, m, H-12b), 1.22 (1H, m, H-14), 1.13 (1H, m, H-15a), 1.72 (1H, dd, *J* = 12.4 , 5.6 Hz, H-15b), 3.81 (1H, s, H-16), 0.82 (3H, s, H-18), 1.79 (3H, s, H-19), 2.59 (1H, qd, *J* = 7.2, 6.8 Hz, H-20), 1.15 (3H, d, *J* = 6.8 Hz, H-21), 4.36 (1H, m, H-22), 4.39 (1H, d, H-23), 1.98 (3H, s, H-27), 2.09 (3H, d, *J* = 0.8 Hz , H-28); ^{13}C NMR data, see Table 1; HRESIMS *m/z* 507.2361 $[M + Na]^+$ (calcd for $C_{28}H_{36}O_7Na$, 507.2359).

Tubocapsanolide D (7): white powder; mp 178−180 °C; $[\alpha]_D^{24.4}$ +100.3 (*c* 0.1, MeOH); UV (MeOH) λ_{max} 220 nm; CD [θ] +16 000 (256 nm); IR (neat) ν_{max} 3491, 2937, 1687, 1382, 1132 cm^{-1}; ^{1}H NMR-(C_5D_5N, 400 MHz) δ 6.03 (1H, dd, *J* = 10.0, 2.4 Hz, H-2), 6.53 (1H, dd, *J* = 10.0, 2.4 Hz, H-3), 5.02 (1H, br s, H-4), 4.43 (1H, dd, *J* = 12.8, 4.8 Hz, H-6), 1.64 (1H, m, H-7a), 2.01 (1H, ddd, *J* = 9.6, 8.8, 4.8 Hz, H-7b), 1.63 (1H, d, *J* = 8.8 Hz, H-8), 1.32 (1H, m, H-9), 1.01 (1H, m, H-11a), 1.56 (1H, m, H-11b), 1.37 (1H, m, H-12a), 1.56 (1H, m, H-12b), 1.26 (1H, m, H-14), 1.36 (1H, m, H-15a), 1.58 (1H, m, H-15b), 1.43 (1H, m, H-16a), 2.27 (1H, m, H-16b), 0.85 (3H, s, H-18), 1.26 (3H, s, H-19), 2.21 (1H, qd, *J* = 7.2, 1.6 Hz, H-20), 1.08 (3H, d, *J* = 7.2 Hz, H-21), 4.61 (1H, ddd, *J* = 12.8, 3.2, 1.6 Hz, H-22), 2.50 (1H, dd, *J* = 18.0, 12.8 Hz, H-23a), 2.36 (1H, dd, *J* = 18.0, 3.2 Hz, H-23b), 1.85 (3H, s, H-27), 1.91 (3H, s, H-28); ^{13}C NMR data, see Table 1; HRESIMS *m/z* 511.2668 $[M + Na]^+$ (calcd for $C_{28}H_{40}O_7Na$, 511.2666).

Tubocapsanolide F (8): white powder; mp 200−202 °C; $[\alpha]_D^{25.0}$ +75.7 (*c* 0.07, MeOH); UV (MeOH) λ_{max} 214 nm; CD [θ] +14 017 (250 nm), +2471 (290 nm), +3098 (340 nm); IR (neat) ν_{max} 3453, 2922, 1682, 1376, 1129, 750 cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MHz) δ 6.36 (1H, d, *J* = 10.0 Hz, H-2), 7.17 (1H, dd, *J* = 9.6, 6.4 Hz, H-3), 3.99 (1H, d, *J* = 6.0, 3.2 Hz ,H-4), 3.20 (1H, br s, H-6), 1.23 (1H, m, H-7a), 2.11 (1H, m, H-7b), 1.60 (1H, m, H-8), 1.01 (1H, m, H-9), 1.71 (1H, m, H-11a), 2.10 (1H, m, H-11b), 1.08 (1H, m, H-12a), 1.66 (1H, m, H-12b), 1.86 (1H, m, H-14), 1.75 (1H, m, H-15a), 1.95 (1H, m, H-15b), 1.89 (1H, m, H-16a), 1.98 (1H, m, H-16b), 0.73 (3H, s, H-18), 1.88 (3H, s, H-19), 2.31 (1H, qd, *J* = 13.6, 6.8 Hz, H-20), 1.19 (3H, d, *J* = 6.8 Hz, H-21), 4.76 (1H, td,*J* = 12.8, 3.2, Hz, H-22), 2.42 (1H, t, *J* = 18.0, 12.8 Hz, H-23a), 2.63 (1H, dd, *J* = 18.0, 3.2 Hz, H-23b), 1.93 (3H, s, H-27), 1.66 (3H, s, H-28); ^{13}C NMR data, see Table 2; HRESIMS *m/z* 493.2564 $[M + Na]^+$ (calcd for $C_{28}H_{38}O_6Na$, 493.2566).

Anomanolide A (11): white powder; mp 170−172 °C; $[\alpha]_D^{24.4}$ +12.9 (*c* 0.09, MeOH); UV (MeOH) λ_{max} 214 nm; IR (neat) ν_{max} 3432, 2952, 1720, 1675, 1380, 1128, 754 cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MHz) δ 6.19 (1H, d, *J* = 10.0 Hz, H-2), 6.93 (1H, dd, *J* = 10.0, 6.0 Hz, H-3), 3.76 (1H, d, *J* = 6.0 Hz, H-4), 3.24 (1H, br s, H-6), 1.35 (1H, dd, *J* = 15.2, 3.2 Hz, H-7a), 2.16 (1H, ddd, *J* = 15.2, 4.0, 3.2 Hz, H-7b), 1.55 (1H, td, *J* = 10.8, 4.0 Hz, H-8), 1.01 (1H, td, *J* = 10.8, 3.2 Hz, H-9), 1.37 (1H, m, H-11a), 1.92 (1H, m, H-11b), 1.42 (2H, m, H-12), 1.43 (1H, m, H-14), 1.23 (1H, m, H-15a), 1.73 (1H, m, H-15b), 1.70 (1H, m, H-16a), 2.04 (1H, d, *J* = 8.4 Hz, H-16b), 0.75 (3H, s, H-18), 1.40 (3H, s, H-19), 2.43 (1H, ddd, *J* = 9.2, 7.6, 1.0 Hz, H-20), 1.31 (1H, dd, *J* = 12.8, 7.6 Hz, H-21a), 2.51 (1H, ddd, *J* = 12.8, 9.2, 2.0 Hz, H-21b), 4.66 (1H, d, *J* = 2.4 Hz, H-22), 2.07 (1H, d, *J* = 13.2 Hz, H-23a), 1.78 (1H, dd, *J* = 13.2, 2.4 Hz, H-23b), 1.45 (3H, s, H-27), 1.18 (3H, s, H-28); ^{13}C NMR data, see Table 2; HRESIMS *m/z* 509.2517 $[M + Na]^+$ (calcd for $C_{28}H_{38}O_7Na$, 509.2510).

Anomanolide C (12): white powder; mp 280−282 °C; $[\alpha]_D^{24.4}$ +3.4 (*c* 0.1, MeOH); UV (MeOH) λ_{max} 214 nm; IR (neat) ν_{max} 3430, 2927, 1702, 1677, 1369, 1130 cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MHz) δ 6.38 (1H, d, *J* = 10.0 Hz, H-2), 7.18 (1H, dd, *J* = 10.0, 6.4 Hz, H-3), 3.98 (1H, dd,*J* = 6.4, 2.4 Hz, H-4), 3.16 (1H, br s, H-6), 1.20 (1H, dd, *J* = 14.0, 11.2 Hz, H-7a), 2.04 (1H, m, H-7b), 1.51 (1H, m, H-8), 1.02 (1H, ddd, *J* = 11.2, 4.0, 2.8 Hz, H-9), 1.51 (1H, m, H-11a), 2.04 (1H, m, H-11b), 1.58 (1H, m, H-12a), 2.01 (1H, m, H-12b), 1.98 (1H, m, H-14), 1.67 (1H, m, H-15a), 1.75 (1H, dd, *J* = 13.6, 7.2 Hz, H-15b), 4.51 (1H, dd, *J* = 7.2, 3.2 Hz, H-16), 0.63 (3H, s, H-18), 1.80 (3H, s, H-19), 2.75 (1H, dd, *J* = 8.4, 8.0 Hz, H-20), 1.98 (1H, m, H-21a), 2.99 (1H, dd, *J* = 9.6, 8.0 Hz, H-21b), 5.47 (1H, brd, *J* = 2.0 Hz, H-22), 2.15 (1H, d, *J* = 12.8 Hz, H-23a), 2.04 (1H, dd, *J* = 12.8, 2.8 Hz, H-23b), 1.67 (3H, s, H-27), 1.34 (3H, s, H-28); ^{13}C NMR data, see Table 2; HRESIMS *m/z* 525.2466 $[M + Na]^+$ (calcd for $C_{28}H_{38}O_8$-Na, 525.2459).

Anomanolide B (13): white powder; mp 168−170 °C; $[\alpha]_D^{24.4}$ +26.5 (*c* 0.1, MeOH); UV (MeOH) λ_{max} 214, 230 nm; IR (neat) ν_{max} 3448, 2960, 1726, 1675, 1369, 1130 cm^{-1}; ^{1}H NMR($CDCl_3$, 400 MHz) δ 5.98 (1H, dd, *J* = 10.0, 2.0 Hz, H-2), 6.45 (1H, dd, *J* = 10.0, 2.0 Hz, H-3), 5.03 (1H, br s, H-4), 4.42 (1H, dd, *J* = 12.8, 4.4 Hz, H-6), 1.72 (1H, m, H-7a), 2.32 (1H, 2.32 dt, *J* = 9.6, 4.4 Hz, H-7b), 1.65 (1H, m, H-8), 1.28 (1H, dd, *J* = 9.6, 3.2 Hz, H-9), 0.96 (1H, d, *J* = 8.0 Hz, H-11a), 1.32 (1H, m, H-11b), 1.35 (1H, m, H-12a), 1.39 (1H, m, H-12b), 1.59 (1H, m, H-14), 1.27 (1H, m, H-15a), 1.73 (1H, m, H-15b), 1.66 (1H, m, H-16a), 2.08 (1H, m, H-16b), 0.72 (3H, s, H-18), 1.24 (3H, s, H-19), 2.40 (1H, q, *J* = 7.6 Hz, H-20), 1.25 (1H, m, H-21a), 2.44 (1H, dd, *J* = 12.8, 2.4 Hz, H-21b), 4.64 (1H, d, *J* = 2.4 Hz, H-22), 2.05 (1H, d, *J* = 14.8 Hz, H-23a), 1.74 (1H, dd, *J* = 14.8, 2.4 Hz, H-23b), 1.43 (3H, s, H-27), 1.15 (3H, s, H-28); ^{13}C NMR data, see Table 2; ESIMS *m/z* 505 $[M + H]^+$.

Anomanolide E (14): white powder; mp 182−184 °C; $[\alpha]_D^{24.4}$ −2.5 (*c* 0.1, MeOH); UV (MeOH) λ_{max} 214 nm; IR (neat) ν_{max} 3455, 2933, 1720, 1675, 1400, 1132 cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MHz) δ 6.12 (1H, dd, *J* = 10.0, 2.4 Hz, H-2), 6.64 (1H, ddd, *J* = 10.0, 3.6, 2.4 Hz, H-3), 2.39 (1H, d, *J* = 19.2 Hz, H-4eq), 3.73 (1H, ddd, *J* = 19.2, 2.4, 2.4 Hz, H-4ax), 4.10 (1H, br s, H-6), 1.85 (1H, dt, *J* = 12.0, 2.8 Hz, H-7a), 2.23 (1H, dt, *J* = 12.0, 2.4 Hz, H-7b), 2.14 (1H, m, H-8), 2.56 (1H, dt, *J* = 11.2, 3.2 Hz, H-9), 1.58 (1H, dq, *J* = 12.0, 3.2 Hz, H-11a), 2.83 (1H, dt, *J* = 12.0, 3.2 Hz, H-11b), 1.71 (1H, m, H-12a), 2.41 (1H, m, H-12b), 2.41 (1H, m, H-14), 1.79 (2H, m, H-15), 4.49 (1H, d, *J* = 6.4 Hz, H-16), 0.76 (3H, s, H-18), 1.67 (3H, s, H-19), 2.79 (1H, dd, *J* = 8.4, 8.0 Hz, H-20), 2.04 (1H, dd, *J* = 12.8, 7.6 Hz, H-21a), 3.02 (1H, ddd, *J* = 12.8, 9.6, 1.2 Hz, H-21b), 5.47 (1H, d, *J* = 2.0 Hz, H-22), 2.15 (1H, d, *J* = 12.0 Hz, H-23a), 2.07 (1H, dd, *J* = 12.0, 2.8 Hz, H-23b), 1.67 (3H, s, H-27), 1.36 (3H, s, H-28); ^{13}C NMR data, see Table 2; HRESIMS *m/z* 527.2618 $[M + Na]^+$ (calcd for $C_{28}H_{40}O_8Na$, 527.2615).

Anomanolide F (15): white powder; mp 190−192 °C; $[\alpha]_D^{26.4}$ +4.76 (*c* 0.07, MeOH); UV (MeOH) λ_{max} 215 nm; CD [θ] +21 513 (250 nm), +2562 (290 nm), −645 (340 nm); IR (neat) ν_{max} 3416, 2929, 2863, 1712, 1668, 1376, 1082, 753 cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MHz) δ 6.12 (1H, dd, *J* = 10.4, 2.4 Hz, H-2), 6.73 (1H, dd, *J* = 10.4, 2.4 Hz, H-3), 5.35 (1H, br s, H-4), 1.22 (1H, m, H-6a), 2.22 (1H, m, H-6b), 1.24 (1H, m, H-7a), 1.78 (1H, m, H-7b), 1.65 (1H, m, H-8), 1.56 (1H, m, H-9), 1.38 (2H, m, H-11), 1.54 (1H, m, H-12a), 1.99 (1H, m, H-12b), 2.16 (1H, m, H-14), 2.09 (2H, m, H-15), 4.42 (1H, br s, H-16), 0.71 (3H, s, H-18), 1.61 (3H, s, H-19), 2.55 (1H, dd, *J* = 8.8, 8.4 Hz, H-20), 1.96 (1H, m, H-21a), 2.71 (1H, d, *J* = 12.0 Hz, H-21b), 5.00 (1H, m, H-22), 2.02 (1H, m, H-23a), 2.05 (1H, m, H-23a), 1.81 (3H, s, H-27), 1.40 (3H, s, H-28); ^{13}C NMR data, see Table 2; HRESIMS *m/z* 527.2620 $[M + Na]^+$ (calcd for $C_{28}H_{40}O_8Na$, 527.2615).

Anomanolide D (16): white powder; mp 196−198 °C; $[\alpha]_D^{24.4}$ +5.6 (*c* 0.1, MeOH); UV (MeOH) λ_{max} 214 nm; IR (neat) ν_{max} 3448, 2948, 1718, 1675, 1378, 1126, 1049 cm^{-1}; ^{1}H NMR(C_5D_5N, 400 MHz) δ 6.16 (1H, dd, *J* = 10.0, 2.0 Hz, H-2), 6.77 (1H, dd, *J* = 10.0, 2.0 Hz, H-3), 5.19 (1H, br s, H-4), 4.64 (1H, dd, *J* = 12.8, 4.4 Hz, H-6), 1.82 (1H, t, *J* = 12.0 Hz, H-7a), 2.21 (1H, dt, *J* = 12.8, 4.0 Hz, H-7b), 1.59 (1H, m, H-8), 1.59 (1H, m, H-9), 1.10 (1H, m, H-11a), 1.37 (1H, m, H-11b), 1.46 (1H, dt, *J* = 9.6, 2.8 Hz, H-12a), 1.94 (1H, m, H-12b), 2.12 (1H, m, H-14), 1.72 (2H, d, *J* = 11.2 Hz, H-15), 4.50 (1H, br.d, *J* = 3.2 Hz, H-16), 0.61 (3H, s, H-18), 1.53 (3H, s, H-19), 2.71 (1H, t, *J* = 8.4 Hz, H-20), 1.93 (1H, m, H-21a), 2.95 (1H, dd, *J* = 11.2, 9.6 Hz, H-21b), 5.45 (1H, d, *J* = 2.0 Hz, H-22), 2.14 (1H, d, *J* = 12.8 Hz, H-23a), 2.02 (1H, dd, *J* = 12.8, 3.2 Hz, H-23b), 1.66 (3H, s, H-27), 1.34 (3H, s, H-28); ^{13}C NMR data, see Table 2; HRESIMS *m/z* 561.2229 $[M + Na]^+$ (calcd for $C_{29}H_{39}ClO_8Na$, 561.2226).

Tubonolide A (17): white powder; mp 200−202 °C; $[\alpha]_D^{26.6}$ +10.2 (*c* 0.1, MeOH); UV (MeOH) λ_{max} 214 nm; IR (neat) ν_{max} 3427, 2938, 1730, 1676, 1371, 985, 752 cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MHz) δ 6.25 (1H, dd, *J* = 8.0, 1.2 Hz, H-2), 6.83 (1H, dd, *J* = 8.0, 2.0 Hz, H-3), 5.23 (1H, m, H-4), 4.69 (1H, dd, *J* = 10.0, 4.0 Hz, H-6), 1.88 (1H, dd, *J* = 10.0, 10.0 Hz, H-7a), 2.26 (1H, ddd, *J* = 10.0, 4.0, 3.2 Hz, H-7b), 1.59 (1H, m, H-8), 1.56 (1H, td, *J* = 8.8, 3.2 Hz, H-9), 1.09 (1H, m, H-11a), 1.43 (1H, m, H-11b), 1.41 (1H, m, H-12a), 1.83 (1H, m, H-12b),

2.13 (1H, m, H-14), 1.72 (1H, m, H-15a), 1.79 (1H, m, H-15b), 4.49 (1H, t, $J = 4.4$ Hz, H-16), 0.75 (3H, s, H-18), 1.62 (3H, s, H-19), 2.62 (1H, m, H-20), 2.67 (1H, dd, $J = 10.4$, 5.6 Hz, H_{ax}-21), 1.64 (1H, d, $J = 10.4$ Hz, H_{eq}-21), 5.09 (1H, br s, H-22), 2.91 (1H, d, $J = 12.4$ Hz, H-23a), 2.15 (1H, m, H-23b), 1.50 (3H, s, H-27), 1.36 (3H, s, H-28), 7.43 (1H, d, $J = 5.6$ Hz, 4-OH), 5.99 (1H, s, 5-OH), 7.28 (1H, d, $J = 3.6$ Hz, 16-OH), 5.41 (1H, s, 17-OH), 6.11 (1H, s, 24-OH); ^{13}C NMR data, see Table 2; HRESIMS *m*/*z* 561.2232 $[M + Na]^+$ (calcd for $C_{28}H_{39}ClO_8Na$, 561.2226).

X-ray Diffraction Analyses of Compounds 1 and 3. Diffraction intensity data were acquired with a Rigaku AFC7S single-crystal X-ray diffractometer with graphite-monochromated Mo Kα radiation ($\lambda = 0.71073$ Å). Crystal data for **1**: $C_{28}H_{36}O_6$ (formula weight 468.57), approximate crystal size, $0.8 \times 0.4 \times 0.1$ mm^3, monoclinic, space group, $P2_1$ (# 4), $T = 293(2)$ K, $a = 7.5150(15)$ Å, $b = 20.187(4)$ Å, $c = 8.1396(16)$ Å, $\beta = 95.64(3)°$, $V = 1228.9(4)$ Å^3, $D_c = 1.266$ Mg/m^3, $Z = 2$, $F(000) = 504$, $\mu_{(MoK\alpha)} = 0.088$ mm^{-1} A total of 2658 reflections were collected in the range $2.02° < \theta < 25.99°$, with 2475 independent reflections [R(int) = 0.0173], completeness to $\theta_{max.}$ was 100%; psi-scan absorption correction applied; full-matrix least-squares refinement on F^2, the number of data/restraints/parameters were 2475/1/318; goodness-of-fit on $F^2 = 1.049$; final R indices [$I > 2\sigma(I)$], $R_1 = 0.0334$, $wR_2 = 0.0824$; R indices (all data), $R_1 = 0.0527$, $wR_2 = 0.0899$, largest difference peak and hole, 0.144 and −0.145 e/Å^3. Crystal data for **3**: $C_{28}H_{37}Cl_1O_6 \cdot 0.5(C_2H_5OH)$ (formula weight 528.06), approximate crystal size, $0.5 \times 0.3 \times 0.3$ mm^3, monoclinic, space group, $P2_1$ (# 4), $T = 298(2)$ K, $a = 7.6195(15)$ Å, $b = 14.984(3)$ Å, $c = 12.540(3)$ Å, $\beta = 93.71(3)°$, $V = 1428.7(5)$ Å^3, $D_c = 1.227$ Mg/m^3, $Z = 2$, $F(000) = 566$, $\mu_{(MoK\alpha)} = 0.175$ mm^{-1} A total of 3065 reflections were collected in the range $2.12° < \theta < 26.00°$, with 2932 independent reflections [R(int) = 0.0243], completeness to $\theta_{max.}$ was 100%; psi-scan absorption correction applied; full-matrix least-squares refinement on F^2, the number of data/restraints/parameters were 2932/3/345; goodness-of-fit on $F^2 = 1.023$; final R indices [$I > 2\sigma(I)$], $R_1 = 0.0646$, $wR_2 = 0.1900$; R indices (all data), $R_1 = 0.0932$, $wR_2 = 0.2140$, largest difference peak and hole, 0.747 and −0.321 e/Å^3. Crystallographic data for the structural analysis have been deposited with the Cambridge Crystallographic Data Centre (deposition number CCDC 617532 for **3** and 617533 for **1**). These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk).

Cytotoxicity Assays. Compounds were assayed for cytotoxicity against Hep G2, Hep 3B, A549, MCF-7, MDA-MB-231, and MRC-5 cells using the MTT method.[25] The IC_{50} is the concentration of agent that reduced cell growth by 50% under the experimental conditions.

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Supporting Information Available: Table S1 with ^{1}H (400 MHz) NMR data of Mosher's esters of compounds **1**, **4**, and **12** in C_5D_5N. The isolation, NMR data, structures, and physical constant data of presumed artifacts of isolation, compounds **18**−**27**, are also available in the Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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