Cucurbitane-Type Triterpenoids from the Stems of *Cucumis melo*

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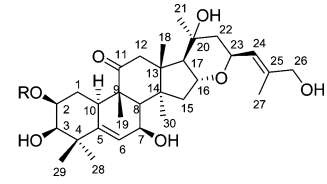
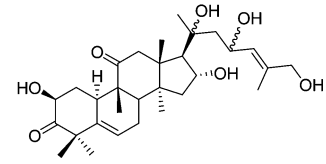
Phytochemical investigation of the stems of *Cucumis melo* led to the isolation and identification of 21 cucurbitane-type triterpenoids, including nine new compounds (**1**−**9**) and 12 known compounds. Their structures were determined on the basis of spectroscopic analyses, chemical methods, and comparison with spectroscopic data in the literature. Two known compounds, cucurbitacin B (**10**) and cucurbitacin A (**11**), showed significant cytotoxic activity against the proliferation of A549/ATCC and BEL7402 cells *in vitro*. Of the new compounds, only compound **7** was weakly cytotoxic. The inhibitory effects of all compounds on the Jak-Stat3 signaling pathway were evaluated, but only cucurbitacin B (**10**) showed significant inhibitory activity of phosphotyrosine STAT3.

Stems of *Cucumis melo* Linn (Cucurbitaceae) have been used in traditional Chinese medicine for the treatment of dyspepsia, jaundice, acute and chronic hepatitis, hepatic cirrhosis, hepatoma, and cancer.[1,2] Previous phytochemical studies have revealed *C. melo* to be a rich source of volatile compounds,[3] triterpenoids,[4−11] sterols,[12] and flavonoids.[13] Cucurbitacins are noted for their cytotoxicity and potential anticancer activity.[14−19] Several published studies have pointed out that the mechanism of activity of cucurbitacins involves their interference with the Jaks−Stat (Janus kinase-signal transducer and activator of transcription) signaling pathway, specifically with the STAT3 signaling pathway.[20−23] Cucurbitacins also exhibit other *in vitro* or *in vivo* pharmacological effects, such as hepatoprotective, cardiovascular, purgative, anti-inflammatory, antimicrobial, anthelmintic, and CNS effects and antifertility activities.[24−27] In our search for new anticancer agents and JAK/STAT3 signaling pathway inhibitors from natural resources, 21 cucurbitane-type triterpenoids, including nine new compounds (**1**−**9**) and 12 known compounds, cucurbitacin B (**10**),[28] 23,24-dihydrocucurbitacin B,[29] cucurbitacin A (**11**),[30] cucurbitacin R,[31] isocucurbitacin R,[32] cucurbitacin G,[33] cucurbitacin H,[33] hexanorcucurbitacin D,[33] arvenin I,[34] arvenin III,[34] dihydroisocucurbitacin B,[35] and 19-norlanosta-5, 24-dien-11-one,[36] were isolated from the stems of *C. melo*.



1 R=H
2 R=glc



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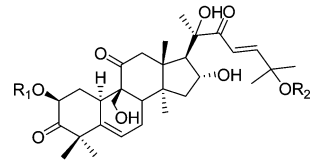
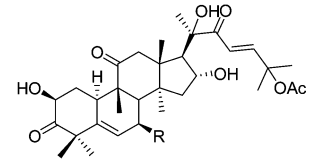
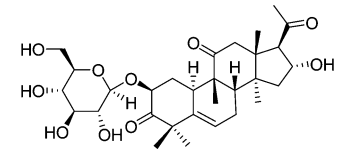


4 R_1=glc, R_2=Ac
5 R_1=H, R_2=H
6 R_1=H, R_2=H (23,24-dihydro)
11 R_1=H, R_2=Ac



7 R=OH
8 R=OH (23,24-dihydro)
10 R=H



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Results and Discussion

Powdered, air-dried stems of *C. melo* (5 kg) collected in Anhui Province, People's Republic of China, were percolated at room temperature with 95% ethanol three times. After evaporation of the ethanol *in vacuo*, the aqueous residue was extracted successively with petroleum ether, $CHCl_3$, ethyl acetate, and 1-butanol. The latter three extracts were subjected to a series of chromatography steps to afford 21 compounds.

Compound **1** was obtained as a white, amorphous powder with the molecular formula $C_{30}H_{46}O_7$, as deduced from HRESIMS and NMR analyses. Its ^{1}H NMR spectrum revealed the existence of two olefinic protons (δ_H 6.22, 1H, d, 7.3 Hz and δ_H 5.85, 1H, d, 5.2 Hz), seven tertiary methyl groups (δ_H 0.97, 1.03, 1.11, 1.23, 1.25, 1.28, 1.70), and seven protons bonded to carbons bearing oxygen. The ^{13}C NMR spectrum displayed 30 signals separated by DEPT experiments into seven methyl, five methylene (one oxygenated methylene), 10 methine (two sp^2 methines and five oxygenated methines), and eight quaternary carbons (one carbonyl carbon, two sp^2 carbons, and one oxygenated carbon). The ^{1}H and ^{13}C NMR data of **1** (Tables 1, 2) were characteristic of the cucurbitacin structure with an additional ring formed by cyclization of the side chain through an ether linkage according to the number of double-bond equivalents.[37] Analyses of its ^{1}H−^{1}H COSY and HSQC spectra led to the fragments C-10−C-1−C-2−C-3; C-6−C-7; C-15−C-16−C-17; and C-22−C-23−C-24. The chemical shifts of C-2 and C-3 and the coupling constant between H-2 and H-3 suggested a 2,3-*cis*-diol structure on ring A.[28,38−41] The HMBC spectrum indicated that the proton signal at δ_H 4.02 was interrelated with C-5 (δ_C 145.2), C-6 (δ_C 123.3), C-9 (δ_C 49.5), and C-14 (δ_C 48.9). The above results indicated that an OH was attached to C-7. Furthermore, a ^{13}C−^{1}H long-range correlation signal between H-16 and C-23 revealed that C-16 and C-23 were linked via an ether bond to form a pyranoid structural element. ^{13}C−^{1}H long-range correlation signals at H-23/C-25 and H-26/C-24 placed the olefinic bond between C-24 and C-25, and an OH on one of the terminal methyl groups. The NOE cross-peaks at H-24/H-16 and CH_3-30/H-17 indicated that H-23, H-17 was α-oriented. NOE cross-peaks at H-2/H-10, H-3/H-1α, CH_3-30/H-7, CH_3-18/H-16, and CH_3-21/H-23 in the ROESY spectrum revealed that OH-2, OH-3, OH-7,

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Table 1. ^{1}H NMR Data (δ) of Compounds **1**−**9** (300 MHz; **1**, **2**, **4**, **9** in CD_3OD and others in $CDCl_3$)

position	1	2	3	4	5	6	7	8	9
1α	1.52 m	1.69 dd (13.2, 10.1)	2.21 m	2.30 m	2.28 m	2.28 m	2.32 m	2.30 m	2.20 m
1β	1.25 m	1.56 dd (13.2, 10.1)	1.20 m	1.20 d (10.1)	1.20 d (13.0)	1.10 d (12.7)	1.24 m	1.26 m	1.32 m
2	3.85 m	4.12 br d (13.2)	4.48 dd (12.9, 8.1)	4.82 dd (10.1, 6.6)	4.36 dd (13.0, 8.0)	4.38 dd (12.7, 7.9)	4.46 dd (12.7, 6.4)	4.42 m	4.92 dd (13.2, 7.5)
3	3.40 d (2.2)	3.56 d (2.2)							
6	5.85 d (5.2)	5.84 d (4.8)	5.80 d (3.5)	5.83 d (5.7)	5.82 br s	5.82 br s	5.96 d (5.2)	5.97 d (5.2)	5.82 d (6.0)
7α	4.02 d (5.2)	4.01 d (4.8)	2.00 m	2.00 m	1.96 m	2.01 m	4.10 d (5.2)	4.10 d (5.2)	2.02 m
7β			2.40 m	2.40 m	2.42 m	2.44 m			2.50 d (15.6)
8	2.10 s	2.09 s	1.96 d (8.1)	2.68 d (8.0)	2.74 d (8.4)	2.74 d (7.8)	2.10 s	2.10 s	2.01 d (15.6)
10	2.49 br dd (13.0, 3.6)	2.52 br d (10.1)	2.75 d (12.8)	3.10 d (10.6)	2.88 d (12.7)	2.87 d (15.0)	2.72 d (10.7)	2.74 d (10.4)	3.08 d (12.2)
12α	3.12 d (15.2)	3.12 d (15.8)	3.18 d (14.1)	3.30 d (13.1)	3.25 d (15.3)	3.24 d (15.0)	3.18 d (14.6)	3.18 d (15.4)	3.50 d (15.6)
12β	2.44 d (15.2)	2.40 d (15.8)	2.42 d (14.1)	2.58 d (13.1)	2.68 d (15.3)	2.68 d (15.0)	2.68 d (14.6)	2.70 d (15.4)	2.44 d (15.6)
15α	1.38 dd (13.6, 3.1)	1.38 dd (13.5, 3.2)	1.54 dd (12.7, 3.2)	1.45 br d (12.7)	1.42 m	1.44 m	1.52 m	1.52 m	1.50 d (13.2)
15β	2.08 br dd (13.6, 9.7)	2.03 br dd (13.5, 9.7)	1.85 br dd (12.7, 7.3)	1.78 br dd (12.7, 9.1)	1.91 m	1.92 m	2.08 m	2.04 m	1.94 m
16	4.65 dt (9.7, 3.1)	4.62 dt (9.7, 3.2)	4.60 t (7.3)	4.58 dd (9.1, 7.3)	4.34 t (7.6)	4.30 t (7.0)	4.38 m	4.30 m	4.84 t (7.7)
17	1.98 d (9.7)	1.97 d (9.7)	2.68 d (7.3)	2.54 d (7.3)	2.54 d (7.6)	2.60 d (7.0)	2.45 d (7.0)	2.48 d (7.0)	3.22 d (7.7)
18	0.97 s	0.95 s	0.85 s	0.98 s	1.04 s	1.05 s	0.99 s	0.99 s	0.66 s
19	1.23 s	1.21 s	1.33 s	3.14 d (10.8)	3.14 d (11.1)	3.12 d (10.6)	1.20 s	1.20 s	1.04 s
				4.20 d (10.8)	4.26 d (11.1)	4.24 d (10.6)			
21	1.28 s	1.28 s	1.40 s	1.40 s	1.40 s	1.43 s	1.42 s	1.41 s	2.20 s
22α	1.82 dd (13.2, 7.3)	1.84 dd (13.3, 6.6)	2.08 m						
22β	1.55 d (13.2)	1.54 d (13.3)	1.56 m						
23	4.70 t (7.3)	4.70 t (6.6)	4.70 t (8.8)	6.82 d (15.9)	6.62 d (15.3)	2.62 m	6.48 d (15.6)	2.50 m	
						2.92 m		2.78 m	
24	6.22 d (7.3)	6.18 d (6.6)	5.50 d (8.8)	6.92 d (15.9)	7.12 d (15.3)	1.80 t (7.6)	7.04 d (15.6)	2.05 m	
26	3.92 s	3.92 s	4.01 s	1.55 s	1.28 s	1.21 s	1.56 s	1.43 s	
27	1.70 s	1.66 s	1.72 s	1.57 s	1.34 s	1.24 s	1.52 s	1.42 s	
28	1.03 s	1.03 s	1.26 s	1.27 s	1.37 s	1.27 s	1.28 s	1.28 s	1.32 s
29	1.25 s	1.25 s	1.05 s	1.30 s	1.34 s	1.33 s	1.38 s	1.38 s	1.28 s
30	1.11 s	1.12 s	1.38 s	1.24 s	1.39 s	1.41 s	1.24 s	1.24 s	1.35 s
Ac				2.01 s			2.01 s	1.94 s	
1′		4.38 d (7.7)		4.32 d (7.7)					4.34 d (7.8)
2′		3.16 m		3.22 m					3.25 m
3′		3.34 m		3.26 m					3.26 m
4′		3.26 m		3.28 m					3.28 m
5′		3.25 m		3.26 m					3.26 m
6′		3.64 dd (11.6,7.0); 3.81 dd (11.6, 2.2)		3.66 dd (11.8, 5.6); 3.88 dd (11.8, 1.3)					3.64 dd (11.0, 6.9); 3.85 dd (11.0, 2.1)

Table 2. ^{13}C NMR Data (δ) of Compounds **1**−**9** (100 MHz; **1**, **2**, **4**, and **9** in CD_3OD and others in $CDCl_3$)

position	1	2	3	4	5	6	7	8	9
1	30.1 t	28.6 t	35.9 t	35.4 t	34.9 t	35.1 t	35.2 t	35.2 t	36.4 t
2	69.3 d	76.9 d	71.5 d	80.3 d	71.6 d	71.8 d	71.4 d	71.4 d	80.0 d
3	80.4 d	77.2 d	213.0 s	213.5 s	212.0 s	212.6 s	212.2 s	212.2 s	213.8 s
4	43.6 s	43.2 s	50.0 s	52.8 s	50.0 s	50.1 s	50.2 s	50.2 s	53.0 s
5	145.2 s	145.1 s	140.3 s	141.9 s	140.0 s	139.9 s	145.0 s	145.0 s	142.2 s
6	123.3 d	123.2 d	120.4 d	122.8 d	121.4 d	121.4 d	121.8 d	121.8 d	121.6 d
7	67.8 d	67.8 d	23.8 t	24.9 t	23.3 t	23.6 t	66.2 d	66.2 d	25.4 t
8	53.8 d	53.8 d	42.6 d	35.1 d	33.0 d	33.2 d	51.8 d	51.7 d	44.8 d
9	49.5 s	49.5 s	48.5 s	55.1 s	53.8 s	53.8 s	47.5 s	47.5 s	50.6 s
10	36.2 d	36.0 d	33.7 d	34.9 d	32.8 d	33.0 d	34.6 d	34.6 d	35.4 d
11	216.4 s	216.4 s	212.7 s	214.4 s	212.4 s	212.3 s	211.8 s	211.8 s	214.5 s
12	49.8 t	50.3 t	48.4 t	51.6 t	48.4 t	48.6 t	48.6 t	48.6 t	48.6 t
13	49.0 s	49.0 s	48.1 s	52.0 s	50.7 s	50.9 s	49.7 s	49.6 s	51.0 s
14	48.9 s	48.8 s	50.2 s	49.8 s	47.7 s	47.9 s	47.0 s	47.3 s	51.8 s
15	42.4 t	42.4 t	44.4 t	47.3 t	45.3 t	45.4 t	45.2 t	45.4 t	46.6 t
16	72.1 d	72.1 d	72.3 d	72.3 d	71.3 d	71.1 d	71.0 d	70.6 d	73.0 d
17	56.9 d	57.0 d	58.2 d	61.7 d	57.2 d	57.9 d	58.1 d	57.7 d	68.4 d
18	20.6 q	20.6 q	20.2 q	20.3 q	18.7 q	18.6 q	19.9 q	19.8 q	20.8 q
19	22.3 q	22.1 q	21.2 q	61.7 t	60.7 t	60.5 t	21.4 q	21.4 q	20.7 q
20	73.8 s	73.9 s	75.7 s	80.8 s	77.9 s	79.2 s	78.2 s	78.8 s	211.1 s
21	30.3 q	30.3 q	27.6 q	26.1 q	23.7 q	24.5 q	23.9 q	24.4 q	32.3 q
22	47.1 t	47.0 t	49.6 t	206.0 s	202.3 s	215.4 s	202.4 s	213.8 s	
23	73.0 d	73.0 d	65.5 d	123.1 d	119.0 d	30.8 t	120.2 d	30.6 t	
24	127.6 d	127.5 d	126.8 d	151.9 d	155.6 d	36.9 t	151.9 d	34.6 t	
25	138.9 s	139.0 s	136.9 s	81.6 s	70.9 s	70.3 s	79.3 s	81.3 s	
26	69.1 t	69.1 t	67.1 t	27.0 q	28.6 q	28.7 q	25.9 q	25.8 q	
27	14.3 q	14.3 q	14.1 q	27.3 q	29.1 q	29.3 q	26.3 q	26.1 q	
28	28.3 q	28.2 q	29.4 q	30.2 q	29.3 q	29.9 q	29.8 q	29.8 q	29.8 q
29	26.5 q	26.7 q	20.0 q	29.7 q	21.0 q	21.2 q	21.2 q	21.2 q	22.3 q
30	22.2 q	22.1 q	18.8 q	22.4 q	19.4 q	19.3 q	19.1 q	19.0 q	19.9 q
$-OCO\underline{C}H_3$				22.3 q			21.9 q	22.4 q	
$-O\underline{C}OCH_3$				172.4 s			170.4 s	170.5 s	
1′		102.4 d		104.8 d					104.8 d
2′		75.6 d		75.9 d					75.9 d
3′		78.7 d		78.7 d					78.7 d
4′		72.1 d		71.9 d					71.9 d
5′		78.3 d		78.4 d					78.4 d
6′		63.3 t		63.4 t					63.4 t

H-16, and OH-20 were β-oriented. The NOE correlation between CH_2-26 and H-24 revealed the *E* configuration of the olefinic bond. Therefore, **1** was characterized as 16α,23α-epoxy-2β,3β,7β,20β,26-pentahydroxy-10α,23α-cucurbit-5,24-(*E*)-dien-11-one.

Compound **2** showed a quasimolecular ion at *m*/*z* 703.3664 [M + Na]$^+$ in the HRESIMS, indicating a molecular formula of $C_{36}H_{56}O_{12}$. Comparison of its ^1H and ^{13}C NMR data with those of **1** revealed that compound **2** was a glycoside of **1**. This observation was confirmed by enzymatic hydrolysis of **2** with β-glucosidase to afford compound **1** and glucose. The specific rotation value of the obtained glucose, $[\alpha]_D^{24}$ +51 (*c* 0.035, H_2O), indicated its D configuration. A significant downfield shift of C-2 from δ_C 69.3 in **1** to δ_C 76.9 in the ^{13}C NMR spectrum suggested that the glucose was at C-2, which was confirmed by the $^{13}C-^1H$ long-range correlations of the anomeric proton at δ_H 4.38 with C-2 (δ_C 76.9) and also H-2 (δ_H 4.12) with C-1$_{glc}$ (δ_C 102.4) in the HMBC spectrum. The sugar unit was deduced to be a β-glycoside from the coupling constant of the anomeric proton (J = 7.7 Hz). Accordingly, the structure of **2** was determined to be 16α,23α-epoxy-2β,3β,7β,20β,26-pentahydroxy-10α,23α-cucurbit-5,24-(*E*)-dien-11-one 2-*O*-β-D-glucopyranoside.

The molecular formula of **3** was determined to be $C_{30}H_{46}O_7$ by HRESIMS (positive-ion mode) ([M + Na]$^+$, *m*/*z* 541.3120), indicating eight degrees of unsaturation. Its ^{13}C NMR spectrum showed 30 resonances, comprising seven methyl, six methylene (one oxygenated), eight methine (two sp^2 and three oxygenated methines), and nine quaternary carbons (two carbonyl and two sp^2 carbons, one oxygenated quaternary carbon). Its ^1H NMR spectrum revealed the existence of two olefinic protons (δ_H 5.80, 1H, d, 3.5 Hz and δ_H 5.50, 1H, d, 8.8 Hz), seven tertiary methyl groups (δ_H 0.85, 1.05, 1.26, 1.33, 1.38, 1.40, 1.72), and five protons bonded to carbons bearing oxygen. Analyses of its $^1H-^1H$ COSY and HSQC spectra led to the fragments C-10−C-1−C-2−C-3; C-6−C-7−C-8; C-15−C-16−C-17; and C-22−C-23−C-24. Comparison of the NMR data of **3** with those of (2β,9β,10α,16α,23*S*)-16,23-epoxy-2,20,26-trihydroxy-9-methyl-19-norlanosta-5,24-(*Z*)-diene-3,11-dione[37] indicated that the structure of **3** was similar to that compound. According to the number of unsaturation equivalents, the 16,23-epoxy function was open, which was supported by the differences of chemical shifts of surrounding carbons and also by the HMBC spectrum, in which $^{13}C-^1H$ long-range correlation signals were not observed at H-16/C-23 and H-23/C-16. $^{13}C-^1H$ long-range correlation signals were observed at H-23/C-25 and H-26/C-24, indicating one double bond at C-24/C-25 and that one of the terminal methyl groups was hydroxylated. The ROESY spectrum of **3** revealed the relative configuration of the tetracyclic skeleton. Furthermore, the NOE correlation between CH_2-26 and H-24 revealed the *E* configuration of the olefinic bond. Compound **3** was thus established to be 2β,16α,20,23,26-pentahydroxy-10α-cucurbit-5,24-(*E*)-diene-3,11-dione.

The positive HRESIMS of **4** showed the quasimolecular ion signal at *m*/*z* 759.3568 [M + Na]$^+$, and in conjunction with the ^{13}C NMR data, its molecular formula was determined to be $C_{38}H_{56}O_{14}$, indicating 11 degrees of unsaturation. The 38 resonances in its ^{13}C NMR spectrum were consistent with a triterpenoid backbone bearing a sugar moiety. Enzymatic hydrolysis of **4** gave glucose and the aglycone, which was identified as cucurbitacin A by comparison with spectroscopic data in the literature.[30] $^{13}C-^1H$ long-range correlation signals at H-1$_{glc}$/C-2 and H-2/C-1$_{glc}$ in its HMBC spectrum indicated linkage of the glucose moiety to C-2 of the aglycone. The sugar moiety was determined to be β-D-glucopyranose by spectroscopic and chemical methods as for

compound **2** {$[\alpha]_D^{24}$ +57 (*c* 0.29, H_2O)}. Thus, compound **4** was cucurbitacin A 2-*O*-*β*-D-glucopyranoside.

Compound **5** possessed the elemental composition $C_{30}H_{44}O_8$ as determined by HRESIMS and NMR analyses. Its ^1H and ^{13}C NMR data were similar to those of cucurbitacin A, except for the absence of an acetoxy moiety along with downfield shifts of C-24 (+3.7 ppm), 26-Me (+2.6 ppm), and 27-Me (+2.8 ppm) and an upfield shift of C-25 to δ 70.1 (−8.4 ppm). The structure of **5** was confirmed by analyses of $^1H-^1H$ COSY, HSQC, HMBC, and ROESY spectra and determined to be 25-deacetylcucurbitacin A.

Compound **6** had the molecular formula $C_{30}H_{46}O_8$ as determined from HRESIMS and NMR analyses, indicating one less unsaturation degree than that of **5**. Its ^1H and ^{13}C NMR data were similar to those of **5** except for the absence of a disubstituted olefinic bond and the emergence of two methylene signals. In contrast to **5**, the signal for C-22 showed a strong downfield shift to δ 215.4 (+13.1 ppm), whereas the two sp^2 methines were missing and seven sp^3 methylenes were observed. Analysis of 2D NMR experiments, including $^1H-^1H$ COSY, HSQC, and HMBC spectra, revealed that compound **6** was 23,24-dihydro-25-deacetylcucurbitacin A.

Compound **7** was obtained as white, amorphous powder with a molecular formula of $C_{32}H_{46}O_9$. Comparison of its ^1H and ^{13}C NMR data with those of cucurbitacin B revealed their structural similarity, and the emergence of a proton signal at δ_H 4.10 and methine carbon at δ 66.2 with the loss of a methylene signal at δ 25.3 indicated the replacement of a methylene by an oxygenated methine in **7**. In the $^1H-^1H$ COSY spectrum of **7**, cross-peaks were found between the proton signal at δ_H 4.10 and the olefinic signal at δ_H 5.96, which indicated the presence of an additional OH group at C-7. Furthermore, the appearance of H-8 (δ_H 2.10) as a singlet provided no coupling constant between H-7 and H-8, corresponding to a dihedral angle of approximately 90°, and led to the conclusion that the 7-OH was *β*-oriented. This was supported by the NOE signal between CH_3-30 and H-7. Thus, compound **7** was determined to be 7*β*-hydroxycucurbitacin B.

Compound **8** had the molecular formula $C_{32}H_{48}O_9$, indicating one less unsaturation degree than that of **7**. Its ^1H and ^{13}C NMR data were similar to those of **7** except for the absence of a disubstituted olefinic bond and the emergence of two methylene signals. In contrast to **7**, the signal for C-22 showed a strong downfield shift to δ 213.8 (−11.4 ppm), whereas the two sp^2 methines were missing and five sp^3 methylenes were observed. The configuration of C-7 was deduced from the coupling constant between H-7 and H-8. Compound **8** was finally identified to be 23,24-dihydro-7*β*-hydroxycucurbitacin B by further analyses of 2D NMR spectra.

Compound **9** had the molecular formula $C_{30}H_{44}O_{10}$. Enzymatic hydrolysis of **9** gave the aglycone, which was identified as hexanorcucurbitacin D by comparison with spectroscopic data in the literature.[33] The sugar moiety was determined to be *β*-D-glucose. $^{13}C-^1H$ long-range correlation signals at H-1_{glc}/C-2 and H-2/C-1_{glc} in its HMBC spectrum indicated linkage of the glucopyranose moiety to C-2 of the aglycone. On the basis of the above data, compound **9** was elucidated as hexanorcucurbitacin D 2-*O*-*β*-D-glucopyranoside.

All 21 cucurbitane-type triterpenoids were evaluated for cytotoxic activity against human non-small-cell lung cancer A549/ATCC and human hepatocellular BEL-7402 cells *in vitro*. Cucurbitacin B (**10**) inhibited the proliferation of A549/ATCC and BEL-7402 cells with IC_{50} values of 0.01 ± 0.001 and 0.008 ± 0.001 *μ*M, respectively, while cucurbitacin A (**10**) inhibited the proliferation of A549/ATCC and BEL-7402 cells with IC_{50} values of 0.4 ± 0.13 and 0.3 ± 0.11 *μ*M, respectively. The new compound **7** showed weak cytotoxicity, with IC_{50} values of 3.21 ± 0.85 and 7.59 ± 0.92 *μ*M, against A549/ATCC and BEL-7402 cell lines. The other compounds exhibited no cytotoxicity at up to 10 *μ*M. Preliminary analyses of the structure−activity relationships of these natural triterpenoids revealed that an *α*,*β*-unsaturated ketone in their side chains and a 25-acetoxy group were important structural requirements for cucurbitacin cytotoxicity, which was in agreement with a previous study.[42] Saturation of the conjugated $\Delta^{23,24}$ olefinic bond eliminated the cytotoxicity.

The effects of all isolated compounds on the Jak/Stat3 signaling pathway were also evaluated, but only cucurbitacin B (**10**) significantly suppressed phosphotyrosine stat3 levels (IC_{50}, 3 *μ*M). None of the other compounds showed such activity at up to 10 *μ*M.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 241MC polarimeter. IR spectra were recorded using a Perkin-Elmer 577 spectrometer. LR-ESIMS were measured using a Finnigan LCQ-DECA instrument, and HR-ESIMS data were obtained on a Mariner spectrometer. NMR spectra were recorded on a Bruker AM 400 with TMS as internal standard, and chemical shifts are expressed in δ (ppm). Preparative HPLC was carried out using a Varian SD-1 instrument, equipped with a Merck NW25 C_{18} column (10 *μ*m, 20 mm × 250 mm) and Prostar 320 UV/vis detector, and the preparative HPLC fractions were analyzed by analytic TLC. Column chromatographic separations were carried out by using MCI gel CHP-20P (75−150 *μ*m; Mitsubishi Chemical Industry Co., Ltd.), silica gel H60 (300−400 mesh), zcx-II (200−300 mesh) (Qingdao Haiyang Chemical Group Corporation, Qingdao, China), and Sephadex LH-20 (Pharmcia Biotech AB, Uppsala, Sweden) as packing materials. HSGF254 silica gel TLC plates (Yantai Chemical Industrial Institute, Yantai, China) and RP-18 WF_{254} TLC plates (Merck) were used for analytical purposes. *β*-Cellulase was manufactured by Lizhu Dongfeng BioTech Co. Ltd., Shanghai, People's Republic of China.

Plant Material. The aerial parts of *Cucumis melo* were purchased from the Yulin Medicinal Materials Market in Guangxi, China, in October 2007, which were collected in Anhui Province, China, and identified by Professor Jingui Shen of Shanghai Institute of Materia Medica, Chinese Academy of Sciences. A voucher specimen (No. SIMM071028) was deposited in the Herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Extraction and Isolation. Powdered air-dried stems of *C. melo* (5 kg) were percolated at room temperature with 95% EtOH (20 L × 3) within 9 days. After evaporation of EtOH *in vacuo*, the aqueous residue (1.5 L) was extracted successively with petroleum ether, $CHCl_3$, ethyl acetate, and 1-butanol (1.5 L × 3 each), yielding petroleum ether (30.0 g), $CHCl_3$ (200.0 g), ethyl acetate (15.0 g), and 1-butanol extracts (45.0 g), respectively. The $CHCl_3$ extract (200.0 g) was chromatographed using MCI gel vacuum liquid chromatography (VLC) (10 cm i.d. × 25 cm) eluted with H_2O, 10%, 20%, 40%, 60%, 70%, 80%, and 100% EtOH (each 1000 mL) to yield fractions C1 (27.8 g), C2 (100 g), C3 (20 g), and C4 (3.3 g). Fraction C1 was separated by VLC on a silica gel column using a petroleum−acetone gradient (10:1 to 0:1) to give subfractions C1A and C1B. Fraction C1A (6.3 g) was subjected to column chromatography (CC) over silica gel eluted with $CHCl_3$−MeOH (8:1) and preparative HPLC eluted with MeOH−H_2O (10 mL/min, 10% to 90% MeOH within 90 min) to afford arvenin I (5 g). Fraction C1B (3 g) was separated by HPLC eluted with a MeOH−H_2O gradient (10% to 100% MeOH within 100 min) to yield arvenin II (500 mg). Fraction C2 (100 g) was subjected to CC over silica gel eluted with petroleum−acetone (10:1, 8:1, 6:1, 5:1, 3:1, 1:1, and 0:1, each 2 L) to give four subfractions: C2A (6.5 g), C2B (3.0 g), C2C (2.4 g), and C2D (3.5 g). Fraction C2A (6.5 g) was subjected to CC over silica gel eluted with $CHCl_3$−MeOH (50:1) to give cucurbitacin R (450 mg) and isocucurbitacin R (400 mg). Fraction C2B (3.0 g) was subjected to CC over silica gel eluted with $CHCl_3$−MeOH (20:1) to give cucurbitacin A (**12**) (300 mg), cucurbitacin G (35 mg), cucurbitacin H (68 mg), and hexanorcucurbitacin D (98 mg), which were further purified by preparative HPLC eluted with a MeOH−H_2O gradient (10% to 100% MeOH within 50 min). Fraction C2C (2.4 g) was subjected to CC over silica gel eluted with $CHCl_3$−MeOH (50:1), then purified by PTLC (eluted with $CHCl_3$−MeOH, 10:1, 20:1, 20:1) and Sephadex LH-20 eluted with EtOH to afford **3** (6 mg), **7** (25 mg), and **8** (8 mg). Fraction C2D (3.5 g) was separated by HPLC eluted with a MeOH−H_2O gradient (10% to 100% MeOH within 100 min) and PTLC (developed with $CHCl_3$−MeOH 10:1) to yield **5** (15 mg) and **6** (9 mg). Fraction

C3 (2.0 g) was subjected to CC over silica gel eluted with petroleum−acetone (10:1) to afford cucurbitacin B (**10**) (125 mg) and dihydrocucurbitacin B (200 mg). Fraction C4 (3.3 g) was also separated by silica gel CC eluted with petroleum−acetone (10:1) to afford dihydroisocucurbitacin B (60 mg). The ethyl acetate fraction (15.0 g) was separated by VLC over a silica gel column using petroleum−acetone (10:1 to 0:1) as eluent to give fractions E1A (3.6 g) and E1B (2.2 g). Fraction E1A was separated by preparative HPLC eluted with a EtOH−H_2O gradient (0% to 100% EtOH within 100 min), then chromatographed on silica gel eluted with $CHCl_3$−MeOH (20:1) to give **1** (20 mg) and **4** (50 mg). The 1-butanol fraction was subjected to CC over MCI gel (10 cm i.d. × 15 cm) with H_2O, 20%, 40%, 60%, 80%, and 100% EtOH (each 1000 mL) as eluent to give fractions B1 (2.1 g), B2 (8.0 g), B3 (4.4 g), and B4 (7.1 g). Fraction B1 (2.1 g) was separated by HPLC eluted with a MeOH−H_2O gradient (10% to 100% MeOH within 100 min), then purified by PTLC (eluted with $CHCl_3$−MeOH, 10:1) to yield **2** (16 mg). Fraction B3 (4.4 g) was chromatographed on silica gel eluted with $CHCl_3$−MeOH (50:1 to 3:1), then purified by HPLC eluted with a MeOH−H_2O gradient (10% to 100% MeOH within 50 min) to give **9** (12 mg). Fraction B4 (7.1 g) was chromatographed on silica gel eluted with $CHCl_3$−MeOH (15:1 to 9:1) to give 19-norlanosta-5,24-dien-11-one (36 mg).

Compound 1: white, amorphous powder; $[\alpha]_D^{23}$ +137 (*c* 0.15, MeOH); IR (KBr) ν_{max} 3415, 2956, 2927, 1685, 1456, 1385, 1207, 1047 cm^{-1}; ^1H NMR and ^{13}C NMR data, Tables 1 and 2; LR-ESIMS (positive-ion mode) *m/z* 541.3 [M + Na]$^+$; HR-ESIMS *m/z* 541.3148 [M + Na]$^+$ (calcd for $C_{30}H_{46}O_7Na$, 541.3141).

Compound 2: white, amorphous powder; $[\alpha]_D^{23}$ +163 (*c* 0.15, MeOH); IR (KBr) ν_{max} 3417, 2931, 1685, 1456, 1381, 1209, 1078, 1041 cm^{-1}; ^1H NMR and ^{13}C NMR data, Tables 1 and 2; LR-ESIMS (positive-ion mode) *m/z* 703.3 [M + Na]$^+$; HR-ESIMS *m/z* 703.3664 [M + Na]$^+$ (calcd for $C_{36}H_{56}O_{12}Na$, 703.3669). Glucose: $[\alpha]_D^{24}$ +51 (*c* 0.035, H_2O).

Compound 3: white, amorphous powder; $[\alpha]_D^{23}$ +86 (*c* 0.15, $CHCl_3$); IR (KBr) ν_{max} 3423, 2970, 2928, 1714, 1689, 1464, 1377, 1022 cm^{-1}; ^1H NMR and ^{13}C NMR data, Tables 1 and 2; LR-ESIMS (positive-ion mode) *m/z* 541.3 [M + Na]$^+$; HR-ESIMS *m/z* 541.3120 [M + Na]$^+$ (calcd for $C_{30}H_{46}O_7Na$, 541.3141).

Compound 4: white, amorphous powder; $[\alpha]_D^{23}$ +28 (*c* 0.13, MeOH); IR (KBr) ν_{max} 3423, 2979, 1718, 1689, 1629, 1371, 1080, 622 cm^{-1}; ^1H NMR and ^{13}C NMR data, Tables 1 and 2; LR-ESIMS (positive-ion mode) *m/z* 759.3 [M + Na]$^+$; HR-ESIMS: *m/z* 759.3548 [M + Na]$^+$ (calcd for $C_{38}H_{56}O_{14}Na$, 759.3568). Glucose: $[\alpha]_D^{24}$ +57 (*c* 0.29, H_2O).

Compound 5: white, amorphous powder; $[\alpha]_D^{23}$ +45 (*c* 0.10, $CHCl_3$); IR (KBr) ν_{max} 3428, 2975, 2929, 1714, 1689, 1629, 1382, 1091, 754 cm^{-1}; ^1H NMR and ^{13}C NMR data, Tables 1 and 2; LR-ESIMS (positive-ion mode) *m/z* 555.3 [M + Na]$^+$; HR-ESIMS *m/z* 555.2927 [M + Na]$^+$ (calcd for $C_{30}H_{44}O_8Na$, 555.2934).

Compound 6: white, amorphous powder; $[\alpha]_D^{23}$ +64 (*c* 0.15, $CHCl_3$); IR (KBr) ν_{max} 3435, 2974, 2929, 1699, 1367, 1209, 1054 cm^{-1}; ^1H NMR and ^{13}C NMR data, Tables 1 and 2; LR-ESIMS (positive-ion mode) *m/z* 557.4 [M + Na]$^+$; HR-ESIMS *m/z* 557.3091 [M + Na]$^+$ (calcd for $C_{30}H_{46}O_8Na$, 557.3090).

Compound 7: white, amorphous powder; $[\alpha]_D^{23}$ +65 (*c* 0.23, $CHCl_3$); IR (KBr) ν_{max} 3446, 2979, 2943, 1718, 1689, 1629, 1464, 1369, 1256, 1126, 1022, 985 cm^{-1}; ^1H NMR and ^{13}C NMR data, Tables 1 and 2; LR-ESIMS (positive-ion mode) *m/z* 597.4 [M + Na]$^+$; HR-ESIMS *m/z* 597.3013 [M + Na]$^+$ (calcd for $C_{32}H_{46}O_9Na$, 597.3040).

Compound 8: white, amorphous powder; $[\alpha]_D^{23}$ +29 (*c* 0.22, $CHCl_3$); IR (KBr) ν_{max} 3448, 2923, 2852, 1716, 1464, 1369, 1255, 1022, 756 cm^{-1}; ^1H NMR and ^{13}C NMR data, Tables 1 and 2; LR-ESIMS (positive-ion mode) *m/z* 599.3 [M + Na]$^+$; HR-ESIMS *m/z* 599.3173 [M + Na]$^+$ (calcd. for $C_{32}H_{48}O_9Na$, 599.3196).

Compound 9: white, amorphous powder; $[\alpha]_D^{23}$ +80 (*c* 0.17, MeOH); IR (KBr) ν_{max} 3425, 2920, 2852, 1695, 1464, 1375, 1080, 1030 cm^{-1}; ^1H NMR and ^{13}C NMR data, Tables 1 and 2; LR-ESIMS (positive-ion mode) *m/z* 587.3 [M + Na]$^+$; HR-ESIMS *m/z* 587.2812 [M + Na]$^+$ (calcd. for $C_{30}H_{44}O_{10}Na$, 587.2832). Glucose: $[\alpha]_D^{24}$ +70 (*c* 0.05, H_2O).

Enzymatic Hydrolysis of Compounds 2, 4, and 9. Compound **2** (6 mg) was dissolved in H_2O (10 mL), and β-cellulase (10 mg) was added to the solution, which was kept at 37 °C for 2 days. The reaction mixture was extracted with ethyl acetate, and the aqueous phase was concentrated *in vacuo*. The residue was subjected to Sephadex LH-20 eluted with EtOH to yield the glucose (1 mg, yield 62.8%), which was compared with authentic sugar samples by co-TLC. Identification of D-glucose in each aqueous layer was carried out by comparing the specific rotation of the liberated glucose with that of authentic D-glucose.[43] Compounds **4** (30 mg) and **9** (6 mg) were hydrolyzed in the same way as for **2**, and the aglycones of **4** (16 mg) and **9** (4 mg) in the organic layer were purified by CC over Si gel eluted with $CHCl_3$−MeOH (25:1).

Bioassay Method. Cytotoxicity of compounds against non-small-cell lung cancer A549/ATCC cells and human hepatocellular BEL-7402 cells was determined using the sulforhodamine B (SRB) assay. Cells were plated in a 96-well plate 24 h before compound treatment and continuously exposed to different concentrations of compounds for another 72 h. After treatment, cells were fixed and stained with SRB as described in Monks et al.[44] Bound SRB was solubilized with 10 mM Tris, and absorbance was measured at 565 nm.

Western Blotting. After drug treatment, cells were washed twice with ice-cold PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 1.8 mM KH_2PO_4, pH 7.4) and lysed in SDS sample buffer. Cell lysates, containing equal amounts of protein, were separated by SDS-PAGE and transferred to polyvinylidine difluoride membranes. After being blocked in 5% nonfat milk in TBST (Tris-buffered saline with 0.1% Tween 20, pH 7.6), membranes were incubated with the primary phospho-Stat3 and β-tubulin antibodies at 4 °C overnight and then exposed to appropriate secondary antibodies for 2 h at room temperature. Immunoreactive proteins were visualized using the enhanced chemiluminescence system from Pierce Chemical (Rockford, IL).

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Supporting Information Available: Figures indicating the main signals in the HMBC and COSY spectra of **1**, **3**, **5**, and **9**, the main NOE correlation signals (↔) in the ROESY spectra of **1**, **3**, **5**, and **7**, and 1D and 2D NMR spectra of compounds **1**−**9** are available free of charge via the Internet at http://pubs.acs.org.

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