Steroidal Alkaloids from the Bulbs of *Fritillaria puqiensis*

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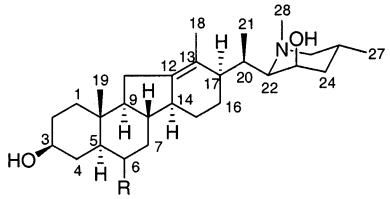
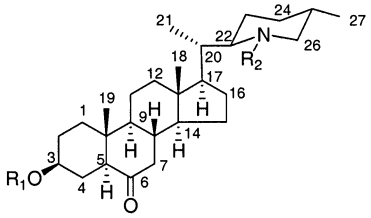
Four new steroidal alkaloids, puqienine A (**1**), puqienine B (**2**), *N*-demethylpuqietinone (**3**), and puqietinonoside (**4**), along with a known steroidal alkaloid, puqietinone, were isolated from the bulbs of *Fritillaria puqiensis*. The structures of these compounds were elucidated on the basis of spectroscopic analysis. The compounds exhibited significant antitussive activity on ammonia liquor-induced cough in mice. Furthermore, the compounds were evaluated for activities against A549 human lung carcinoma cell line, BGC-823 human stomach adenocarcinoma cell line, SMMC-7721 human hepatocarcinoma cell line, and HL-60 human promyelocytic leukemia cell line.

The bulbs of several *Fritillaria* species (Liliaceae), Chinese name "Beimu", have been used as an antitussive and expectorant in traditional Chinese medicine for centuries.[1] Pharmacological investigations suggested that steroidal alkaloids were responsible for the bioactivities of this herbal drug.[2,3] *F. puqiensis* G. D. Yu et. G. Y. Chen was cultivated and utilized therapeutically as an important substitute for "Beimu" in China.[4] Two steroidal alkaloids, puqietinone and puqiedinone, were isolated from the plant,[5,6] and both showed antitussive and antitumor activities.[7,8] In continuation of our studies on *F. puqiensis*, we isolated and identified four new steroidal alkaloids (compounds **1**−**4**), together with a known steroidal alkaloid, puqietinone, from the dried ground bulbs of this plant. In this paper we describe the extraction, isolation, and structure elucidation of four new alkaloids (**1**−**4**).



1 R = ⁻OH

2 R = =O



3 R_1 = H R_2 = H

4 R_1 = Glu R_2 = Me

Compound **1**, obtained as colorless needles from acetone, gave a positive Dragendorff test. The HRESIMS exhibited a quasimolecular ion $[M + H]^+$ at *m/z* 446.3625, corresponding to the molecular formula $C_{28}H_{47}NO_3$. In the EIMS

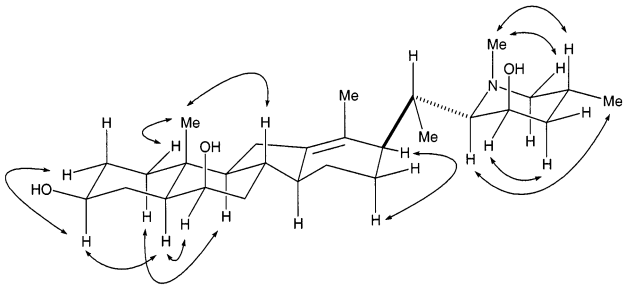


Figure 1. Key NOESY correlations of compound **1**.

the base peak at *m/z* 128 was assigned to the characteristic fragment arising from the cleavage between C-20 and C-22.[9] The IR spectrum showed a hydroxyl absorption at 3414 cm^{-1}. The ^1H NMR spectrum of **1** (Table 1) displayed two tertiary methyl signals at δ 1.59 (3H, s, H-18) and 0.90 (3H, s, H-19), two secondary methyl signals at δ 0.75 (3H, d, J = 6.6 Hz, H-27) and 0.70 (3H, d, J = 6.8 Hz, H-21), and an *N*-methyl signal at δ 2.47 (3H, s, H-28). The ^{13}C NMR spectrum showed 28 signals. The above data suggested that compound **1** was a veratramine-type alkaloid.[10] In the ^{13}C NMR spectrum (Table 2) two downfield signals at δ 138.9 and 125.9 were assigned to two quaternary olefinic carbons, C-13 and C-12, respectively, supported by a deshielded methyl group at δ 17.0 (C-18) and no signal for an olefinic proton. In addition, the shielded signal at δ 18.1 (C-25) was due to the γ -gauche interaction of the hydroxyl group at C-23 with C-25. The configurational assignments were determined through the NOESY spectrum of **1** (Figure 1). Thus, in the NOESY spectrum the correlation between H-16α and H-17 revealed that the side chain was β oriented. In the piperidine part of the molecule, NOEs were observed between H-22/H-27, H-23/H-24α, H-26β/H-28, and H-25/H-28, which suggested compound **1** possessed 22α-H, 23β-OH, and 25α-CH_3. The key correlations of the isosteroidal part were H-2α/H-3, H-3/H-5, H-5/H-6, H-1α/H-9, H-1β/H-19, and H-19/H-8, which further proved A/B *trans*, B/C *trans*, 3β, and 6β-OH. Therefore, combined with the X-ray analysis (Figure 2), puqienine A was elucidated as (20*R**,22*R**)-*N*-methyl-5α,17β,25α-veratraman-3β,6β,23β-triol (**1**).

Compound **2** was crystallized from acetone as colorless needles and gave a positive reaction to the Dragendorff reagent. The molecular formula, $C_{28}H_{45}NO_3$, was assigned on the basis of the quasimolecular ion peak at *m/z* 444.3478 $[M + H]^+$ in its HRESIMS. The IR spectrum displayed

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Table 1. ^{1}H NMR Data of Compounds **1**−**4**[a] (δ)

	1 (DMSO-d_6)	**2** (DMSOd_6)	**3** ($CDCl_3$)	**4** (pyridine-d_5)
1	1.04 m, 1.37 m	1.32 m, 1.51 m	1.24 m, 1.78 m	0.85 m, 1.37 m
2	1.32 m, 1.65 m	1.21 m, 1.65 m	1.40 m, 1.84 m	1.36 m, 1.85 m
3	3.41 br, m	3.37 br, m	3.56 br, m	3.77 br, m
4	1.49 m, 1.60 m	1.31 m, 2.18 m	1.48 m, 1.90 m	1.52 m, 2.18 m
5	1.03 m	2.29 dd (11.5, 2.6)	2.19 dd (12.5, 2.8)	1.85 dd (12.3, 2.8)
6	3.67 br, m			
7	1.89 m, 1.18 m	2.24 m, 2.30 m	1.94 t (12.7), 2.32 dd (12.7, 4.5)	1.82 t (12.7) 2.16 dd (12.7, 4.6)
8	1.35 m	1.29 m	1.79 m	1.50 m
9	1.10 m	1.82 m	1.22 m	0.95 m
11	1.93 m, 2.21 m	1.94 m, 2.33 m	1.37 m, 1.62 m	0.98 m, 1.27 m
12			1.21 m, 2.03 m	0.94 m, 1.75 m
14	1.62 m	1.85 m	1.22 m	1.90 m
15	0.92 m, 1.67 m	0.89 m, 1.66 m	1.36 m, 1.78 m	1.26 m
16	1.29 m, 1.71 m	1.29 m, 1.69 m	1.09 m, 1.54 m	1.26 m
17	2.30 m	2.30 m	1.26 m	1.02 m
18	1.59 s	1.58 s	0.68 s	0.41 s
19	0.90 s	0.60 s	0.75 s	0.43 s
20	2.29 m	2.27 m	1.51 m	1.71 m
21	0.70 d (6.8)	0.66 d (6.8)	0.90 d (6.8)	0.79 d (6.6)
22	2.78 dd (10.4, 2.6)	2.76 dd (13.0, 2.8)	2.45 m	2.55 m
23	3.80 br, m	3.77 br, m	1.12 m, 1.48 m	1.02 m, 1.45 m
24	1.17 m, 1.86 m	1.14 m, 1.84 m	0.97 m, 1.80 m	0.62 m, 1.53 m
25	2.14 br, m	2.12 br, m	1.43 m	1.44 m
26	2.21 m, 2.29 m	2.18 m, 2.26 m	2.27 m	1.43 m
27	0.75 d (6.6)	0.73 d (6.6)	0.81 d (6.6)	0.58 d (5.2)
28	2.47 s	2.45 s		1.92 s
1′				4.84 d (7.7)
2′				3.82 dd (8.7, 7.7)
3′				4.05 t (8.7)
4′				4.00 t (8.7)
5′				3.78 m
6′				4.19 dd (11.6, 5.5) 4.40 dd (11.6, 1.8)

[a] J values in Hz are in parentheses. Assignments were made using HMQC and HMBC techniques.

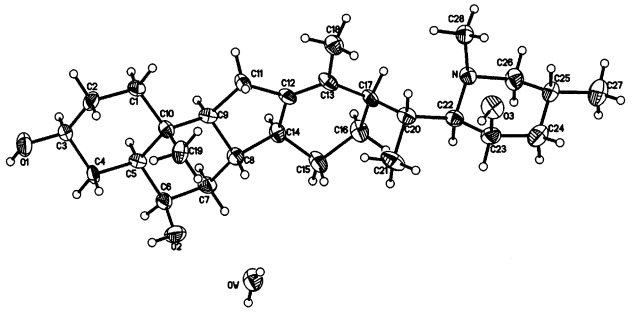

Figure 2. X-ray structure of compound **1** with 30% probability ellipsoids.

absorption bands for hydroxyl (3427 cm^{-1}) and carbonyl (1696 cm^{-1}) groups. The ^{1}H NMR spectrum of **2** (Table 1) indicated the presence of two tertiary methyl signals [δ 0.60 (3H, s, H-19) and 1.58 (3H, s, H-18)], two secondary methyl groups [δ 0.66 (3H, d, J = 6.8 Hz, H-21) and 0.73 (3H, d, J = 6.6 Hz, H-27)], a methyl group located at the nitrogen atom [δ 2.45 (3H, s, H-28)], and two hydrogen signals [δ 3.37 (1H, br, m, H-3α) and 3.77 (1H, br, m, H-23α)]. The ^{13}C NMR spectrum of **2** (Table 2) also showed the presence of a carbonyl carbon [δ 210.5 (C-6)] and two quaternary olefinic carbons [δ 137.5 (C-13) and 127.1 (C-12)]. All the 1D and 2D NMR data were in good accordance with those of compound **1** except for the signals of the A and B rings because of the carbonyl group instead of the hydroxyl group at C-6. Thus, compound **2** was established as (20*R**,22*R**)-*N*-methyl-5α,17β,25α-veratraman-3β,23β-diol-6-one, named puqienine B.

Compound **3** was obtained as colorless needles from acetone and gave a positive reaction to the Dragendorff reagent. The HRESIMS displayed a quasimolecular ion peak at *m/z* 416.3530 [M + H]$^+$, consistent with the molecular formula $C_{27}H_{45}NO_2$. In the EIMS spectrum, the base peak at *m/z* 98 belonged to the characteristic fragment ion of the piperidine side chain of a steroidal alkaloid.[5] The IR spectrum indicated the presence of hydroxyl (3487 cm^{-1}) and carbonyl (1702 cm^{-1}) groups. The ^{1}H NMR spectrum of **3** (Table 1) showed two tertiary signals [δ 0.68 (3H, s, H-18) and 0.75 (3H, s, H-19)], two secondary methyl protons [δ 0.81 (3H, d, J = 6.6 Hz, H-27) and 0.90 (3H, d, J = 6.8 Hz, H-21)], and an oxygenated methine [δ 3.56 (br, m, H-3α)]. The ^{13}C NMR spectrum of **3** (Table 2) also displayed the presence of four methyl carbons at δ 19.5 (C-27), 13.6 (C-21), 13.2 (C-19), and 12.0 (C-18), one carboxyl carbon at δ 210.6 (C-6), and one oxygenated carbon at δ 70.7 (C-3). Comparison of the NMR data of **3** with those of puqietinone[5] showed the absence of an *N*-methyl group, while the remaining signals were in good accordance. Furthermore, the signals of C-22 and C-26 were shifted upfield to δ 59.0 and 55.4 due to the lack of the *N*-CH_3, respectively. Also, the configurations of C-22 and C-25 were deduced to be similar to puqietinone from biogenesis. Thus, compound **3** was tentatively determined as (22*R*,25*S*)-22,26-epiminocholest-3β-ol-6-one, named *N*-demethylpuqietinone.

Compound **4** was crystallized from acetone as colorless needles. The molecular formula, $C_{34}H_{57}NO_7$, was confirmed from a quasimolecular ion peak at *m/z* 592.4203 [M + H]$^+$ by HRESIMS, suggesting that **4** might be an alkaloid glycoside. Acid hydrolysis of **4** afforded puqietinone and glucose. The IR spectrum showed the absorption bands for hydroxyl (3437 cm^{-1}) and carbonyl (1705 cm^{-1}) groups. The ^{1}H NMR spectrum of **4** (Table 1) showed two tertiary

Table 2. ^{13}C NMR Data of Compounds **1**−**4**[a] (δ)

carbon	**1** (DMSO-d_6)		**2** (DMSO-d_6)		**3** (DMSO-d_6)		**4** (pyridine-d_5)	
1	39.1	CH_2	36.8	CH_2	36.8	CH_2	36.5	CH_2
2	31.3	CH_2	30.2	CH_2	30.8	CH_2	29.2	CH_2
3	70.3	CH	69.2	CH	70.7	CH	76.5	CH
4	35.4	CH_2	30.6	CH_2	30.1	CH_2	26.7	CH_2
5	48.1	CH	55.8	CH	56.9	CH	56.1	CH
6	70.6	CH	210.5	C	210.6	C	209.4	C
7	38.7	CH_2	45.4	CH_2	46.7	CH_2	46.5	CH_2
8	41.2	CH	47.0	CH	38.0	CH	37.6	CH
9	52.0	CH	53.4	CH	54.1	CH	53.6	CH
10	35.0	C	38.1	C	40.9	C	40.6	C
11	28.1	CH_2	27.8	CH_2	21.6	CH_2	21.4	CH_2
12	125.9	C	127.1	C	39.7	CH_2	39.6	CH_2
13	138.9	C	137.5	C	43.2	C	43.1	C
14	47.4	CH	47.6	CH	56.7	CH	56.1	CH
15	25.4	CH_2	25.2	CH_2	27.6	CH_2	24.0	CH_2
16	22.7	CH_2	22.5	CH_2	24.0	CH_2	23.9	CH_2
17	36.0	CH	36.0	CH	53.2	CH	53.0	CH
18	17.0	CH_3	17.1	CH_3	12.0	CH_3	11.7	CH_3
19	14.5	CH_3	12.3	CH_3	13.2	CH_3	12.7	CH_3
20	31.3	CH	31.4	CH	40.9	CH	35.2	CH
21	13.0	CH_3	13.0	CH_3	13.6	CH_3	12.4	CH_3
22	63.3	CH	63.4	CH	59.0	CH	65.8	CH
23	65.9	CH	65.9	CH	25.4	CH_2	27.2	CH_2
24	42.6	CH_2	42.7	CH_2	34.0	CH_2	33.6	CH_2
25	18.1	CH	18.1	CH	32.8	CH	31.4	CH
26	63.3	CH_2	63.2	CH_2	55.4	CH_2	66.0	CH_2
27	19.2	CH_3	19.3	CH_3	19.5	CH_3	19.5	CH_3
28	37.2	CH_3	37.2	CH_3			42.7	CH_3
1′							101.9	CH
2′							75.0	CH
3′							78.3	CH
4′							71.6	CH
5′							78.2	CH
6′							62.7	CH_2

[a] Assignments were made using HMQC and HMBC techniques.

signals [δ 0.41 (3H, s, H-18) and 0.43 (3H, s, H-19)], two secondary methyl protons [δ 0.58 (3H, d, J = 5.2 Hz, H-27) and 0.79 (3H, d, J = 6.6 Hz, H-21)], and a methyl group located at the nitrogen atom [δ 1.92 (3H, s, H-28)]. The ^{13}C NMR signals (Table 2) at δ 101.9, 78.3, 78.2, 75.0, 71.6, and 62.7, along with an anomeric proton signal at δ 4.84 (1H, d, J = 7.7 Hz) in the ^{1}H NMR spectrum, confirmed the presence of a glucopyranosyl unit in **4**. In addition, in the ^{13}C NMR spectrum, the chemical shifts for all the carbons of the aglycone part corresponded closely to those of puqietinone except for the C-2, C-3, and C-4 signals, supporting that **4** possessed puqietinone as its aglycone. The connection of the sugar unit and aglycone could be confirmed by HMBC. Thus, in the HMBC the cross-peak between the anomeric proton (H-1′) at δ 4.84 and C-3 at δ 76.5 was observed. Hence, compound **4** was established to be 3-*O*-β-D-glucopyranosylpuqietinone, named puqietinonoside.

All four new steroidal alkaloids, along with puqietione, were evaluated for in vivo antitussive activity on mouse cough model induced with ammonia liquor. These compounds prolonged the latent period and reduced the cough times significantly at dosage levels of 5 and 10 mg/kg, respectively (Table 3).

Furthermore, the compounds were tested for their antitumor activity using A549 human lung carcinoma cell line, BGC-823 human stomach adenocarcinoma cell line, SMMC-7721 human hepatocarcinoma cell line, and HL-60 human promyelocytic leukemia cell line. At concentrations of 1×10^{-7} and 1×10^{-6} mol/L, all the compounds were inactive. Only at a concentration of 1×10^{-5} mol/L did compound **3** show weak activity against the HL-60 cell line (51% inhibition). Compared with a previous in vivo study,[8] puqietione did not show antitumor activity in vitro.

Experimental Section

General Experimental Procedures. The melting points were measured on a X_4 micro-melting point apparatus and were uncorrected. Optical rotations were determined in MeOH on a PE-241 MC digital polarimeter at 20 °C. IR spectra were recorded in KBr disks on a Nicolet Impact 410 spectrophotometer. NMR spectra were obtained in DMSO, $CDCl_3$, or pyridine-d_5 containing TMS as an internal standard on a Bruker Am-300, 500 NMR spectrometer. HRESIMS and EIMS were recorded on a Bruker APEXIII 7.0 TESLA FTMS and a JEOL DX-300 UGZAB-HS spectrometer, respectively. Column chromatography was performed with silica gel (200−300 mesh, Qingdao Marine Chemical Factory, China). TLC was conducted on silica gel G plates (0.25 mm thick, Qingdao Marine Chemical Factory, China). X-ray crystallographic data collection for compound **1** was carried out on a Nonius CAD4/PC single-crystal X-ray diffractometer.

Plant Material. The bulbs of *F. puqiensis* were collected from Puqi, Hubei Province, People's Republic of China, in May 2000, and authenticated by one of the authors (P.L.). A voucher specimen (No. 255830) was deposited at the Herbarium of the Department of Pharmacognosy, China Pharmaceutical University.

Extraction and Isolation. Dried ground bulbs (10 kg) were extracted with 70% EtOH by soaking repeatedly (20 L × 3). The concentrated extract was dissolved in 2% HCl (pH 3.5) and partitioned with ether. The pH of the aqueous solution was readjusted with NH_4OH to 9.2 and extracted with $CHCl_3$. The $CHCl_3$ extract (crude alkaloid, 50 g) was chromatographed over Si gel using $CHCl_3$−CH_3OH of increasing polarity as eluent to obtain five fractions, A−E. Fractions B−D were further separated by repeated column chromatography and eluted with petroleum ether−Me_2CO−diethylamine (88:12:2, 80:20:2, and 75:25:2, respectively). Fraction B afforded **2** (150 mg) and puqietinone (500 mg), fraction C yielded **3** (20 mg), and fraction D gave **1** (180 mg) and **4** (200 mg).

Puqienine A (1): colorless needles (from Me_2CO); mp 260−261 °C; $[\alpha]_D^{20}$ −31.1° (*c* 0.2, MeOH); IR (KBr) ν_{max} 3414, 2909, 1040 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz), see Table 1; ^{13}C

Table 3. Antitussive Effect of Compounds **1**−**4** and Puqietione on Mice Cough Induced by Ammonia Liquor (mean ± SD, n = 10)

	high dosage (10 mg/kg)		low dosage (5 mg/kg)	
	latent period (second)	cough times (in 3 min)	latent period (second)	cough times (in 3 min)
codeine[a]	72.9 ± 35.0***	5 ± 4***		
0.5% CMC-Na[b]	2.4 ± 0.9	39 ± 17		
1	30.7 ± 21.9***	14 ± 8***	15.7 ± 12.3**	18 ± 7**
2	44.2 ± 15.3***	10 ± 5***	28.5 ± 14.0***	12 ± 4***
3	19.9 ± 8.0***	18 ± 5**	9.3 ± 5.3***	23 ± 5*
4	33.8 ± 20.9***	13 ± 5***	19.9 ± 17.0***	17 ± 6**
puqietione	10.4 ± 4.8***	11 ± 6***	10.6 ± 6.0***	17 ± 8**

[a] Positive control. [b] Negative control. *p < 0.05, ** p < 0.01, *** p < 0.001 vs CMC-Na.

NMR (DMSO-d_6, 125 MHz), see Table 2; EIMS *m/z* 445 $[M]^+$ (3), 195 (4), 155 (8), 128 (100), 105 (4), 84 (9); HRESIMS *m/z* 446.3625 $[M + H]^+$ (calcd for $C_{28}H_{48}NO_3$, 446.3629).

X-ray Crystallography of 1.[11] A crystal, 0.4 × 0.3 × 0.2 mm, was obtained from Me_2CO. Cell parameters: $a = 11.213(2)$ Å, $b = 14.543(3)$ Å, $c = 16.498(3)$ Å, $V = 2690.3(9)$ Å^3, crystal system orthorhombic, space group $P2_12_12_1$, $Z = 4$, $D_{calc} = 1.145$ Mg/m^3, $\lambda = 0.71073$ Å, μ(Mo Kα) = 0.075 mm^{-1}, $F(000) = 1024$, $T = 293(2)$ K. Data collection yielded 2957 reflections; 2957 were independent. All non-hydrogen and water-hydrogen atoms were identified with difference Fourier maps, and other hydrogen atoms were yielded theoretically.[12] Full-matrix least-squares refinement on F^2 led to a final R[I > 2σ(I)], R(all), and GOF of 0.0665, 0.2021, and 0.996. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.244 and −0.254 e Å^{-3}, respectively. It was observed that the unit cell was composed of four molecules and one water molecule with inter-hydrogen bonds between 3-OH(1)/H_2O, 6-OH(2)/H_2O, 23-OH(3)/H_2O, and N(4)/H_2O ["(1), (2), (3), (4)" represent four different molecules, respectively].

Puqienine B (2): colorless needles (from Me_2CO); mp 226−227 °C; $[\alpha]_D^{20}$ −31.2° (*c* 0.1, MeOH); IR (KBr) ν_{max} 3427, 2912, 1696, 1053 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz), see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz), see Table 2; EIMS *m/z* 443 $[M]^+$ (2), 155 (2), 128 (100), 105 (2), 84 (3); HRESIMS *m/z* 444.3478 $[M + H]^+$ (calcd for $C_{28}H_{46}NO_3$, 444.3472).

***N*-Demethylpuqietinone (3):** colorless needles (from Me_2CO); mp 285−286 °C; $[\alpha]_D^{20}$ −10.4° (*c* 0.1, MeOH); IR (KBr) ν_{max} 3487, 2946, 1702 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; EIMS *m/z* 121 (2), 112 (3), 98 (100), 55(9); HRESIMS *m/z* 416.3530 $[M + H]^+$ (calcd for $C_{27}H_{46}NO_2$, 416.3523).

Puqietinonoside (4): colorless needles (from Me_2CO); mp 327−328 °C; $[\alpha]_D^{20}$ −22.7° (*c* 0.1, MeOH); IR (KBr) ν_{max} 3437, 2943, 1705, 1024 cm^{-1}; ^{1}H NMR (pyridine-d_5, 300 MHz), see Table 1; ^{13}C NMR (pyridine-d_5, 75 MHz), see Table 2; HRESIMS *m/z* 592.4203 $[M + H]^+$ (calcd for $C_{34}H_{58}NO_7$, 592.4208).

Acid Hydrolysis of 4. A solution of **4** (30 mg) in 2% H_2SO_4 (5 mL) was heated in a boiling water bath for 5 h. After cooling, the mixture was neutralized with 10% aqueous NH_3, and extracted with $CHCl_3$. The $CHCl_3$ layer was evaporated and chromatographed on a Si gel column with petroleum ether−Me_2CO−diethylamine (85:15:2) to give puqietinone (10 mg), which was identified by NMR spectra and compared with an authentic sample. The H_2O layer was detected by TLC (n-BuOH−Me_2CO−H_2O, 4:5:1) to reveal the presence of glucose.

Antitussive Evaluation. The in vivo antitussive activity was investigated on a classical mouse cough model induced by ammonia liquor.[13] Briefly, mice were administered orally with alkaloids once a day for three consecutive days. On the third day, 2 h after the alkaloids were administered, mice were placed in a special glass chamber and exposed for 5 s to a 28% NH_4OH aerosol which was produced through a nebulizer by compressed air at a pressure of about 400 mmHg. Then the latent period (the seconds from the beginning of spray to induced cough) and the times of cough within 3 min (from the beginning of spray) were observed. Codeine (10 mg/kg) was used as positive control, and the vehicle 0.5% sodium carboxymethyl cellulose (0.2 mL/mouse) as negative control.

Antitumor Evaluation. Inhibition of human cancer cell growth was assessed using the MTT assay as previously described.[14,15] The inhibition percent of the A549, BGC-823, SMMC-7721, or HL-60 at three concentrations of 1×10^{-7}, 1×10^{-6}, and 1×10^{-5} mol/L of each test compound was determined and compared to untreated cells. Mitomycin C was used as a positive control for A549, SMMC-7721, and HL-60, and cisplatinum for BGC-823.

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Supporting Information Available: Tables of X-ray crystallographic data for compound **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) The Committee of China Materia Medica. *China Materia Medica*; Shanghai Scientific and Technological Press: Shanghai, 1999; Vol. 8, pp 7169−7174.
(2) Li, P.; Ji, H.; Xu, G. J.; Xu, L. S. *J. China Pharm. Univ.* **1993**, *24*, 360−362.
(3) Yao, L. N.; Sun, H. Q.; Jiang. Z.; Wei, Y. D.; Lu, C. B. *Acta Univ. Med. Tongji.* **1993**, *22*, 47−49.
(4) Yu, G. D.; Li, P.; Xu, G.. J.; Lu, Y. Q. *J. Nanjing Coll. Pharm.* **1985**, *16*, 25−32.
(5) Li, P.; Li, X. G.; Xu, G. J.; Kaneko, K. *J. China Pharm. Univ.* **1990**, *21*, 198−200.
(6) Lin, G.; Ho, Y.-P.; Li, P.; Li, X. G. *J. Nat. Prod.* **1995**, *58*, 1662−1667.
(7) Ji, H.; Li, P.; Yao, L.; Zhou, S.; Xu, G. J. *J. China Pharm. Univ.* **1993**, *24*, 95−97.
(8) Li, P.; Wang, Y. X.; Xu, G. J.; Xu, L. S. *J. Chin Pharm. Sci.* **1995**, *4*, 217−219.
(9) Xu, D. M.; Xu, M. L.; Wang, S. Q.; Hung, E. X.; Wen, X. G. *J. Nat. Prod.* **1990**, *53*, 549−552.
(10) Li, Q. H.; Wu, Z. H.; Zhang, L. L.; Shao, L. *Acta Pharm. Sin.* **1988**, *23*, 415−421.
(11) Crystallographic data (excluding structure factors) for compound **1** have been deposited with the Cambridge Crystallographic Data Centre and allocated the deposition number CCDC 242451. 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).
(12) Sheldrick, G. *SHELXTL*, version 5.1; Bruker Analytical X-ray Systems: Madison, WI, 1997. SHELXS (a structure solution program via Patterson or direct methods) and SHELXL (structure refinement software).
(13) Xu, S. Y.; Bian, R. L.; Chen, X. *Pharmacological Experiment Methodology*; People's Medical Publishing House: Beijing, 1991; p 1167.
(14) Ekwall, B.; Johanson, A. *Toxicol. Lett.* **1980**, *5*, 299−307.
(15) Verlag: C. W.; Ekwall, B. *Toxicol. Lett.* **1980**, *5*, 309−317.

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