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Preparation and antitussive, expectorant, and antiasthmatic activities of verticinone's derivatives

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Preparation and antitussive, expectorant, and antiasthmatic activities of verticinone's derivatives

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To prepare verticinone derivatives with significant antitussive, expectorant, and antiasthmatic activities, the compounds 3β-acetylverticinone (**1**), 3-ketoverticinone (**2**), 3β-benzoylverticinone (**3**), 3β-propionylverticinone (**4**), 3β-butyrylverticinone (**5**), and 3β-butoxycarbonylverticinone (**6**) have been prepared. All of these are new compounds. Among them, **1**–**6** exhibited potent antitussive and expectorant activities; **1** and **3**–**6** displayed various antiasthmatic activities. The antitussive activity of **1**–**6**, the expectorant activity of **1**–**2** and **4**–**6**, and the antiasthmatic activity of **1** are higher than those of verticinone. The results demonstrated that **1** had dominant biological activities, suggesting that it would be a potential antitussive, expectorant, and antiasthmatic agent.

Keywords: verticinone; 3β-acetylverticinone derivative; antitussive; expectorant; antiasthmatic

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1. Introduction

Fritillaria hupehensis Hsiao and K.C. Hsia [1], a well-known medicinal plant grown in the northwest of Hubei province, China, is used as an antitussive by local folks. It has been recorded in the Pharmacopoeia of the People's Republic of China, named Hubeibeimu [2]. Phytochemical investigation has led to the isolation of 11 C-nor-D-homo steroidal alkaloids and six non-basic chemical constituents [3,4]. Verticinone is the main alkaloid from the bulbs of *F. hupehensis* Hsiao and K.C. Hsia, whose content is about 0.3% of the crude herb. Pharmacological investigations indicated that verticinone had significant antitussive, expectorant, and antiasthmatic activities [5]. In this paper, verticinone was regarded as a leading compound and its derivatives were prepared, whose antitussive, expectorant, and antiasthmatic activities of the derivatives were investigated.

2. Results and discussion

Derivative **1**, obtained as colorless needles from acetone, had a quasi-molecular ion peak at *m/z* 472 $[M + H]^+$ in the positive FAB-MS, which was 42 amu more than that of verticinone. Its ^1H and ^{13}C NMR spectra were similar to those of verticinone, except for the additional methyl signal at δ_H 1.98 (3H, s) and δ_C 21.2 (CH_3), and the additional carboxylic signal at δ_C 170.4. The HMBC spectrum showed the cross-peaks between the methyl protons (δ_H 1.98) and the carboxylic carbon (δ_C 170.4), indicating the presence of

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an acetoxyl group in **1**. The chemical shift of C-2 [δ_C 26.5 (CH_2)] and C-4 [δ_C 26.1 (CH_2)] of **1** is upfield of about 4 ppm compared with the C-2 [δ_C 30.5 (CH_2)] and C-4 [δ_C 30.1 (CH_2)] of verticinone, and the chemical shift of C-3 [δ_C 72.9 (CH)] of **1** was downfield of about 2 ppm compared with C-3 [δ_C 70.9 (CH)] of verticinone. The acylation shift indicated that acetoxyl group is connected with the hydroxyl of C-3. Long-range coupling was also observed from H-3 [δ_H 4.64 (1H, m)] to the carboxylic carbon (δ_C 170.4). Thus, the acetoxyl group was attached to the C-3 of verticinone (Figure 1).

Alkaline hydrolysis of **1** with 5% NaOH–MeOH yielded compound **1a**, whose spectral characteristics were identical to those of verticinone. This was further confirmed by the melting point of mixture and TLC analysis with verticinone. Thus, the structure of compound **1** was determined as (20*S*,25*S*)-5α,14α-cevanine-3β-acetoxyl-20β-hydroxy-6-one.

Derivative **2**, obtained as colorless needles from acetone, exhibited its quasi-molecular ion peak at *m/z* 428 $[M + H]^+$, which was 2 amu less than that of verticinone. The ^1H NMR spectrum revealed the presence of three methyl groups, among which H-19 at δ_H 0.91 (3H, s) is downfield shifted due to the existence of C-3 carbonyl. The ^{13}C NMR spectrum showed two ketone carbonyls. The chemical shift of C-2 [δ_C 38.2 (CH_2)] and C-4 [δ_C 37.3 (CH_2)] of **2** were downfield compared with C-2 [δ_C 30.5 (CH_2)] and C-4 [δ_C 30.1 (CH_2)] of verticinone. Thus, one of the ketone carbonyls was located at C-3. All pieces of evidence illuminated the structure of **2** as (20*S*,25*S*)-5α,14α-cevanine-20β-hydroxy-3,6-dione.

Derivative **3** had a quasi-molecular ion peak at *m/z* 534 $[M + H]^+$, which was 104 amu more than that of verticinone. Its ^1H and ^{13}C NMR spectra were similar to those of verticinone, except for the additional carboxylic signal at δ_C 160.0 and the additional monosubstituted benzene signals at δ_H 8.02 (2H, d, $J = 7.2$ Hz), 7.55 (1H, m), and 7.42 (2H, m) in the ^1H NMR spectrum and δ_C 130.7 (s), 129.5 (d), 128.2 (d), and 132.7 (d) in the ^{13}C NMR spectrum. The HMBC cross-peaks of H-3/C-1′ and H-3′/C-1′ and the acylation shift of C-2, C-4, and C-3 of **3** indicated that the benzoyl group is connected with the hydroxyl of C-3. Thus, the structure of compound **3** was determined as (20*S*,25*S*)-5α,14α-cevanine-3β-benzoyl-20β-hydroxy-6-one.

Comparison of the ^1H and ^{13}C NMR spectra of derivative **4** with **1**, combined with the quasi-molecular ion peak at *m/z* 486 $[M + H]^+$, suggested that they are similar except that **4** possesses one more methylene group. The HMBC cross-peaks of H-3/C-1′, H-2′/C-1′, and H-3′/C-1′ and the acylation shift of C-2, C-4, and C-3 of **4** indicated that the propionyl group is connected with the hydroxyl of C-3. Thus, the structure of compound **4** was determined as (20*S*,25*S*)-5α,14α-cevanine-3β-propionyl-20β-hydroxy-6-one.

Derivative **5** differed from **4** in the presence of one more methylene. The HMBC cross-peaks of H-3/C-1′, H-2′/C-1′, and

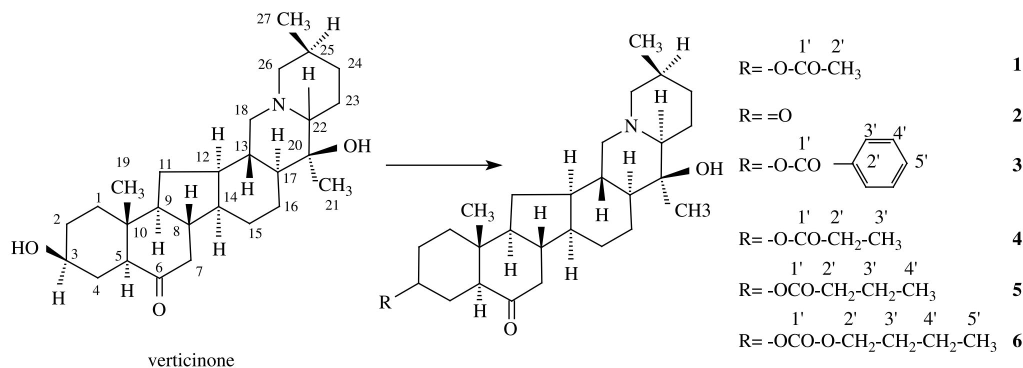

Figure 1. Structures of verticinone and **1**–**6**.

H-3′/C-1′ and the acylation shifts of C-2, C-4, and C-3 of **5** indicated that the butyryl group is connected with the hydroxyl of C-3. Thus, the structure of compound **5** was determined as (20*S*,25*S*)-5α,14α-cevanine-3β-butyryl-20β-hydroxy-6-one.

Derivative **6** had a quasi-molecular ion peak at *m/z* 529 $[M + H]^+$, which was 30 amu more than that of **5**. Its ^{1}H and ^{13}C NMR spectra were similar to those of **5**, except for the additional oxygenated methylene signal at δ_C 67.6. The HMBC cross-peaks of H-3/C-1′ and H-2′/C-1′ and the acylation shifts of C-2, C-4, and C-3 of **6** indicated that the butoxycarbonyl group is connected with the hydroxyl of C-3. Combined with the analysis of its acylation reactant and reaction condition, the structure of compound **6** was determined as (20*S*,25*S*)-5α,14α-cevanine-3β-butoxycarbonyl-20β-hydroxy-6-one.

The antitussive, expectorant, and antiasthmatic activities of the derivatives were studied according to the reported methods [5]. Compounds **1–6** exhibited potent antitussive and expectorant activities; **1** and **3–6** displayed various antiasthmatic activities. The antitussive activity of **1–6**, the expectorant activity of **1–2** and **4–6**, and the antiasthmatic activity of **1** are higher than those of verticinone (as shown in Tables 2–4).

The acute dosing toxicities in mice of verticinone and **1–6** were also examined, and the intraperitoneal LD_{50} values were 6.86, 14.39, 9.98, 23.21, 14.47, 18.41, and 16.49 mg kg^{-1}, respectively. The results indicated that **1** had more significant biological activity and lower toxicity than those of verticinone, suggesting that it would be a potential antitussive, expectorant and antiasthmatic agent.

cAMP plays an important role in the antiasthmatic mechanism. The content of cAMP in cell depends on the dynamic balance of AC catalyzing ATP to change into cAMP, and PDE catalyzing cAMP to change into 5′-AMP in cell [6]. The experiment showed that these derivatives had little effect on AC, but **1** and **3–6** had significant inhibiting activity on PDE. Thus, **1** and **3–6** could increase the content of cAMP in cell, which were parallel to the fact that **1** and **3–6** had significant antiasthmatic activities (as shown in Table 5).

3. Experimental

3.1 General experimental procedures

Melting points (uncorrected) were measured on XT-4 micromelting point apparatus. Optical rotations were obtained using a PerkinElmer model-341 polarimeter in MeOH. IR spectra were measured on Shimadzu IR-460 spectrophotometer. Mass spectra were determined on a VG Auto spec-300 mass spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM-400D NMR spectrometer, with TMS as an internal standard. Silica gel H (160–200 mesh, Qingdao Marine Chemical Group Co., Qingdao, China) was used for column chromatography and SiO_2 GF_{254} (Qingdao Marine Chemical Group Co.) for TLC. Spots on the plate were observed and visualized by spraying with Dragendorff's reagent.

3.2 Plant material

The bulbs of *F. hupehensis* were collected from Enshi County, Hubei province, China, April 2004, and identified by Professor Jizhou Wu (Faculty of Pharmaceutical Sciences, Tongji Medical College). A voucher specimen (JZ-0401) has been deposited in the Herbarium of Faculty of Pharmaceutical Sciences, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China.

3.3 Isolation of verticinone

The powdered bulbs (100 kg) of *F. hupehensis* Hsiao and K.C. Hsia were extracted with 95% EtOH, and the extract was dissolved in 2% HCl. The acidic solution was basified with ammonia up to pH > 11, and the precipitate was dissolved in chloroform to produce the total alkaloids yield of 1120 g, after the removal of chloroform. The total alkaloids were further fractionated by repeated column chromatography over silica

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gel to obtain verticinone (315 g), using petroleum–Me_2CO–Et_2NH as a solvent.

3.4 Preparation of 1

Verticinone (100 mg, 0.23 mmol) was dissolved in anhydrous pyridine (5 ml); acetic anhydride (5 ml) was added and the mixture stirred for 24 h at room temperature followed by TLC analysis (petroleum–Me_2CO–Et_2NH, 7:3:1). Aqueous copper sulfate solution (3 ml) was added to destroy the pyridine, and the product was recovered with ethyl acetate, which was washed with aqueous sodium bicarbonate solution, brined (60% w/v), and dried over anhydrous sodium sulfate. Removal of the solvent on a rotavapor afforded the derivative **1** (75 mg, yield 68%) as colorless needles from acetone; mp 179.5–180.5°C; $[\alpha]_D^{20}$ −73.5 (*c* 0.5, MeOH); ^{1}H NMR: δ_H 1.98 (3H, s, H-1′), 0.74 (3H, s, H-19), 0.98 (3H, s, H-21), 1.04 (3H, d, $J = 7.0$ Hz, H-27), 4.64 (1H, m, H-3); ^{13}C NMR spectral data: see Table 1; (+)FAB-MS *m/z*: 472 [M + H]$^+$.

3.5 Preparation of 2

Verticinone (100 mg, 0.23 mmol) was dissolved in dichloromethane (10 ml). PCC (200 mg) was added to the solution in portions with stirring. The mixture was stirred for 48 h at room temperature followed by TLC (petroleum–Me_2CO–Et_2NH, 7:3:1). The removal of the solvent on a rotary evaporator afforded **2** (53 mg, yield 53%) as colorless needles from acetone [7]; mp 177.5–178.5°C; $[\alpha]_D^{20}$ +11.3 (*c* 0.5, MeOH); ^{1}H NMR: δ_H 0.91 (3H, s, H-19), 0.99 (3H, s, H-21), 1.02 (3H, d, $J = 7.0$ Hz, H-27); ^{13}C NMR spectral data: see Table 1; (+)FAB-MS *m/z*: 428 [M + H]$^+$.

3.6 Preparation of 3

Verticinone (100 mg, 0.23 mmol) was dissolved in anhydrous pyridine (5 ml), and the benzoyl chloride (5 ml) was added. The mixture was treated by the same method as described for the preparation of **1** [8]. Compound **3** (82 mg, yield 66%) was obtained as colorless needles from acetone; mp 133.5–134.5°C; $[\alpha]_D^{20}$ −77.3 (*c* 0.5, MeOH); ^{1}H NMR: δ_H 0.83 (3H, s, H-19), 1.02 (3H, s, H-21), 1.08 (3H, d, $J = 7.0$ Hz, H-27), 4.92 (1H, m, H-3), 8.02 (2H, d, $J = 7.2$ Hz, H-3′ and H-7′), 7.55 (1H, m, H-5′), 7.42 (2H, m, H-4′ and H-6′); ^{13}C NMR spectral data: see Table 1; (+)FAB-MS *m/z*: 534 [M + H]$^+$.

3.7 Preparation of 4

Verticinone (100 mg, 0.23 mmol) was dissolved in anhydrous pyridine (5 ml), and then propionic anhydride (5 ml) was added. The mixture was treated by the same method as described for the preparation of **1** [7]. Compound **4** (78 mg, yield 68%) was obtained as colorless cubic crystals from acetone; mp 172–173°C; $[\alpha]_D^{20}$ −44.6 (*c* 0.5, MeOH); ^{1}H NMR: δ_H 0.74 (3H, s, H-19), 0.98 (3H, s, H-21), 1.03 (3H, d, $J = 7.0$ Hz, H-27), 4.65 (1H, m, H-3), 1.08 (3H, t, $J = 6.0$ Hz, H-3′), 2.25 (2H, q, $J = 6.0$ Hz, H-2′); ^{13}C NMR spectra data: see Table 1; (+)FAB-MS *m/z*: 486 [M + H]$^+$.

3.8 Preparation of 5

Verticinone (100 mg, 0.23 mmol) was dissolved in anhydrous pyridine (5 ml), and then butyric anhydride (5 ml) was added, and the mixture was treated by the same method as described for the preparation of **1** [7]. Compound **5** (71 mg, yield 60%) was obtained as colorless arris crystals from acetone; mp 128–129°C; $[\alpha]_D^{20}$ −55.7 (*c* 0.5, MeOH); ^{1}H NMR: δ_H 0.75 (3H, s, H-19), 0.99 (3H, s, H-21), 1.04 (3H, d, $J = 7.0$ Hz, H-27), 4.66 (1H, m, 3-H), 0.91 (3H, t, $J = 5.8$ Hz, H-4′), 2.22 (2H, t, H-2′); ^{13}C NMR spectral data: see Table 1; (+)FAB-MS *m/z*: 500 [M + H]$^+$.

3.9 Preparation of 6

Verticinone (100 mg, 0.23 mmol) was dissolved in anhydrous pyridine (5 ml). Butyrylchloro carbonate (5 ml) was added, and the mixture was treated by the same method as described for the preparation of **1** [7]. Compound **6** (71 mg, yield 60%) was obtained as colorless needles from acetone; mp 66–68°C; $[\alpha]_D^{20}$ −92.6 (*c* 0.5, MeOH);

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Table 1. ^{13}C NMR spectral data for verticinone and **1–6** ($CDCl_3$, 100 MHz).

No.	Verticinone	**1**	**2**	**3**	**4**	**5**	**6**
1	37.1 (t)	36.7 (t)	37.4 (t)	36.8 (t)	36.7 (t)	36.7 (t)	37.0 (t)
2	30.5 (t)	26.5 (t)	38.2 (t)	26.8 (t)	26.6 (t)	26.6 (t)	26.6 (t)
3	70.9 (d)	72.9 (d)	209.1 (s)	73.6 (d)	72.7 (d)	72.6 (d)	76.8 (d)
4	30.1 (t)	26.1 (t)	37.3 (t)	26.4 (t)	26.2 (t)	26.2 (t)	26.1 (t)
5	56.5 (d)	56.3 (d)	56.0 (d)	56.4 (d)	56.3 (d)	56.3 (d)	56.3 (d)
6	211.0 (s)	210.2 (s)	210.8 (s)	210.1 (s)	210.3 (s)	210.5 (s)	209.8 (s)
7	46.0 (t)	45.9 (t)	45.7 (t)	46.0 (t)	45.9 (t)	45.9 (t)	45.9 (t)
8	42.1 (d)	42.0 (d)	42.1 (d)	42.2 (d)	42.0 (d)	41.9 (d)	42.1 (d)
9	56.7 (d)	56.3 (d)	57.1 (d)	56.5 (d)	56.3 (d)	56.3 (d)	56.3 (d)
10	38.4 (s)	38.2 (s)	38.5 (s)	38.4 (s)	38.2 (s)	38.3 (s)	38.3 (s)
11	29.4 (t)	29.0 (t)	29.1 (t)	29.1 (t)	29.0 (t)	29.0 (t)	29.1 (t)
12	41.1 (d)	40.8 (d)	40.8 (d)	41.0 (d)	40.8 (d)	40.8 (d)	41.0 (d)
13	39.3 (d)	39.2 (d)	39.2 (d)	39.3 (d)	39.2 (d)	39.3 (d)	39.2 (d)
14	43.5 (d)	43.9 (d)	43.9 (d)	44.0 (d)	43.9 (d)	43.9 (d)	43.9 (d)
15	24.7 (t)	24.5 (t)	24.6 (t)	24.6 (t)	24.5 (t)	24.5 (t)	24.6 (t)
16	20.6 (t)	20.5 (t)	20.5 (t)	20.6 (t)	20.5 (t)	20.5 (t)	20.6 (t)
17	48.8 (d)	48.7 (d)	48.7 (d)	48.8 (d)	48.7 (d)	48.7 (d)	48.7 (d)
18	61.8 (t)	62.2 (t)	62.2 (t)	62.3 (t)	62.2 (t)	62.2 (t)	62.3 (t)
19	12.8 (q)	12.6 (q)	12.1 (q)	12.8 (q)	12.6 (q)	12.6 (q)	12.9 (q)
20	71.0 (s)	70.8 (s)	70.9 (s)	70.7 (s)	70.8 (s)	70.8 (s)	70.4 (s)
21	20.4 (q)	20.2 (q)	20.4 (q)	20.3 (q)	20.2 (q)	20.2 (q)	20.3 (q)
22	70.3 (d)	70.2 (d)	70.3 (d)	70.3 (d)	70.2 (d)	70.2 (d)	70.3 (d)
23	19.1 (t)	19.0 (t)	19.0 (t)	19.0 (t)	19.0 (t)	19.0 (t)	19.0 (t)
24	29.2 (t)	29.3 (t)	29.3 (t)	29.4 (t)	29.3 (t)	29.3 (t)	29.3 (t)
25	27.7 (d)	27.6 (d)	27.6 (d)	27.6 (d)	27.6 (d)	27.6 (d)	27.6 (d)
26	62.3 (t)	61.7 (t)	61.7 (t)	61.8 (t)	61.7 (t)	61.7 (t)	61.8 (t)
27	17.3 (q)	17.1 (q)	17.2 (q)	17.2 (q)	17.1 (q)	17.2 (q)	17.2 (q)
1′		170.4 (s)		160.0 (s)	173.8 (s)	173.1 (s)	154.7 (s)
2′		21.2 (q)		130.7 (s)	27.8 (t)	36.5 (t)	67.6 (t)
3′				129.5 (d)	9.1 (q)	18.5 (t)	19.0 (t)
4′				128.2 (d)		13.6 (q)	30.7 (t)
5′				132.7 (d)			18.9 (q)

^{1}H NMR: δ_H 0.70 (3H, s, H-19), 0.95 (3H, s, H-21), 1.00 (3H, d, $J = 7.0$ Hz, H-27), 4.46 (1H, m, H-3), 0.87 (3H, t, $J = 7.4$ Hz, H-5′), 4.04(2H, t, $J = 5.0$ Hz, H-2′); ^{13}C NMR spectral data: see Table 1; (+)FAB-MS *m/z*: 530 $[M + H]^+$.

3.10 Activity assay

3.10.1 Antitussive evaluation

Kunming mice (half of each sex, Experimental Animal Center, Tongji Medical College, Huazhong University of Science and Technology) weighing 18–22 g were used [5]. The animals were housed in groups of 10 per cage under 12 h light–dark cycles with food and water available continuously. Animal studies were conducted according to the Institute Animal Ethics Committee regulations approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals. The animals were randomly divided into 9 groups with 10 animals in each and received different treatments, as shown in Table 2, by intraperitoneal injections. One hour later, every animal was put into an inverted 500 ml beaker with a cotton ball containing 0.2 ml ammonia water. The cough latency period and cough number during 3 min were recorded by a trained observer.

3.10.2 Expectorant evaluation

The Kunming mice (half of each sex) were randomly divided into nine groups with 10 animals in each and received different

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Table 2. The antitussive activity of verticinone and **1**–**6** ($n = 10$).

Group	Dosage (mg/kg)	Cough latency (s)	Cough times (in 3 min)
Negative control	0	46.1 ± 26.8	18.0 ± 9.8
Positive control	30	82.7 ± 43.0***	7.7 ± 4.9***
Verticinone	3	50.6 ± 14.7	14.8 ± 8.0
1	3	92.8 ± 43.0***†††	8.5 ± 7.0***†††
	1	83.3 ± 38.5***†††	8.8 ± 6.7***†††
2	3	62.4 ± 46.8*	8.4 ± 4.7***†††
3	3	57.1 ± 41.0	10.6 ± 6.0***††
4	3	25.1 ± 12.1	7.6 ± 3.8***†††
5	3	44.7 ± 19.8	10.9 ± 5.3***†
6	3	71.2 ± 41.2**††	12.0 ± 10.2**

Values are mean ± SD. Positive control, codeine phosphate; negative control, distilled water with no sample. Compared with the negative: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Compared with the verticinone: †$p < 0.05$, ††$p < 0.01$, †††$p < 0.001$.

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treatments, as shown in Table 3, by intraperitoneal injections [5]. At the end of half an hour after the administration, 0.5 ml of 0.5% phenol red solution was administrated to every animal. Thirty minutes later, the animals were killed and the windpipe between the thyroid cartilage and the windpipe crotch was separated, which was put into the tube with 2.0 ml of 0.5% sodium bicarbonate. The lavage fluid was centrifuged at 4000 rpm for 20 min at 4°C. Then the absorbability was assayed at 433 nm with WFZ800-D3B ultraviolet spectrophotometer.

Table 3. The expectorant activity of verticinone and **1**–**6** ($n = 10$).

Group	Dosage (mg/kg)	Absorption
Negative control	0	0.0852 ± 0.0338
Positive control	30	0.1874 ± 0.0922***
Verticinone	3	0.0964 ± 0.0293
1	3	0.1502 ± 0.0509***†††
2	3	0.1268 ± 0.0693**††
3	3	0.1077 ± 0.0501*
4	3	0.2145 ± 0.0613***†††
5	3	0.1361 ± 0.0296***†††
6	3	0.1187 ± 0.0587**†

Values are mean ± SD. Positive control, guaiamar; negative control, distilled water with no sample. Compared with the negative: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Compared with verticinone: †$p < 0.05$, ††$p < 0.01$, †††$p < 0.001$.

3.10.3 *Antiasthmatic evaluation*

Dunkin–Hartley guinea pigs (half of each sex, Experimental Animal Center, Tongji Medical College, Huazhong University of Science and Technology), weighing 200–250 g (juveniles) were used [5]. The animals were housed in groups of eight per cage under a 12 h light–dark cycle with food and water available continuously. The animals were randomly divided into nine groups with eight animals in each and received different treatments, as

Table 4. The antiasthmatic activity of verticinone and **1**–**6** ($n = 8$).

Group	Dosage (mg/kg)	The convulsion latency period difference of before and after administration (s)
Negative control	0	− 5.6 ± 7.5
Positive control	125	74.8 ± 23.0***
Verticinone	3	18.5 ± 9.7***
1	3	45.6 ± 21.2***†††
2	3	− 3.2 ± 2.9
3	3	7.0 ± 8.4***
4	3	3.6 ± 10.4**
5	3	12.0 ± 8.6***
6	3	8.8 ± 13.5***

Values are mean ± SD. Positive control, aminophylline injection; negative control, distilled water with no sample. Compared with the negative: **$p < 0.01$, ***$p < 0.001$. Compared with the verticinone: †$p < 0.05$, ††$p < 0.01$, †††$p < 0.001$.

Table 5. Effect of verticinone's derivatives on AC and PDE of guinea pig's bronchus smooth muscle ($n = 8$).

Group	Dosage (mg/kg)	PDE (nmol/min per milligram protein)	AC (nmol/min per milligram protein)
Negative control	0	5.7393 ± 2.0412	3.7763 ± 2.0288
Verticinone	3	4.5806 ± 1.6789**	3.6153 ± 2.3975
1	3	4.0370 ± 2.7470**	3.2114 ± 1.0886
2	3	5.0450 ± 2.0790	3.3956 ± 2.0395
3	3	4.5540 ± 2.3878*	3.5071 ± 1.7974
4	3	4.7723 ± 2.2573*	3.6370 ± 1.9872
5	3	2.7647 ± 2.9482***††	3.3374 ± 2.8389
6	3	4.2387 ± 2.0322**	3.6925 ± 3.6322

Values are mean ± SD. Negative control, distilled water with no sample. Compared with negative control: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. Compared with the verticinone: $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$.

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shown in Table 4, by intraperitoneal injections. The guinea pigs were placed individually in a transparent Perspex chamber (dimensions, 16 cm × 16 cm × 16 cm) and exposed to a nebulized balanced mixture of 2% acetylcholine chloride and 0.1% histamine by a constant pressure airflow of 53 kPa (400 mmHg). The animals were continuously watched by a trained observer and convulsion latency period and convulsion number of the animals during 6 min were recorded.

3.10.4 Effects on PDE and AC

Dunkin–Hartley guinea pigs used in antiasthmatic evaluation were killed immediately [6]. The tracheal tissue below the porta pulmonis was separated for homogenization with 0.32 M saccharobiose solution in the ratio of 1:10. The protein concentration was measured by the BCA method. The homogenate was centrifuged at 10,000 rpm for 15 min, and the supernatants and the sediments were separated for phosphodiesterase (PDE) and adenyl cyclase (AC) assay. Two hundred microliters of the supernatants and the sediments were suspended with saccharobiose solution and added to the centrifuge tubes. The tubes were bathed at 37°C for 5 min, and then 200 μl PDE or AC substrates (bathed at 37°C before) were added. After joggling for 1 min and incubating at 37°C for 20 (for the supernatants) or 15 min (for the sediments), respectively, the tubes were placed in a water bath at 90°C for 3 min to quit the enzyme reaction. All the tubes were centrifuged at 8000 rpm for 10 min. Two hundred microliters of the supernatants were taken for cAMP isolation with a 0.5 g Al_2O_3 column chromatograph (1 × 10 cm). The absorbability at 260 nm was assayed. The AC or PDE activities were expressed by the producing or consuming amount of cAMP.

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