

Article

Cytotoxic and Antimalarial Amaryllidaceae Alkaloids from the Bulbs of *Lycoris radiata*

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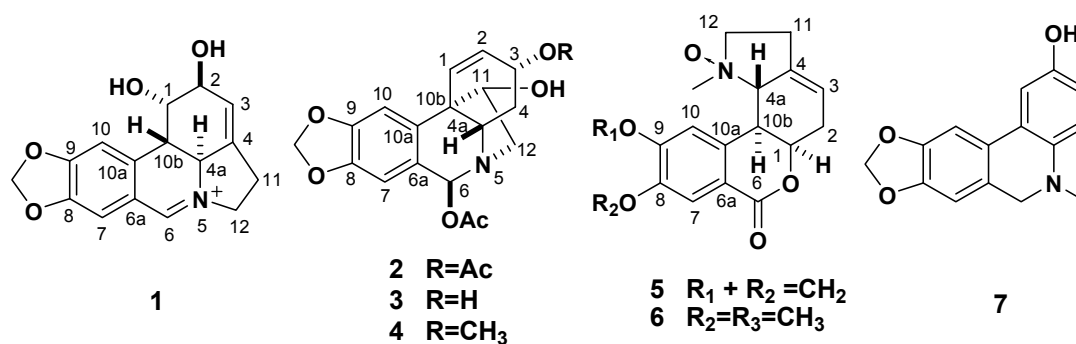
Abstract: Phytochemical investigation of the 80% ethanol extract of the bulbs of *Lycoris radiata* resulted in the isolation of five new Amaryllidaceae alkaloids: (+)-5,6-dehydrolycorine (**1**), (+)-3 α ,6 β -diacetyl-bulbispermine (**2**), (+)-3 α -hydroxy-6 β -acetyl-bulbispermine (**3**), (+)-8,9-methylenedioxyhomolycorine-*N*-oxide (**5**), and 5,6-dihydro-5-methyl-2-hydroxyphenanthridine (**7**), together with two known compounds, (+)-3 α -methoxy-6 β -acetylbulbispermine (**4**) and (+)-homolycorine-*N*-oxide (**6**). Structural elucidation of all the compounds were performed by spectral methods such as 1D and 2D (¹H-¹H COSY, HMQC, and HMBC) NMR spectroscopy, in addition to high resolution mass spectrometry. Alkaloid **1** showed potent cytotoxicity against astrocytoma and glioma cell lines (CCF-STTG1, CHG-5, SHG-44, and U251), as well as HL-60, SMMC-7721, and W480 cell lines with IC₅₀ values of 9.4–11.6 μ M. Additionally, compound **1** exhibited antimalarial activity with IC₅₀ values of 2.3 μ M for D-6 strain and 1.9 μ M for W-2 strain of *Plasmodium falciparum*.

Keywords: *Lycoris radiata*; Amaryllidaceae; alkaloids; cytotoxic; antimalarial

1. Introduction

The genus *Lycoris* (Amaryllidaceae) consists of more than 20 species which are mainly distributed in the temperate woodlands of eastern Asia, particularly in China and Japan [1,2]. The alkaloids, the major chemical constituents of this plant genus, are known to have various chemical structures and a wide range of biological activities [3–7]. Alkaloids affect the central nervous system and have acetylcholinesterase-inhibitory, analgesic, anti-inflammatory, antiviral, antimalarial, antitumor, or antineoplastic activity [8–14]. Galantamine hydrobromide, derived from galanthamine, which is found in numerous Amaryllidaceae, has been clinically used for the treatment of Alzheimer's disease [15]. *Lycoris radiata*, a perennial monocot, is endemic in China, Japan and Korea [16]. It is commonly known as Shi Shuan and used in China as a traditional folk medicine, from which more than ten indole alkaloids have been isolated [17,18]. The previous phytochemical studies revealed that *L. radiata* contained crinine-, galanthamine-, lycorine-, homolycorine- and montanine-type alkaloids [19,20]. The present studies on chemical constituents of the EtOH extract of *L. radiata* afforded five new Amaryllidaceae alkaloids, (+)-5,6-dehydrolycorine (**1**), (+)-3 α ,6 β -diacetyl-bulbispermine (**2**), (+)-3 α -hydroxy-6 β -acetylbulbispermine (**3**), (+)-8,9-methylenedioxy-homolycorine-*N*-oxide (**5**), and 5,6-dihydro-5-methyl-2-hydroxyphenanthridine (**7**), and two known compounds, (+)-3 α -methoxy-6 β -acetylbulbispermine (**4**) and (+)-homolycorine-*N*-oxide (**6**) (Figure 1). In this paper, we describe the isolation and structure elucidation on the basis of spectroscopic methods of the new compounds. Furthermore, all the alkaloids were evaluated *in vitro* for their cytotoxic and antimalarial properties.

Figure 1. The structures of compounds **1**–**7**.



2. Results and Discussion

Compound **1** was obtained as a yellow amorphous powder. The ESIMS afforded a quasimolecular ion peak at m/z 286, and its HR-ESI-MS revealed the $[M]^+$ peak at m/z 286.1075 (calcd. for C₁₆H₁₆NO₄⁺, 286.1074), corresponding to the molecular formula C₁₆H₁₆NO₄⁺. Its UV absorption at $\lambda_{\max}$ 374, 309, 253, and 212 nm showed an extended chromophore and a methylenedioxy substituted benzene ring. The IR absorption bands at 3,410, 3,355, 1,645, 1,605 and 923 cm^{−1} indicated OH groups and phenyl functions. The ¹H-NMR spectrum of **1** exhibited two singlets for two *para*-located aromatic protons at δ_H 7.28 (H-7) and 7.18 (H-10), a methylenedioxy signal at δ_H 6.17 and a downfield singlet corresponding to the proton of an iminium salt (δ_H 8.84) [21]. The ¹³C-NMR spectrum showed 16 carbon signals [OCH₂O $\times$ 1, CH₂ (sp³) $\times$ 2, CH (sp³) $\times$ 4, CH (sp²) $\times$ 4 and C (sp²) $\times$ 5, Table 1]. The above data suggested that **1** was an amaryllidaceae alkaloid similar to lycorine [22], except for an imine moiety

located between *N*-5 and C-6 (δ_C 163.2) in **1**, as supported by HMBCs of δ_H 8.84 (H-6) with δ_C 60.4 (C-4a), 113.0 (C-7), 128.3 (C-10a) and 59.0 (C-12) (Figure 2). The relative configuration of H-4a and H-10b in the amaryllidaceae alkaloids isolated from the genus *Lycoris* were always α - and β -orientations, respectively [22]. The relative configuration of **1** was elucidated by a ROESY experiment. The ROESY correlations of H-10b/H-1 and H-4a/H-2 indicated the β -orientation of H-1 and α -orientation of H-2, which was further supported by its positive specific rotation ($[\alpha]_D^{23.3} = +438.1$) [22]. Therefore, compound **1** was identified as (+)-5,6-dehydrolycorine.

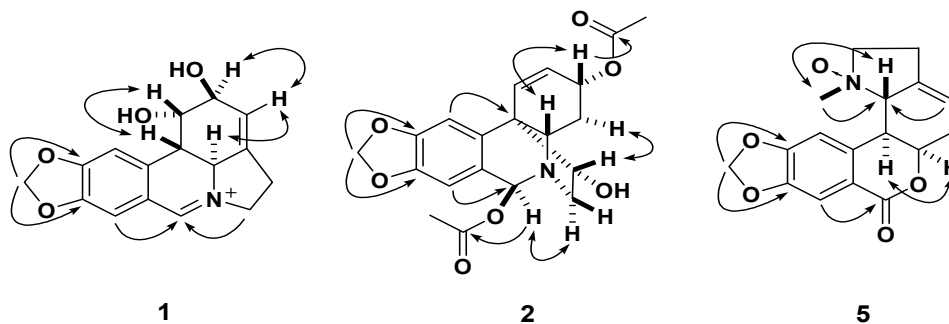
Compound **2** was obtained as a colorless oil. The HRESIMS displayed a pseudomolecular ion at m/z 410.1212 $[M+Na]^+$ (calcd for $C_{20}H_{21}NO_7Na$, 410.1216) consistent with a molecular formula of $C_{20}H_{21}NO_7$, corresponding to 11 degrees of unsaturation. The IR absorption bands at 3385 and 1713 cm^{-1} are ascribable to the OH and the ester C=O groups, respectively. The 1H -NMR spectrum exhibited two singlets at δ_H 6.64 (s) and 6.83 (s) assigned to two *para*-position aromatic protons, a broad singlet at δ_H 5.88 ascribed to a methylenedioxy, two olefinic signals at δ_H 6.36 and 5.85 assigned to the H-1 and H-2, and two singlets at δ_H 2.11 (s) and 2.07 (s) ascribed to two acetoxy Me groups. The ^{13}C -NMR spectrum displayed 20 carbon resonances, including a phenyl (δ_C 102.9, 108.9, 124.7, 137.1, 146.4, 148.1), two Ac (δ_C 21.2 and 170.4; 20.2 and 170.1), a methylenedioxy (δ_C 101.2), three oxygenated methins (δ_C 67.3, 78.3 and 87.0), two olefinic carbons (δ_C 127.5 and 129.9), two CH_2 (sp^3), one CH (sp^3) and a quaternary carbon (sp^3) (Table 1). The above data resembled those of (+)-3 α -methoxy-6 β -acetylbulbispermine (**4**) [23] except for an acetoxy group in **2** instead of the methoxyl group at C-3 in **4**, which was confirmed by HMBC correlations of H-3 (δ_H 3.91) with carbonyl group (δ_C 170.4) of acetoxy group. The HMBC of the signal of proton at δ_H 4.51 (H-6) with δ_C 170.1 suggested that the other AcO group was located at C-6. The ROESY correlations of H-4a/H-3 and H-11/H-4a suggested both H-3 and H-11 to be β -orientation, and the ROESY correlation of H-6/H-12 α indicated α -orientation for H-6 (Figure 2). Accordingly, the structure of **2** was established as (+)-3 α ,6 β -diacetylbulbispermine.

Compound **3** corresponded to the molecular formula $C_{18}H_{19}NO_6$, which was established by a quasimolecular ion peak in the HRESIMS. The general features of its IR and NMR spectra closely resembled those of **2**, except that the acetyl at C-3 in **2** were replaced by a hydroxyl in **3**, and an upfield shift of the signals for C-3 relative to that of **2** was observed. On the basis of the observation of NOESY data similar to those of **2**, the stereochemistry of **3** was expected to be the same. Accordingly, compound **3** was elucidated to be (+)-3 α -hydroxy-6 β -acetylbulbispermine.

Compound **5** was obtained as a colorless oil. Its positive HRESIMS spectrum showed a quasimolecular ion peak at m/z 316.1183 $[M+H]^+$, consistent with the molecular formula $C_{17}H_{17}NO$, accounting for 10 degrees of unsaturation. The IR absorption bands at 1705 and 1655 cm^{-1} indicated the existence of ketones, while the UV absorption bands at 265 and 212 nm suggested a conjugated moiety. The 1H -NMR spectrum showed singlet signals for two aryl protons (δ_H 7.18 and 7.58), one olefinic proton (δ_H 5.80), one methylenedioxy (δ_H 6.07), and two vicinal methylenes [δ_H 2.70 (H-11) and 3.51, 3.70 (H-12)]. The ^{13}C -NMR spectrum displayed one *N*-CH₃, four CH_2 and six CH groups (including three sp^3 carbons and three sp^2 carbons), and six sp^2 quaternary carbons. The NMR data and the characteristic downfield signals of the carbon resonances for C-4a (δ_C 79.1), C-12 (δ_C 71.0), and *N*-CH₃ (δ_C 56.2) indicated that **5** was a derivative of homolycorine- α -*N*-oxide [21,24]. The methylenedioxy was positioned between C-8 and C-9, and the methyl at *N*-5, respectively, based on the HMBC correlations of the proton signal of methylenedioxy (δ_H 6.07) with C-8 (δ_C 151.8) and C-9 (δ_C 147.9),

and of the methyl signal (δ_{H} 2.97) with C-4a and C-12, respectively (Figure 2). The ROESY correlation $N\text{-CH}_3/\text{H-4a}$ suggested α -orientation of the N -oxide. Thus, the structure of **5** was assigned the name (+)-8,9-methylenedioxy-homolycorine- N -oxide.

Figure 2. The key HMBC ($\curvearrowright$) and ^1H - ^1H COSY ($\curvearrowleft$) correlations of compounds **1**, **2**, and **5**.



Compound **7**, colorless needles, was assigned a molecular formula of $\text{C}_{15}\text{H}_{13}\text{NO}_3$, based on the HRESIMS spectrum which showed a pseudomolecular ion at m/z 256.0977 [$M+\text{H}$] $^+$ (calcd. 256.0974). Its ^{13}C -NMR spectrum showed 15 carbon signals [$\text{NCH}_3 \times 1$, CH_2 (sp^3) $\times 2$, CH (sp^2) $\times 5$ and C (sp^2) $\times 7$, Table 1]. The ^1H -NMR spectrum showed singlet signals for two aryl protons (δ_{H} 7.02 and 6.69), one $N\text{-CH}_3$ (δ_{H} 2.75), and an ABX system [δ_{H} 6.48 (1H, d, $J = 3.2$ Hz, H-1), 6.32 (1H, dd, $J = 8.2, 3.2$ Hz, H-3), and 6.63 (1H, d, $J = 8.2$ Hz, H-4)]. These spectral data showed similarities to those of 5,6-dihydro-5-methylphenanthridine [25]. The HMBC of C-2 (δ_{C} 154.1) with H-4 (δ_{H} 6.63) together the characteristic signals of the ABX system positioned the hydroxy group at C-2, which was further supported by the observation of downfield chemical shift of C-2 in **7**. Therefore, compound **7** was identified as 5,6-dihydro-5-methyl-2-hydroxyphenanthridine.

The cytotoxic activities of the isolated alkaloids were determined against eight human tumor cell lines, BEN-MEN-1 (meningioma), CCF-STTG1 (astrocytoma), CHG-5 (glioma), SHG-44 (glioma), U251 (glioma), HL-60 (human myeloid leukemia), SMMC-7721 (hepatocellular carcinoma), and W480 (colon cancer) using the modified MTT method. The *in vitro* cytotoxic activities of these compounds against human cell lines are summarized in Table 2. Among the tested compounds, lycorine-type alkaloid **1** exhibited the most potent cytotoxic potential against all tested tumor cell lines, with IC_{50} values of 9.4–11.6 μM , except against BEN-MEN-1. Crinine-type alkaloids **2–4** showed significant cytotoxicities against HL-60 ($\text{IC}_{50} < 10$ μM), and moderate cytotoxicities against astrocytoma and glioma cell lines, CCF-STTG1, CHG-5, SHG-44 and U251 (10 $\mu\text{M} < \text{IC}_{50} \leq 30$ μM). Homolycorine-type alkaloids **5–6** and **7** had no cytotoxic activities ($\text{IC}_{50} > 80$ μM).

Malaria is one of the most common vector-borne infectious diseases. This disease is caused by parasites of the genus *Plasmodium* and causes such symptoms as anemia, fever, chills, nausea, and in severe cases, coma and death. The effects of isolated alkaloids *in vitro* antimalarial activity were evaluated by using the drug-resistant D-6 strain and the drug-sensitive W-2 strain of *P. falciparum*. Lycorine-type alkaloid **1** possessed high antimalarial activities with low IC_{50} values (D-6: 2.3 μM ; W-2 strain: 1.9 μM) (Table 3). Crinine-type alkaloids **2–4** showed moderate antimalarial activities, with values of 18.9, 17.9 and 21.3 μM for the D-6 strain and of 20.1, 19.3 and 23.4 μM for the W-2 strain, respectively. Homolycorine-type alkaloids **5–6** and **7** had no antimalarial activities.

Table 1. ^1H -NMR data of compounds **1–3** and **5** in CDCl_3 (δ in ppm and J in Hz).

No.	δ ^1H (Hz)				δ ^{13}C			
	1	2	3	5	1	2	3	5
1	4.66 (<i>dd</i> , 3.8, 3.2)	6.36 (<i>d</i> , 10.2)	6.38 (<i>d</i> , 10.2)	4.96 (<i>m</i>)	72.2	127.5	127.7	78.1
2	4.32 (<i>dd</i> , 5.2, 3.2)	5.85 (<i>dd</i> , 10.2, 5.0)	6.16 (<i>dd</i> , 10.2, 5.0)	2.53, 2.90 (<i>m</i>)	70.2	129.9	132.8	31.6
3	5.36 (<i>d</i> , 5.2)	3.91 (<i>m</i>)	4.27 (<i>m</i>)	5.80 (<i>dd</i> , 5.6, 5.0)	118.5	67.3	64.4	126.0
4	-	2.37 (<i>m</i>)	2.38 (<i>m</i>)	-	145.6	31.0	32.8	141.5
4a	4.46 (<i>d</i> , 13.6)	3.38 (<i>dd</i> , 13.8, 3.2)	3.40 (<i>dd</i> , 13.8, 3.2)	4.15 (<i>d</i> , 13.2)	60.4	58.5	57.8	79.1
6	8.84 (<i>s</i>)	4.51 (<i>br s</i>)	4.52 (<i>br s</i>)	-	163.2	87.0	87.1	166.8
6a	-	-	-	-	140.9	124.7	124.8	117.9
7	7.28 (<i>s</i>)	6.64 (<i>s</i>)	6.65 (<i>s</i>)	7.58 (<i>s</i>)	113.0	108.9	108.9	108.9
8	-	-	-	-	149.1	146.4	146.5	151.8
9	-	-	-	-	157.4	148.1	148.2	147.9
10	7.18 (<i>s</i>)	6.83 (<i>s</i>)	6.85 (<i>s</i>)	7.18 (<i>s</i>)	106.6	102.9	103.1	109.8
10a	-	-	-	-	128.3	137.1	137.2	136.3
10b	3.28 (<i>dd</i> , 13.6, 3.8)	-	-	3.63 (<i>dd</i> , 13.2, 2.8)	45.6	49.8	50.0	38.2
11	2.65, 2.88 (<i>m</i>)	3.96 (<i>m</i>)	3.97 (<i>m</i>)	2.70 (<i>m</i>)	34.4	78.3	78.4	26.5
12	4.23, 4.38 (<i>m</i>)	2.80, 3.32 (<i>m</i>)	2.81, 3.33 (<i>m</i>)	3.51, 3.70 (<i>m</i>)	59.0	58.8	58.9	71.0
OCH ₂ O	6.17 (<i>br s</i>)	5.88 (<i>br s</i>)	5.90 (<i>br s</i>)	6.07 (<i>br s</i>)	104.7	101.2	101.2	102.1
<i>N</i> -CH ₃	-	-	-	2.97 (<i>s</i>)	-	-	-	56.2
OCH ₃	-	-	-	-	-	-	-	-
OCH ₃	-	-	-	-	-	-	-	-
CO ₂ CH ₃	-	-	-	-	-	170.4	170.6	-
CO ₂ CH ₃	-	2.11 (<i>s</i>)	2.12 (<i>s</i>)	-	-	21.2	21.6	-
CO ₂ CH ₃	-	-	-	-	-	170.1	-	-
CO ₂ CH ₃	-	2.07 (<i>s</i>)	-	-	-	20.2	-	-

Table 2. The cytotoxicity of compounds **1–7** against eight human tumor cell lines ^a.

Cell lines								
	BEN-MEN-1	CCF-STTG1	CHG-5	SHG-44	U251	HL-60	SMMC-7721	W480
1		10.3 ± 0.9	10.2 ± 1.6	9.4 ± 1.3	11.8 ± 0.7	10.8 ± 1.6	10.5 ± 0.9	11.6 ± 1.1
2		27.1 ± 5.1	30.1 ± 4.4	27.1 ± 3.2	17.4 ± 2.1	7.3 ± 1.1	63.2 ± 11.8	51.1 ± 10.9
3		29.4 ± 4.1	29.4 ± 5.3	28.3 ± 2.7	15.8 ± 1.7	7.1 ± 0.9	66.8 ± 9.4	53.5 ± 12.4
4		29.7 ± 5.4	29.6 ± 6.3	29.1 ± 3.8	16.7 ± 2.6	8.6 ± 1.4	68.2 ± 12.3	50.1 ± 7.8
5	-	83.2 ± 13.7	-	-	-	-	86.2 ± 17.4	-
6	-	-	93.0 ± 21.1	-	-	-	85.0 ± 16.2	-
7	-	-	-	-	-	81.3 ± 15.7	-	-
Doxorubicin	17.8	24.7	21.8	33.7	28.4		37.6	14.1

^a Doxorubicin are expressed as IC₅₀ values in nM, and compound **1–7** are expressed as IC₅₀ values in μM. (-) IC₅₀ > 100 μM.

Table 3. *In vitro* antimalarial activity against *Plasmodium falciparum* of compounds **1–7** ^a.

	D-6	W-2
1	2.3	1.9
2	18.9	20.1
3	17.9	19.3
4	21.3	23.4
5	-	-
6	-	-
7	-	-
Chloroquine	9.8	6.7

^a Chloroquine data are expressed as IC₅₀ values in nM, and compounds **1–7** are expressed as IC₅₀ values in μM. (-) IC₅₀ > 100 μM.

3. Experimental

3.1. General

Optical rotations were determined with a JASCO P2000 digital polarimeter (Tokyo, Japan). Ultraviolet (UV) and infrared (IR) spectra were obtained on JASCO V-650 and JASCO FT/IR-4100 spectrophotometers (Tokyo, Japan), respectively. The NMR spectra were measured in CDCl₃ on a Bruker AM-600 spectrometer (Fällanden, Switzerland). Chemical shifts were reported using residual CDCl₃ (δ_{H} 7.26 and δ_{C} 77.0 ppm) and CD₃OD (δ_{H} 3.30 and δ_{C} 49.0 ppm) as internal standard. High resolution ESIMS spectra were obtained on a LTQ Orbitrap XL (Thermo Fisher Scientific, Waltham, MA, USA) spectrometer. Silica gel 60 (230–400 mesh, Merck, Darmstadt, Germany), LiChroprep RP-18 (Merck, 40–63 μm), and Sephadex LH-20 (Amersham Pharmacia Biotech, Roosendaal, The Netherlands) were used for column chromatography (CC). HPLC separation was performed on an instrument consisting of a Waters 600 controller, a Waters 600 pump, and a Waters 2487 dual λ absorbance detector, with a Prevail (250 $\times$ 10 mm i.d.) preparative column packed with C18 (5 μm). Precoated silica gel plates (Merck, Kieselgel 60 F254, 0.25 mm) and precoated RP-18 F_{254s} plates (Merck) were used for analytical thin-layer chromatography analyses.

3.2. Plant Material

The bulbs of *L. radiata* were collected in April of 2011 in Lishui, a city of Zhejiang Province in China, and identified by one of the authors (Q.-J. Zhao). A specimen (201104001L) was deposited in the Herbarium of School of Pharmacy, Second Military Medical University, Shanghai, China.

3.3. Extraction and Isolation

The bulbs of *L. radiata* (10.5 kg) were cut into small pieces and were extracted with 80% ethanol (10 L) three times under reflux for 15 h and then concentrated under reduced pressure to give a crude extract (618.5 g). The crude extract was partitioned between equal volumes of chloroform and water to provide a chloroform-soluble fraction (110.6 g) and an aqueous layer. The chloroform-soluble fraction was further fractionated through a silica gel column (200–300 mesh) using increasing volumes of acetone in petroleum ether (100:1, 50:1, 30:1, 15:1, 10:1, 7:1, 5:1, 3:1, 1:1, V/V) as eluents to give 12 fractions according to TLC analysis. Fraction 5 (5.3 g) was applied to an ODS MPLC column (100 g) and eluted with MeOH-H₂O (20:80, 30:70, 40:60, each 500 mL) to yield four subfractions (Fr. 5-1 and Fr. 5-4). Subfraction 5-2 (358 mg) was purified by a preparative RP-HPLC (ODS column, 250 $\times$ 20 mm) using MeOH/H₂O (26:74) as mobile phase to obtain **2** (73 mg) and **7** (64 mg). Subfraction 5-3 (515 mg) was chromatographed by a Sephadex LH-20 column eluted with MeOH/H₂O (50:50), and purified by a preparative RP-HPLC (ODS column, 250 $\times$ 20 mm) using MeOH/H₂O (30:70) as mobile phase to yield **4** (73 mg) and **5** (68 mg). Fraction 6 (3.3 g) was applied to an ODS column eluted with MeOH/H₂O (30:70, 40:60, 50:50) to provide 4 Subfraction (Fr. 6-1 and Fr. 6-4). Subfraction 6-2 (119 mg) was purified by a preparative RP-HPLC (ODS column, 250 $\times$ 20 mm) eluted with MeOH/H₂O (22:78) to get **6** (57 mg). Subfraction 6-3 (MeOH-H₂O 20:80, 303 mg) was repeatedly chromatographed on silica gel (chloroform:methanol, 20:1 $\rightarrow$ 10:1) and then purified by a Sephadex LH-20 column eluted with

MeOH/H₂O (50:50) to afford **1** (68 mg). Subfraction Subfraction 6-4 was purified by a preparative RP-HPLC (ODS column, 250 × 20 mm) eluted with MeOH/H₂O (23:77) to get **3** (73 mg).

(+)-5,6-Dehydrolycorine (**1**): Yellow amorphous powder. $[\alpha]_D^{23.3} = +438.1$ ($c = 0.11$, MeOH). UV (CDCl₃) $\lambda_{\max}(\log \epsilon)$: 374 (4.05), 309 (3.83), 253 (4.22), 212 (4.65) nm. IR (KBr) $\nu_{\max}$ 3410, 3355, 1645, 1605, 1590, 1502, 1275, 1035, 923 cm⁻¹. ¹H-NMR and ¹³C-NMR data, see Table 1. ESI-MS m/z : 286 ($[M]^+$). HR-ESI-MS (pos.) m/z : 286.1075 ($[M]^+$, C₁₆H₁₆NO₄⁺. calc. 286.1074).

(+)-3 α ,6 β -Diacetylbulbispermine (**2**): Colorless oil. $[\alpha]_D^{23.3} = +33.7$ ($c = 0.16$, MeOH). UV (CDCl₃) $\lambda_{\max}(\log \epsilon)$: 292 (3.73), 240 (3.90) nm. IR (KBr) $\nu_{\max}$ 3385, 2902, 1713, 1483, 1250, 1060, 933, 870 cm⁻¹. ¹H-NMR and ¹³C-NMR data, see Table 1. EI-MS m/z : 387 ($[M]^+$). HR-ESI-MS (pos.) m/z : calc. 410.1212 ($[M + Na]^+$, C₂₀H₂₁NO₇Na. calc. 410.1216).

(+)-3 α -Hydroxy-6 β -acetylbulbispermine (**3**): Colorless oil. $[\alpha]_D^{23.3} = +23.6$ ($c = 0.10$, MeOH). UV (CDCl₃) $\lambda_{\max}(\log \epsilon)$: 291 (3.78), 240 (3.87) nm. IR (KBr) $\nu_{\max}$ 3386, 2898, 1712, 1485, 1248, 1055, 930 cm⁻¹. ¹H-NMR and ¹³C-NMR data see Table 1. EI-MS m/z : 345 ($[M]^+$). HR-ESI-MS (pos.) m/z : calc. 368.1112 ($[M + Na]^+$, C₁₈H₁₉NO₆Na. calc. 368.1110).

(+)-8,9-Methylenedioxy-homolycorine-N-oxide (**5**): Colorless oil. $[\alpha]_D^{23.3} = +143.9$ ($c = 0.14$, MeOH). UV (CDCl₃) $\lambda_{\max}(\log \epsilon)$: 380 (2.72), 308 (3.55), 265 (3.78), 212 (4.56) nm. IR (KBr) $\nu_{\max}$ 2945, 1705, 1655, 1600, 1453, 1311, 1224, 1064, 911 cm⁻¹. ¹H-NMR and ¹³C-NMR data, see Table 1. EI-MS m/z : 315 ($[M]^+$). HR-ESI-MS (pos.) m/z : calc. 316.1183 ($[M + H]^+$, C₁₇H₁₈NO₅. calc. 316.1185).

5,6-Dihydro-5-methyl-2-hydroxyphenanthridine (**7**): Colorless needles. $[\alpha]_D^{23.3} = +43.1$ ($c = 0.05$, MeOH). UV (CDCl₃) $\lambda_{\max}(\log \epsilon)$: 380 (2.65), 308 (3.55), 263 (3.70), 210 (4.40) nm. IR (KBr) $\nu_{\max}$ 3410, 1710, 1640, 1602, 1460, 1255, 1035, 915 cm⁻¹. ¹H-NMR δ_H : 6.48 (1H, d, $J = 3.2$ Hz, H-1), 6.32 (1H, dd, $J = 8.2, 3.2$ Hz, H-3), 6.63 (1H, d, $J = 8.2$ Hz, H-4), 4.21 (2H, br s, H-6), 7.02 (1H, s, H-7), 6.69 (1H, s, H-10), 2.75 (3H, s, *N*-Me), 5.99 (2H, s, OCH₂O); ¹³C-NMR δ_C : 113.6 (C-1), 154.1 (C-2), 117.2 (C-3), 113.0 (C-4), 138.9 (C-4a), 63.8 (C-6), 133.7 (C-6a), 109.9 (C-7), 147.7 (C-8), 147.6 (C-9), 107.3 (C-7), 110.4 (C-10), 131.0 (C-10a), 117.5 (C-10b), 31.1 (*N*-Me). EI-MS m/z : 255 ($[M]^+$). HR-ESI-MS (pos.) m/z : 256.0977 ($[M + H]^+$, C₁₅H₁₄NO₃. calc. 256.0974).

3.4. Cytotoxicity Assay in Vitro

The cytotoxic activities of the isolated compounds were determined using the revised MTT method [26,27] against BEN-MEN-1 (meningioma), CCF-STTG1 (astrocytoma), CHG-5 (glioma), SHG-44 (glioma), U251 (glioma), HL-60 (human myeloid leukemia), SMMC-7721 (hepatocellular carcinoma), and W480 (colon cancer). Doxorubicin was used as the positive control. Cancer cells (4×10^3 cells) suspended in 100 μ L/well of DMEM medium containing 10% fetal calf serum were seeded onto a 96-well culture plate. After 24 h pre-incubation at 37 °C in a humidified atmosphere of 5% CO₂/95% air to allow cellular attachment, various concentrations of test solution were added and cells were incubated for 48 h under the above conditions. At the end of the incubation, 10 μ L of tetrazolium reagent was added into each well followed by further incubation at 37 °C for 4 h. The supernatant was decanted, and DMSO (100 μ L/well) was added to allow formosan solubilization. The concentrations of

the assayed compounds were 0.04, 0.2, 1.0, 5, 25, and 125 μM , respectively. The optical density (OD) of each well was detected using a microplate reader at 550 nm and for correction at 595 nm. Each determination represented the average mean of six replicates. The 50% inhibition concentration (IC_{50} value) was determined by non-linear regression with Graphpad Prism software version 4.0 (GraphPad Software, Inc., San Diego, CA, USA) and was used as criteria to judge the cytotoxicity. All the IC_{50} results represent an average of a minimum of three experiments and were expressed as means $\pm$ standard deviation (SD). All cell lines were purchased from the Cell Bank of the Shanghai Institute of Biochemistry & Cell Biology, Chinese Academy of Sciences (Shanghai, China). Other reagents were purchased from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd (Shanghai, China).

3.5. Assay for in Vitro Antimalarial Activity against *Plasmodium Falciparum*

P. falciparum strains D-6 (drug-resistant) and W-2 (drug-sensitive) were cultured in human erythrocytes in RPMI medium (RPMI-1640 with 25 mM HEPES buffer, 24 mM NaHCO_3 , 0.2% glucose, 0.05% L-glutamine, 50 $\mu\text{g/mL}$ hypoxanthine, and 25 $\mu\text{g/mL}$ gentamicin) supplemented with 10% human plasma at 37 °C, under 93% N_2 , 4% CO_2 , and 3% O_2 [4,10]. Antimalarial activity of the test compound was determined from the dose–response curve using the parasite lactate dehydrogenase (pLDH) assay according to the method of Makler [4,10]. The concentrations of the assayed compounds were 0.02, 0.1, 0.5, 2.5, 12.5, and 62.5 μM , respectively. One hundred and ninety microliters of asynchronous parasites (2.0% hematocrit and 0.5 or 1% parasitemia) was seeded into a 96-well microplate and 10 μL of test compound solution (dissolved in 25% ethanol or 5% DMSO) was added. After incubating at 37 °C for 72 h under 93% N_2 , 4% CO_2 , and 3% O_2 , the microplate was immediately frozen at -20 °C for 18 h. Then, the microplate was thawed at 37 °C and 20 μL of the hemolyzed parasite suspension was transferred to another microplate containing 100 μL of Malstat reagent. The plate was further incubated for 15 min at room temperature, and 20 μL of a 1:1 mixture of nitroblue tetrazolium and phenazine ethosulfate (2 mg and 0.1 mg/mL, respectively) was added to each well. After incubation for 2 h at room temperature in the dark, the blue formazan product was measured at 655 nm with iEMS microplate reader MF (LabSystems, Helsinki, Finland). The 50% inhibitory concentration (IC_{50}) value was determined by non-linear regression with Graphpad Prism software version 4.0 (GraphPad Software, Inc.). It was used as criteria to judge the activity (active: $\text{IC}_{50} \leq 10$ μM ; moderately active: 10 $\mu\text{M} < \text{IC}_{50} \leq 30$ μM ; not active: $\text{IC}_{50} > 30$ μM). *P. falciparum* strains were purchased from the Cell Bank of the Shanghai Institute of Biochemistry & Cell Biology, Chinese Academy of Sciences.

4. Conclusions

Five new Amaryllidaceae alkaloids: (+)-5,6-dehydrolycorine (**1**), (+)-3 α ,6 β -diacetylbulbispermine (**2**), (+)-3 α -hydroxy-6 β -acetylbulbispermine (**3**), (+)-8,9-methylenedioxy-homolycorine-*N*-oxide (**5**), and 5,6-dihydro-5-methyl-2-hydroxyphenanthridine (**7**), and two known compounds, (+)-3 α -methoxy-6 β -acetylbulbispermine (**4**) and (+)-homolycorine-*N*-oxide (**6**), were isolated from the 80% ethanol extract of the bulbs of *Lycoris radiata*. All the alkaloids were evaluated *in vitro* for cytotoxic properties against BEN-MEN-1 (meningioma), CCF-STTG1 (astrocytoma), CHG-5 (glioma), SHG-44 (glioma), U251 (glioma), HL-60 (human myeloid leukemia), SMMC-7721 (hepatocellular carcinoma), and W480

(colon cancer) and antimalarial activity against two strains of *P. falciparum* (D-6: drug-resistant and W-2: drug-sensitive). Alkaloids **1–4** showed significant cytotoxic activities against HL-60 (IC₅₀ values < 10 µM). Lycorine-type alkaloid **1** exhibited the most potent cytotoxicities and crinine-type alkaloids **2–4** possessed moderate cytotoxicities against astrocytoma and glioma cell lines, CCF-STTG1, CHG-5, SHG-44 and U251 (10 µM < IC₅₀ value ≤ 30 µM). Homolycorine-type alkaloids **5–6** and **7** had no cytotoxic activities (IC₅₀ values > 80 µM). In addition, lycorine-type alkaloid **1** has antimalarial activities against the two strains of *P. falciparum*.

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Sample Availability: Samples of the compounds **1–7** available from the authors.