

Cytotoxic Sesquiterpene Lactones from *Eupatorium lindleyanum*

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Ten new guaiane type sesquiterpene lactones, namely, eupalinilides A–J (**1–10**), as well as nine known compounds, eupachinilide C (**11**), eupachinifolin D (**12**), eupachinilide E (**13**), 2 α -hydroxyeupatolide (**14**), 3-deacetyeupalinin A (**15**), heliangine (**16**), 8 β -tigloyloxy-2,3-seco-6 β H,7 α H-helianga-4Z,11(13)-diene-3,10 β ;6, 12-diolid-2-oic acid (**17**), 8 β -(4'-hydroxytigloyloxy)-3 β ,14-dihydroxy-6 β H,7 α H-germacra-1(10)Z,4Z,11(13)-trien-6,12-olide (**18**), and 8 β -tigloyloxy-3 β ,14-dihydroxy-6 β H,7 α H-germacra-1(10)Z,4E,11(13)-trien-6,12-olide (**19**), were isolated from the whole plant of *Eupatorium lindleyanum*. Eupalinilides B (**2**), C (**3**), E (**5**), F (**6**), and I (**9**) have been tested for cytotoxicity against P-388 and A-549 tumor cell lines. The results showed that eupalinilides B (**2**) and E (**5**) demonstrated potent cytotoxicity. The structures of these compounds were determined by spectroscopic methods including 1D and 2D NMR spectra.

The plant *Eupatorium lindleyanum* DC., which showed antihistamine and antibacterial activities,¹ is indigenous to China. The whole plant called “Ye-Ma-Zhui” by local residents is used for the treatment of cough and tracheitis, and has bitter and acerbity tastes.¹ In a previous study, eupalinins A–D isolated from *E. lindleyanum* DC. showed remarkable inhibitory activity against KB cell culture.² In the current research, 10 new guaiane type sesquiterpenoids, namely, eupalinilides A–J (**1–10**), along with nine known ones were isolated from the whole plant of *E. lindleyanum* DC. Their structures and configuration were elucidated mainly by spectroscopic methods, especially 2D NMR techniques. We report herein the isolation and structural elucidation of these sesquiterpene lactones and some of their cytotoxic activities against P-388 and A-549 tumor cell lines.

Results and Discussion

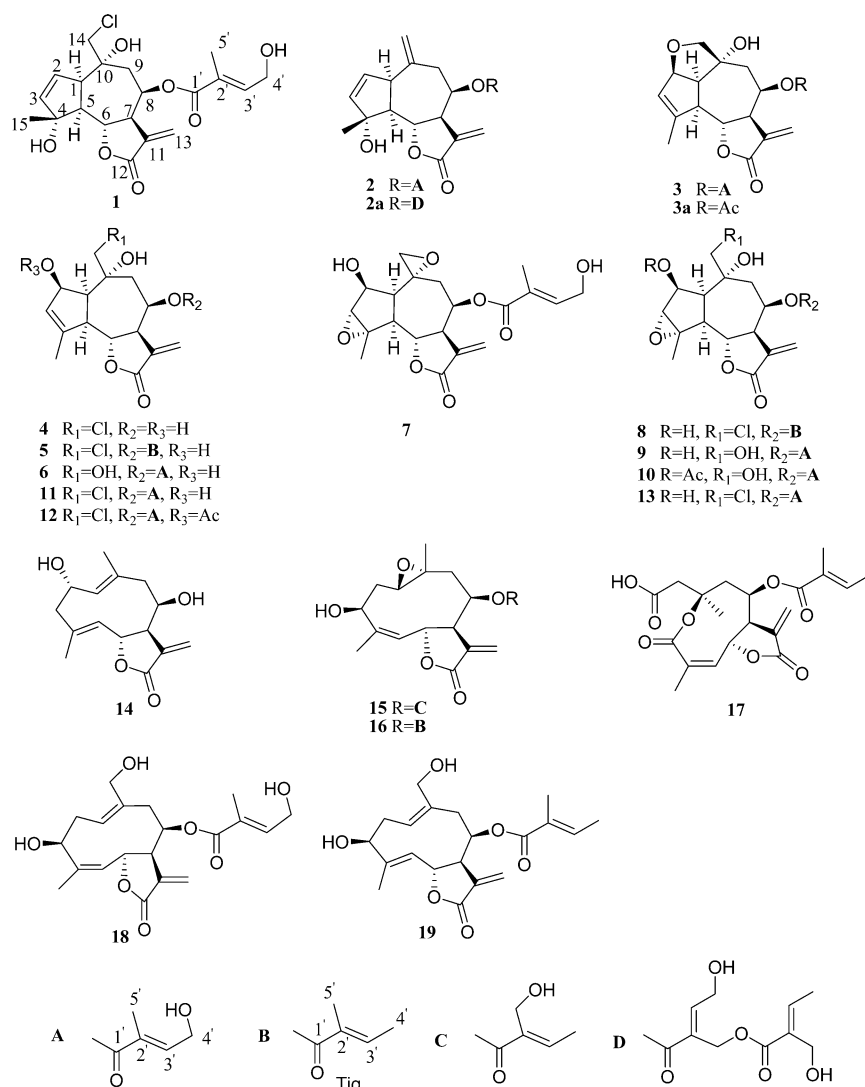
Eupalinilide A (**1**) had a molecular formula of C₂₀H₂₅ClO₇ as determined by HREIMS at *m/z* 412.1285 [M]⁺ (calcd 412.1289), inferring eight degrees of unsaturation. The ¹H and ¹³C NMR and DEPT spectra showed the presence of two methyls, four secondary carbons, eight tertiary carbons, and six quaternary carbons (Tables 1 and 2). The characteristic features of the ¹H and ¹³C NMR implied that **1** was a guaiane type sesquiterpenoid. IR absorption bands at 3431, 1751, 1713, and 1647 cm⁻¹ indicated the existence of hydroxyls, a five-membered lactone, an ester group, and double bonds. In the ¹³C NMR, the carbon signals at δ_C 168.6 were assignable to the carbonyl of the lactone group, and the carbon signals at δ_C 134.0 and 122.6 were attributable to a terminal double bond, indicating the presence of an α -methylene- γ -lactone moiety, which was confirmed by the HMBC correlations (Figure 1a). A chlorinated methylene was distinguished by its shielded carbon signal at δ_C 52.3 (C-14)^{3,4} and somewhat downfield shifted proton signals at δ_H 3.63 (d, *J* = 10.9 Hz, H-14a) and 3.57 (d, *J* = 10.9 Hz, H-14b)^{3,5,6} compared with an oxygenated methylene. The proton signals at δ 1.82 (3H, br s, H-5'), 4.35 (2H, d, *J* = 6.0 Hz, H-4'), and 6.72 (br t, *J* = 6.0 Hz, H-3') and carbon signals at δ 166.2 (ester carbonyl), 127.7 and 141.4 (double bond) obviously showed the presence of a 4'-hydroxytigloyl moiety,^{4,7,8} which was confirmed by HMBC correlations and NOESY correlations (Figure 2). The proton signal at δ 5.70 and the carbon signal at δ 65.8 were

respectively assigned to H-8 and C-8 of one oxygenated methine. The downfield shifted H-8 proton signal indicated that the 4'-hydroxytigloyloxy was attached to C-8, which was confirmed by HMBC correlation between H-8 and C-1'. The other oxygenated methine proton signal at δ 4.71 (dd, *J* = 11.9, 9.8 Hz) was assigned to H-6 on the basis of HMBC correlation pairs of H-6/C-4 (δ 83.9), H-6/C-7 (δ 48.6), and H-5/C-6 (δ 76.5). The downfield shifted proton signal H-6 was caused by the deshielding effect of an ester carbonyl, suggesting that the lactone group was formed between C-6 and C-12. A disubstituted double bond inferred by the carbon signals at δ 130.7 (C-2) and 139.4 (C-3) and the proton signals at δ 6.00 (dd, *J* = 5.8, 2.6 Hz, H-2) and 5.87 (d, *J* = 5.8 Hz, H-3) was only assignable to a $\Delta^{2,3}$ double bond, which was confirmed by the correlations between H-1 and H-2 and between H-2 and H-3 in the ¹H–¹H COSY spectrum and also the important correlations in the HMBC. Two oxygenated carbon signals at δ 83.9 and 74.7 were respectively assigned to C-4 and C-10 by HMBC correlations with the nearby proton signals.

The relative configuration of **1** was determined via a NOESY experiment (Figure 2), in which the H-7 (δ 3.13, br d, *J* = 9.8 Hz), correlating with both H-5 and H-1, indicated that H-5 and H-1 were α -oriented since H-7 is α -oriented in all guaiane type sesquiterpenoids.⁹ The coupling constant between H-5 and H-6 (*J*_{5,6} = 11.9 Hz) indicated an antiperiplanar arrangement and hence that H-6 adopted a β -orientation. The correlations between H-6/H₂-14 and H-6/H₃-15 suggested that the chlorinated methylene and C-15 methyl were both in β -orientations because H-6 was β -oriented. The strong NOESY correlation between H-7 and H-8 indicated that H-8 was also α -oriented. Thus, the structure of **1** was unambiguously elucidated as 8 β -(4'-hydroxytigloyloxy)-14-chloro-4 β ,10 β -dihydroxy-1 α H,5 α H,6 β H,7 α H-guai-2,11(13)-dien-6,12-olide.

Eupalinilide B (**2**) showed a molecular formula C₂₀H₂₄O₆ as determined by HREIMS at *m/z* 360.1566 (calcd 360.1573). The proton signals at δ 1.77 (3H, s, H-5'), 4.30 (2H, br d, *J* = 6.0 Hz, H-4'), and 6.73 (br t, *J* = 6.0 Hz, H-3') and the carbon signals at δ 166.6 (ester carbonyl), 127.7 and 141.6 (double bond), 59.5 (methylene), and 12.6 (methyl) showed the presence of a 4'-hydroxytigloyl moiety,^{7,8} similar to that of **1**. Comparison of the ¹H and ¹³C NMR data of **2** with those of hymenopappolide¹⁰ (**2a**) indicated that both compounds have the same sesquiterpenoid moiety, and the only

Chart 1



difference occurs at the C-8 bearing ester groups. The structure of **2** was therefore determined as 8 β -(4'-hydroxytigloyloxy)-4 α -hydroxy-1 α H,5 α H,6 β H,7 α H-guai-2,10-(14),11(13)-trien-6,12-olide.

Eupalinilide C (**3**) gave the molecular formula C₂₀H₂₄O₇ as determined by HREIMS at *m/z* 376.1520 (calcd 376.1522), inferring the presence of nine degrees of unsaturation. The ¹H and ¹³C NMR spectra (Tables 1 and 2) and DEPT experiment showed the presence of two carbonyl groups, one terminal double bond, and two trisubstituted double bonds. Three sets of double bonds and two carbonyl groups accounted for five of the nine degrees of unsaturation, and the remaining four degrees of unsaturation were assumed for the existence of a tetracyclic ring system in **3**. The characteristic proton signals at δ 1.78 (3H, s, H-5'), 4.34 (2H, br d, *J* = 6.0 Hz, H-4'), and 6.71 (br t, *J* = 6.0 Hz, H-3') and the carbon signals at δ 166.4 (ester carbonyl), 127.7 and 141.3 (double bond), 59.7 (methylene), and 12.9 (methyl) were the features of a 4'-hydroxytigloyl moiety as in **1** and **2**. Comparison of the ¹H and ¹³C NMR spectroscopic data (Table 1) of **3** with those of the reported **3a**¹⁰ suggested that both **3** and **3a** shared a common guaianolide type sesquiterpenoid scaffold, and the only difference occurred at the C-8 bearing ester groups: the former is a 4'-hydroxytigloyloxy, and the latter is an acetoxy. The structure of **3** was thus elucidated to be 8 β -(4'-hydroxyti-

gloyloxy)-2 β ,14-epoxy-10 α -hydroxy-1 α H,5 α H,6 β H,7 α H-guai-3,11(13)-dien-6,12-olide.

Eupalinilide D (**4**) was formulated as C₁₅H₁₉ClO₅ by HREIMS at *m/z* 314.0931 (calcd 314.0921). The ¹H and ¹³C NMR spectrum combined with a DEPT experiment showed that **4** was composed of one methyl, three methylenes (one chlorinated and one olefinic), seven methines (three oxygenated and one olefinic), and four quaternary carbons (one oxygenated, two olefinic, and one carbonyl). The presence of an α -methylene- γ -lactone moiety was evidenced by the carbon signals at δ 170.9 (carbonyl) and 135.1 and 122.0 (terminal double bond). Comparison of the ¹H NMR data of **4** with those of eupachifolin D⁹ (**12**) showed good similarity, except for H-2 (δ 4.54, dd, *J* = 4.1, 1.7 Hz) and H-8 (δ 4.42, td, *J* = 8.1, 4.0 Hz) of **4**, which were upfield shifted, indicating that **4** is the dediacyl derivative of **12**. The structure of **4** was thus established as 14-chloro-2 β ,8 β ,10 α -trihydroxy-1 α H,5 α H,6 β H,7 α H-guai-3,11(13)-dien-6,12-olide.

Eupalinilide E (**5**) had a molecular formula of C₂₀H₂₅ClO₆ on the basis of HREIMS at *m/z* 396.1342 (calcd 396.1340). The proton signals at δ 6.70 (br q, *J* = 8.0 Hz, H-3'), 1.74 (3H, d, *J* = 8.0 Hz, H-4'), and 1.72 (3H, s, H-5') in the ¹H NMR showed the typical feature of a tigloyl moiety. An extensive analysis of its ¹H and ¹³C NMR data (Tables 1 and 2) permitted the establishment of the

Table 1. ¹H NMR Data of Compounds **1–10** (400 MHz, measured in CDCl₃)

	1 (multi, <i>J</i> (Hz))	2 (multi, <i>J</i> (Hz))	3 (multi, <i>J</i> (Hz))	4 (multi, <i>J</i> (Hz))	5 (multi, <i>J</i> (Hz))
1	3.55*	3.37 (br d, 9.3)	3.00 (t like, 8.8, 7.3)	2.45*	2.61 (br s)
2	6.00 (dd, 5.8, 2.6)	5.76 (dd, 5.6, 2.6)	5.25 (dd, 7.3, 1.7)	4.54 (dd, 4.1, 1.7)	4.59*
3	5.87 (d, 5.8)	5.94 (d, 5.6)	5.48 (d, 1.7)	5.72 (br s)	5.74 (br s)
5	2.86 (dd, 11.9, 10.4)	2.42 (dd, 10.8, 9.3)	3.16 (t like, 9.2, 8.8)	2.73 (dd, 11.0, 7.3)	2.76 (dd, 10.9, 7.4)
6	4.71 (dd, 11.9, 9.8)	4.97 (dd, 10.8, 9.2)	4.62 (t like, 10.2, 9.2)	4.49 (dd, 11.0, 8.7)	4.59 (dd, 10.9, 8.7)
7	3.13 (br d, 9.8)	3.23 (m)	3.49 (m)	3.75 (dd, 8.7, 4.0)	3.93*
8	5.70 (m)	5.65 (m)	5.82 (m)	4.42 (td, 8.1, 4.0)	5.64 (td, 8.4, 4.7)
9	2.71 (dd, 15.4, 3.8)	2.81 (dd, 14.3, 3.2)	2.41 (dd, 14.7, 5.5)	2.31 (m)	2.43 (m)
	1.82*	2.35 (dd, 14.3, 3.3)	1.67 (dd, 14.7, 8.9)	2.42*	2.50 (m)
13	6.34 (d, 3.4)	6.31 (d, 3.4)	6.33 (d, 3.6)	6.40 (d, 3.8)	6.26 (d, 3.7)
	5.60 (d, 3.0)	5.63 (d, 3.0)	5.56 (d, 3.2)	5.58 (d, 3.2)	5.44 (d, 3.3)
14	3.63 (d, 10.9)	4.96 (br s)	3.67 (d, 9.8)	3.95 (d, 11.1)	3.92 (d, 11.1)
	3.57 (d, 10.9)	4.92 (br s)	3.54 (d, 9.8)	3.67 (d, 11.1)	3.66 (d, 11.1)
15	1.50 (3H, s)	1.56 (3H, s)	1.93 (3H, d, 1.4)	2.00 (3H, s)	2.02 (3H, s)
3'	6.72 (br t, 6.0)	6.73 (br t, 6.0)	6.71 (br t, 6.0)		6.70 (br q, 8.0)
4'	4.35 (2H, d, 6.0)	4.30 (2H, br d, 6.0)	4.34 (2H, br d, 6.0)		1.74 (3H, d, 8.0)
5'	1.82 (3H, s)	1.77 (3H, s)	1.78 (3H, s)		1.72 (3H, s)
	6^a (multi, <i>J</i> (Hz))	7 (multi, <i>J</i> (Hz))	8 (multi, <i>J</i> (Hz))	9^a (multi, <i>J</i> (Hz))	10 (multi, <i>J</i> (Hz))
1	2.42 (m)	1.71 (dd, 8.5, 5.0)	2.43*	2.29*	2.57 (dd, 7.7, 4.4)
2	4.55 (br s)	4.38 (d, 5.0)	4.34 (d, 4.5)	4.22*	5.29 (d, 4.4)
3	5.73 (br s)	3.31 (br s)	3.28 (br s)	3.25 (br s)	3.12 (br s)
5	2.71 (dd, 10.7, 7.3)	2.50 (dd, 11.5, 8.5)	2.56 (dd, 11.3, 7.8)	2.46 (dd, 11.4, 7.6)	2.64 (dd, 10.9, 7.7)
6	4.70 (dd, 10.7, 8.3)	4.84 (dd, 11.5, 8.2)	4.77 (dd, 11.3, 8.5)	4.83 (dd, 11.4, 8.8)	4.70 (dd, 10.9, 8.8)
7	4.04 (dd, 8.3, 4.3)	3.54 (dd, 8.2, 3.9)	3.96 (dd, 8.5, 4.3)	4.08 (dd, 8.8, 4.0)	4.05 (dd, 8.8, 3.9)
8	5.66 (td, 8.4, 4.3)	5.56 (ddd, 8.7, 7.4, 3.9)	5.60 (td, 8.3, 4.3)	5.62 (td, 8.2, 4.0)	5.63 (m)
9	2.35 (m)	2.79 (dd, 15.0, 7.4)	2.49 (dd, 14.8, 8.3)	2.46*	2.44 (dd, 14.8, 9.2)
		2.13 (dd, 15.0, 8.7)	2.43*		1.87 (dd, 14.8, 7.4)
13	6.17 (d, 3.8)	6.33 (d, 3.6)	6.26 (d, 3.7)	6.16 (d, 3.8)	6.27 (d, 3.0)
	5.45 (d, 3.3)	5.55 (d, 3.0)	5.43 (d, 3.3)	5.44 (d, 3.3)	5.45 (d, 2.7)
14	3.63 (d, 11.1)	2.73 (d, 4.9)	3.74 (d, 11.3)	3.51 (d, 11.2)	3.43 (d, 11.0)
	3.48 (d, 11.1)	2.64 (d, 4.9)	3.60 (d, 11.3)	3.40 (d, 11.2)	3.36 (d, 11.0)
15	1.99 (3H, s)	1.68 (3H, s)	1.70 (3H, s)	1.64 (3H, s)	1.70 (3H, s)
3'	6.68t6.0	6.69 (br t, 5.8)	6.72 (br q, 7.7)	6.69 (br t, 5.9)	6.71 (t, 5.6)
4'	4.21 (2H, d, 6.0)	4.32 (2H, d, 5.8)	1.75 (3H, d, 7.7)	4.22 (2H)*	4.32 (2H, br s)
5'	1.74 (3H, s)	1.75 (3H, s)	1.74 (3H, s)	1.75 (3H, s)	1.76 (3H, s)
COMe					2.06 (3H, s)

^a Measured in CD₃OD, *signal overlapped.**Table 2.** ¹³C NMR Data of Compounds **1–10** (100 MHz, measured in CDCl₃)

C	1	2	3	4	5	6^a	7	8	9^a	10
1	55.9	52.4	55.6	54.4	52.1	53.5	49.4	48.0	48.9	45.5
2	130.7	133.3	87.6	74.5	75.0	76.1	73.3	72.2	72.8	74.2
3	139.4	138.8	126.6	128.5	128.6	130.9	64.5	64.3	65.9	61.1
4	83.9	82.6	145.6	149.0	150.3	150.1	66.0	65.9	66.8	65.3
5	55.4	54.3	54.3	51.7	54.9	56.7	50.3	50.0	51.5	49.3
6	76.5	76.8	77.3	81.7	82.0	84.7	76.7	77.6	80.0	77.0
7	48.6	48.5	46.6	49.0	47.3	48.9	48.3	48.1	49.5	47.5
8	65.8	67.3	66.4	63.7	66.5	69.4	67.6	66.9	69.5	67.1
9	42.2	43.2	37.7	38.8	36.3	36.3	36.2	36.6	36.3	35.0
10	74.7	142.7	81.8	73.2	73.5	76.1	55.6	73.7	76.1	73.8
11	134.0	134.9	133.8	135.1	134.4	137.4	133.7	134.6	137.4	134.3
12	168.6	169.3	169.4	170.9	169.8	172.2	169.1	170.0	171.9	169.5
13	122.6	122.2	123.1	122.0	122.1	122.5	122.8	122.3	122.2	122.1
14	52.3	117.9	78.7	54.9	55.2	71.2	56.5	55.7	71.5	69.5
15	24.4	28.6	15.4	17.7	18.0	18.7	18.5	19.4	20.0	18.7
1'	166.2	166.6	166.4		167.3	168.8	166.3	167.6	168.8	166.8
2'	127.7	127.8	127.7		128.0	129.2	127.7	128.3	129.2	127.6
3'	141.4	141.6	141.3		138.2	143.3	140.7	138.6	143.3	141.5
4'	59.7	59.5	59.7		12.0	60.2	59.7	12.4	60.2	59.6
5'	12.7	12.6	12.9		14.3	13.2	12.9	14.7	13.2	12.8
COMe										169.8
COMe										21.2

^a Measured in CD₃OD.

backbone of this sesquiterpenoid, which is actually identical to that of **4**. Compared with **4**, the proton signal of H-8 (δ 5.64, td, *J* = 8.4, 4.7 Hz) in **5** was downfield shifted, indicating that the tigloyloxy moiety resided at C-8. Thus, the structure of **5** was established as 8 β -tigloyloxy-14-chloro-2 β ,10 α -dihydroxy-1 α H,5 α H,6 β H,7 α H-guai-3,11(13)-dien-6,12-olide.

Eupalinilide F (**6**) showed a molecular formula of C₂₀H₂₆O₈ as determined by HREIMS at *m/z* 394.1637 [M]⁺ (calcd 394.1628). The ¹H and ¹³C NMR spectroscopic data (Tables 1 and 2) showed six quaternary carbons, eight methines, four methylenes, and two methyls. The presence of an α -methylene- γ -lactone moiety was distinguishable by the carbon signals at δ 172.2 (carbonyl) and 137.4 and 122.5

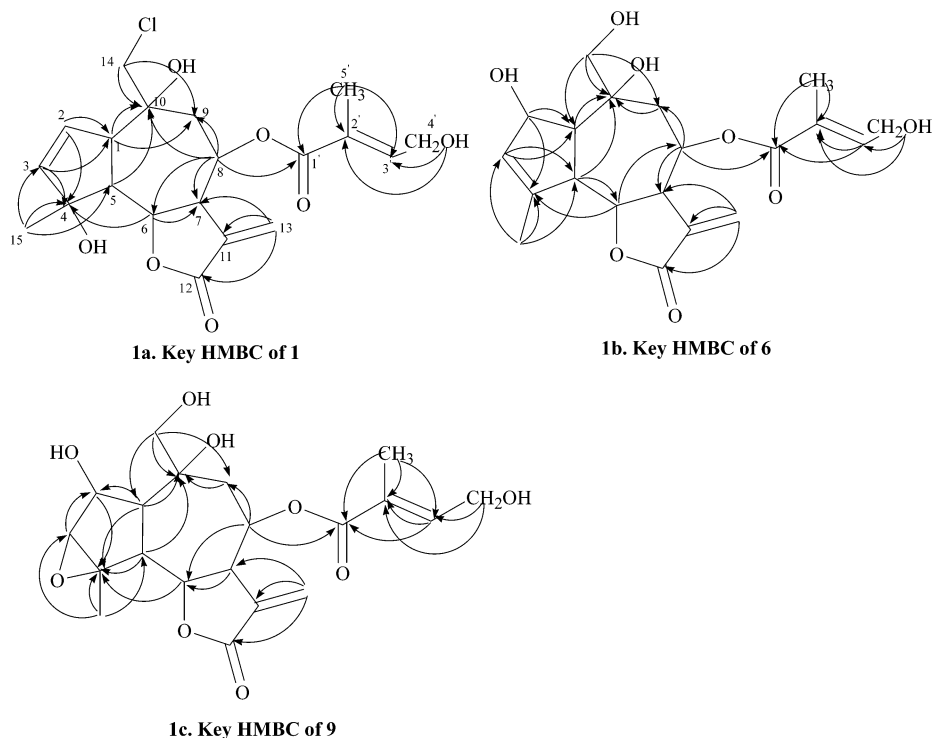


Figure 1. Selected HMBC correlations (H → C) of compounds **1**, **6**, and **9**.

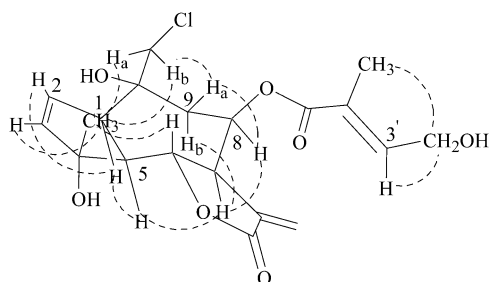


Figure 2. Key NOESY interactions (---) of compound **1**.

(terminal double bond) in the ^{13}C NMR spectrum, in which a guaiane type sesquiterpene lactone feature of **6** was also inferred. A tertiary carbon at δ_{C} 130.9 (C-3) and a quaternary carbon at δ_{C} 150.1 (C-4) were assigned to a Δ^3 double bond, which was confirmed by the correlation between H-3 at δ 5.73 (br s) and H-2 at δ 4.55 (br s) in the ^1H – ^1H COSY spectrum. By comparison of the ^1H and ^{13}C NMR spectroscopic data of **6** with those of eupachifolin D⁹ (**12**), both compounds exhibited very high similarity, except for the presence of an oxygenated C-14 methylene in **6**, instead of the chlorinated C-14 methylene in **12**. As a consequence, the proton signals of H₂-14 at δ 3.63 (d, J = 11.1 Hz) and 3.48 (d, J = 11.1 Hz) of **6** shifted slightly upfield, and the carbon signal of C-14 at δ 71.2 of **6** showed a significant downfield shift. The acyl group at C-8 was identified as a 4'-hydroxytigloyloxy moiety by ^1H and ^{13}C NMR spectroscopic data. The structural assignment of **6** was finally confirmed by 2D NMR techniques, such as HMBC (Figure 1b). The structure of **6** was established as 8 β -(4'-hydroxytigloyloxy)-2 β ,10 α ,14-trihydroxy-1 α H,5 α H,6 β H,7 α H-guai-3,11(13)-dien-6,12-olide.

Eupalinilide G (**7**) showed a molecular formula of $\text{C}_{20}\text{H}_{24}\text{O}_8$ as determined by HREIMS at m/z 392.1468 [M]⁺ (calcd 392.1471). The analysis of the ^1H and ^{13}C NMR data (Tables 1 and 2) indicated that **7** had also the backbone of a guaiane type sesquiterpenoid. Comparison of the ^1H and ^{13}C NMR data of **7** with those of the known compounds

eupachifolin E¹¹ and graminiliatrin⁵ indicated that the structure of **7** was similar except for the ester group at C-8. The proton signals at δ 1.75 (3H, s), 4.32 (2H, d, J = 5.8 Hz), and 6.69 (br t, J = 5.8 Hz) and carbon signals at δ 166.3 (carbonyl), 127.7, 140.7 (double bond), 59.7 (methylene), and 12.9 (methyl) were characteristic of a 4'-hydroxytigloyl moiety. Thus, the structure of **7** was identified as 8 β -(4'-hydroxytigloyloxy)-3 α ,4 α :10 α ,14-diepoxy-2 β -hydroxy-1 α H,5 α H,6 β H,7 α H-guai-11(13)-en-6,12-olide.

Eupalinilide H (**8**) had a molecular formula of $\text{C}_{20}\text{H}_{25}\text{ClO}_7$ by HREIMS at m/z 412.1285 [M]⁺ (calcd 412.1289). The ^1H and ^{13}C NMR data (Tables 1 and 2) of **8** were evidence of a guaiane type sesquiterpenoid. The ^1H and ^{13}C NMR data of **8** showed that its structure was closely related to the known compound graminichlorin,⁵ the only difference was the ester moiety at C-8. The ester group at C-8 for **8** was assigned as a tigloyloxy moiety by ^1H and ^{13}C NMR data. Thus, the structure of **8** was elucidated as 8 β -tigloyloxy-14-chloro-3 α ,4 α -epoxy-2 β ,10-dihydroxy-1 α H,5 α H,6 β H,7 α H-guai-11(13)-en-6,12-olide.

Eupalinilide I (**9**) showed a molecular ion in its HREIMS at m/z 410.1579 [M]⁺ corresponding to the molecular formula of $\text{C}_{20}\text{H}_{26}\text{O}_9$ (calcd 410.1577). The ^1H and ^{13}C NMR data (Tables 1 and 2) of **9** also showed the framework of a guaiane type sesquiterpenoid. Comparison of the ^1H and ^{13}C NMR data of **9** with those of eupachinilide E³ (**13**) implied that the structures of the two compounds were closely related, and the only difference was the occurrence of an oxygenated methylene at C-14 in **9** instead of the chlorinated one in **13**. The oxygenated methylene was distinguished by both the ^1H and ^{13}C NMR spectra, in which the carbon signal at δ 71.5 was assignable to C-14, and the proton signals at δ 3.51 and 3.40 (each 1H, d, J = 11.2 Hz) were attributable to H-14a and H-14b, respectively. The structure of **9** was thus elucidated as 8 β -(4'-hydroxytigloyloxy)-3 α ,4 α -epoxy-2 β ,10 α ,14-trihydroxy-1 α H,5 α H,6 β H,7 α H-guai-11(13)-en-6,12-olide, and this structure was further confirmed by the ^1H – ^1H COSY, HMQC, and HMBC spectra (Figure 1c).

Table 3. Cytotoxic Activities of Compounds **2**, **3**, **5**, **6**, and **9** against P-388 and A-549 Tumor Cell Lines

	IC ₅₀ (μg/mL)	
	P-388	A-549
2	0.21	0.75
3	1.20	11.0
5	NA	0.028
6	NA	NA
9	NA	NA
PAB ^a	0.32	0.86

^a Pseudolaric acid B was used as positive control; NA stands for not active.

Eupalinilide **J** (**10**) had a molecular formula of C₂₂H₂₈O₁₀ as determined by HREIMS at *m/z* 452.1680 [M]⁺ (calcd 452.1682). The EIMS and ¹H and ¹³C NMR data (Tables 1 and 2) indicated that **10** had one more acetyl group (δ_C 169.8, 21.2 and δ_H 2.06, s) than **9**. Further analysis of the ¹H and ¹³C NMR data of both **9** and **10** showed high similarity, except that the chemical shift of H-2 at δ 5.29 (d, *J* = 4.4 Hz) in **10** was shifted downfield, indicating that the acetoxy group in **10** was linked to C-2. Thus, the structure of **10** was elucidated to be 8 β -(4'-hydroxytigloyloxy)-3 α ,4 α -epoxy-2 β -acetoxy-10 α ,14-dihydroxy-1 α H,5 α H,6 β H,7 α H-guai-11(13)-en-6,12-olide. The structure of **10** was further confirmed by the ¹H-¹H COSY, HMQC, and HMBC spectra.

The nine known sesquiterpenoids were identified as eupachinilide C (**11**),³ eupachifolin D (**12**),¹¹ eupachinilide E (**13**),³ 2 α -hydroxyeupatolide (**14**),¹³ 3-deacetyeupalinin A (**15**),² heliangine (**16**),¹³ 8 β -tigloyloxy-2,3-seco-6 β H,7 α H-helianga-4Z,11(13)-diene-3,10 β ;6,12-diolid-2-oic acid (**17**),¹⁵ 8 β -(4'-hydroxytigloyloxy)-3 β ,14-dihydroxy-6 β H,7 α H-germacra-1(10)Z,4Z,11(13)-trien-6,12-olide (**18**),⁷ and 8 β -tigloyloxy-3 β ,14-dihydroxy-6 β H,7 α H-germacra-1(10)Z,4E,11(13)-trien-6,12-olide (**19**)¹⁴ by comparison of their spectroscopic data with those reported.

Cytotoxicity Evaluation of Eupalinilides B (2), C (3), E (5), F (6), and I (9). Eupalinilides B (**2**), C (**3**), E (**5**), F (**6**), and I (**9**) were tested for cytotoxicity against P-388 and A-549 tumor cell lines according to standard protocols,¹⁶ and pseudolaric acid B¹⁷ was used as positive control. The results showed that eupalinilides B (**2**) and E (**5**) demonstrated potent cytotoxicity (Table 3).

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 341 polarimeter (Na filter, λ = 589 nm). IR spectra were recorded on a Perkin-Elmer 577 spectrometer with KBr disk. ¹H and ¹³C NMR spectra were recorded on a Bruker AM-400 spectrometer with TMS as internal standard. EIMS (70 eV) spectra were recorded on a Finnigan MAT 95 instrument. All solvents used were of analytical grade (Shanghai Chemical Plant). Silica gel (200–300 mesh) and Sephadex LH-20 were used for column chromatography, and precoated silica gel GF₂₅₄ plates (Qingdao Haiyang Chemical Plant, Qingdao, People's Republic of China) used for TLC. C18 reversed-phase silica gel (250 mesh, Merck) and MCI gel (CHP20P, 75–150 μm, Mitsubishi Chemical Industries Ltd.) were also used for column chromatography.

Plant Material. The whole plant of *Eupatorium lindleyanum* DC. was collected from Songyang County of Zhejiang Province, People's Republic China, and was identified by Prof. Zeng-Tao Wang and Dr. Mian Zhang of Shanghai Traditional Chinese Medical University. A voucher specimen has been deposited in the Herbarium of Shanghai Institute of Materia Medica (accession number Eup-2001-2Y).

Extraction and Isolation. The dried powder of the whole plant (2.2 kg) of *E. lindleyanum* was percolated with 95% EtOH. After removal of the EtOH under reduced pressure, a dark green residue (64.5 g) was obtained, which was then subjected to silica gel column chromatography using a gradient solvent system of petroleum–acetone (100:1 to 0:100; v/v) to obtain six major fractions, 1–6. Fraction 3 (1.37 g) was separated by silica gel column chromatography eluted by a gradient of MeOH in CHCl₃ (CHCl₃–MeOH, 100:1 to 20:1) to yield **5** (73 mg) and **8** (46 mg), sequentially. Fraction 4 was fractionated by silica gel column chromatography eluted also by a gradient of MeOH in CHCl₃ (CHCl₃–MeOH, 100:1–10:1) to collect major subfractions 4a–4h. Each of the subfractions 4a–4h was purified by reverse-phased C-18 silica gel column eluted with 40% H₂O in MeOH to afford **1** (12 mg), **2** (10 mg), **4** (12 mg), **11** (583 mg), **12** (8 mg), **13** (13 mg), **14** (20 mg), and **16** (27 mg), respectively. Fraction 5 was further fractionated by MCI gel column chromatography eluted with 45% H₂O in MeOH to give nine major subfractions, 5a–5i. Each of the subfractions 5a–5i was subjected to reverse-phased C-18 silica gel column chromatography eluted with 40% H₂O in MeOH and then a silica gel column (CHCl₃–MeOH, 10:1) to yield **3** (15 mg), **6** (74 mg), **7** (7 mg), **9** (19 mg), **10** (12 mg), **15** (50 mg), **17** (8 mg), **18** (7 mg), and **19** (9 mg), respectively.

Eupalinilide A (1): colorless gum; [α]_D²⁰ –34.3° (*c* 0.47, CHCl₃); IR (KBr) ν_{\max} 3431, 2924, 1751, 1713, 1647, 1448, 1379, 1252, 1130, 1011 cm⁻¹; ¹H NMR, see Table 1; ¹³C NMR, see Table 2; EIMS *m/z* 414 [C₂₀H₂₅³⁷ClO₇]⁺ (1), 412 [M]⁺ (3), 397 (5), 361 (4), 278 (13), 260 (10), 242 (10), 229 (30), 211(12), 129 (11), 99 (100), 71 (45), 69 (11); HREIMS *m/z* 412.1285 (calcd for C₂₀H₂₅ClO₇, 412.1289).

Eupalinilide B (2): colorless gum; [α]_D²⁰ –84.9° (*c* 0.67, CHCl₃); IR (KBr) ν_{\max} 3439, 2968, 2933, 1763, 1709, 1651, 1323, 1257, 1138, 1007, 733 cm⁻¹; ¹H NMR, see Table 1; ¹³C NMR, see Table 2; EIMS *m/z* [M]⁺ 360 (4), 345 (8), 260 (13), 242 (14), 229 (9), 213 (19), 197 (7), 162 (5), 135 (4), 129 (6), 99(34), 83 (100), 71 (30); HREIMS *m/z* 360.1566 (calcd for C₂₀H₂₄O₆, 360.1573).

Eupalinilide C (3): white powder; [α]_D²⁰ –7.0° (*c* 1.0, CHCl₃); IR (KBr) ν_{\max} 3380, 3313, 2925, 2880, 1761, 1705, 1655, 1435, 1327, 1271, 1124, 1003, 959, 908, 820, 735 cm⁻¹; ¹H NMR, see Table 1; ¹³C NMR, see Table 2; EIMS *m/z* [M]⁺ 376- (5), 361 (6), 346 (9), 328 (24), 260 (9), 242 (11), 230 (40), 229 (25), 212 (10), 185 (5), 131 (5), 123 (19), 107 (14), 99 (100), 71 (45), 69 (11); HREIMS *m/z* 376.1520 (calcd for C₂₀H₂₄O₇, 376.1522).

Eupalinilide D (4): colorless gum; [α]_D²⁰ –59.4° (*c* 0.8, CHCl₃); IR (KBr) ν_{\max} 3415, 2929, 1751, 1643, 1437, 1383, 1271, 1151, 1022 cm⁻¹; ¹H NMR, see Table 1; ¹³C NMR, see Table 2; EIMS *m/z* 316 [C₁₅H₁₉³⁷ClO₅]⁺ (1), 314 [M]⁺ (3), 260 (2), 248 (5), 236 (8), 220 (36), 213 (30), 195 (100), 177 (80), 159 (85), 135 (40), 107 (36), 93 (42), 81 (38), 69 (34); HREIMS *m/z* 314.0931 (calcd for C₁₅H₁₉ClO₅, 314.0921).

Eupalinilide E (5): white powder; [α]_D²⁰ –56.2° (*c* 1.0, CHCl₃); IR (KBr) ν_{\max} 3647, 3481, 3348, 2912, 1751, 1709, 1651, 1398, 1275, 1144, 1014, 949, 818, 731, 581 cm⁻¹; ¹H NMR, see Table 1; ¹³C NMR, see Table 2; EIMS *m/z* 398 [C₂₀H₂₅³⁷ClO₆]⁺ (1), 396 [M]⁺ (3), 378 [M – H₂O]⁺ (1), 342 (3), 329 (10), 278 (16), 229 (33), 199 (34), 83 (100), 55 (85); HREIMS *m/z* 396.1342 (calcd for C₂₀H₂₅ClO₆, 396.1340).

Eupalinilide F (6): colorless gum; [α]_D²⁰ –38.0° (*c* 1.3, CH₃OH); IR (KBr) ν_{\max} 3383, 2926, 1755, 1699, 1651, 1259, 1136, 1013, 754 cm⁻¹; ¹H NMR, see Table 1; ¹³C NMR, see Table 2; EIMS *m/z* [M]⁺ 394 (3), 376 (4), 361 (10), 346 (20), 328 (90), 277 (4), 230 (18), 99 (100), 95 (8), 71 (45); HREIMS *m/z* 394.1637 (calcd for C₂₀H₂₆O₈, 394.1628).

Eupalinilide G (7): colorless gum; [α]_D²⁰ –44.3° (*c* 0.47, CHCl₃); IR (KBr) ν_{\max} 3439, 2926, 2854, 1759, 1713, 1655, 1456, 1325, 1259, 1126, 1014, 960, 825, 756, 567 cm⁻¹; ¹H NMR, see Table 1; ¹³C NMR, see Table 2; EIMS *m/z* [M]⁺ 392 (6), 375 (2), 363 (5), 259 (16), 231(10), 202 (12), 187 (25), 159 (14), 123 (17), 99 (100), 71 (70), 69 (36); HREIMS *m/z* 392.1468 (calcd for C₂₀H₂₄O₈, 392.1471).

Eupalinilide H (8): colorless gum; $[\alpha]_D^{20} -45.0^\circ$ (*c* 1.5, CHCl₃); IR (KBr) ν_{\max} 3462, 2935, 1751, 1705, 1649, 1267, 1140, 1007, 824, 733, 501 cm⁻¹; ¹H NMR, see Table 1; ¹³C NMR, see Table 2; EIMS *m/z* 414 [C₂₀H₂₅³⁷ClO₇]⁺ (1), 412 [M]⁺ (3), 394 (2), 376 (7), 359 (10), 329 (5), 312 (15), 294 (20), 259 (17), 187 (15), 157 (13), 123 (25), 83 (100), 55 (40); HREIMS *m/z* 412.1285 (calcd for C₂₀H₂₅ClO₇, 412.1289).

Eupalinilide I (9): colorless gum; $[\alpha]_D^{20} -43.4^\circ$ (*c* 1.0, CH₃-OH); IR (KBr) ν_{\max} 3419, 2935, 1740, 1701, 1651, 1425, 1335, 1265, 1142, 999, 825, 557 cm⁻¹; ¹H NMR, see Table 1; ¹³C NMR, see Table 2; EIMS *m/z* [M]⁺ 410 (6), 392 (10), 379 (10), 361 (16), 294 (5), 229 (11), 203 (15), 187 (14), 159 (20), 123 (30), 99 (70), 95 (100), 71 (65); HREIMS *m/z* 410.1579 (calcd for C₂₀H₂₆O₉, 410.1577).

Eupalinilide J (10): colorless gum; $[\alpha]_D^{20} -46.7^\circ$ (*c* 0.68, CHCl₃); IR (KBr) ν_{\max} 3439, 2935, 1749, 1713, 1651, 1373, 1236, 1134, 1020, 824, 523 cm⁻¹; ¹H NMR, see Table 1; ¹³C NMR, see Table 2; EIMS *m/z* [M]⁺ 452 (5), 421 (1), 403 (2), 362 (2), 361 (6), 343 (5), 336(3), 305 (2), 276 (5), 258 (10), 245 (17), 217 (7), 201 (3), 175(5), 167 (6), 154 (8), 125 (11), 123 (22), 99 (42), 95 (100), 71 (28); HREIMS *m/z* 452.1680 (calcd for C₂₂H₂₈O₁₀, 452.1682).

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