Cytotoxic Sesquiterpenoids from *Eupatorium chinense*

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Ten new sesquiterpenoids, namely, eupachinilides A−J (**1**−**10**), together with seven known sesquiterpenoids, eupachifolin D (**11**), budlein B (**12**), 8*β*-(4′-hydroxytiglyloxy)-2*β*-hydroxy-1α*H*,5α*H*,6*βH*,7α*H*-guai-3,10(14),11(13)-trien-6,12-olide (**13**), 1,10-hydrobahia (**14**), 2α-hydroxyeupatolide (**15**), eupaserrin (**16**), and mollisorin B (**17**), were isolated from the whole plant of *Eupatorium chinense*. Their structures were elucidated mainly by spectral methods, especially 2D NMR techniques. Eupachinilides A (**1**), E (**5**), F (**6**), and I (**9**) exhibited moderate cytotoxic activities against several tumor cell lines. The structures assigned previously for eupachifolins B (**11a**), C (**13a**), and D (**11**) were revised by spectral analysis and 2D NMR techniques.

Plants of *Eupatorium chinense* L. (Compositae) and its two varieties, *E. chinense* var. *simplicifolium* and *E. chinense* var. *tripartitum*, are indigenous to China,[1] and its hot water extract is traditionally used for the treatments of cold, snakebite, and inflammation.[2] The methanolic extract of this plant showed anticancer activity against Hela cells *in vivo*.[2] The intriguing applications in traditional Chinese medicine and the reported anticancer activity of this plant have driven us to conduct the current investigation.

The *E. chinense* plant material used in the present study was collected from Songyang County of Zhejiang Province, China, where it is known as "Da-Fa-San", used for cold treatment by local residents and has a very bitter taste. Seven guaiane-type sesquiterpenoids were isolated previously from *E. chinense* L. var. *hakonense*,[3] and a few sesquiterpenoids, eupachifolins A−E, were obtained from *E. chinense* var. *simplicifolium*.[4] In the current research, 10 new sesquiterpenoids, namely, eupachinilides A−J (**1**−**10**), as well as seven known sesquiterpenoids, eupachifolin D (**11**), budlein B (**12**), 8*β*-(4′-hydroxytiglyloxy)-2*β*-hydroxy-1α*H*,5α*H*,6*βH*,7α*H*-guai-3,10(14),11(13)-trien-6,12-olide (**13**), 1,10-hydrobahia (**14**), 2α-hydroxyeupatolide (**15**), eupaserrin (**16**), and mollisorin B (**17**), were isolated from the whole plant of *E. chinense*. Herein, we report the isolation and structural elucidation of these sesquiterpenoids and their cytotoxic activities. The revision of the structures assigned previously for eupachifolins B (**11a**), C (**13a**), and D (**11**) is also briefly discussed in this paper.

Results and Discussion

Eupachinilide A (**1**) was obtained as a white powder. The HREIMS spectrum of **1** showed the molecular ion at *m*/*z* 378.1633 to establish the molecular formula of $C_{20}H_{26}O_7$ (calcd 378.1679) with eight degrees of unsaturation. The ^{1}H and ^{13}C NMR (Tables 1 and 2) revealed the presence of three methyls (δ_H 1.89, 3H, dd, J = 7.2, 1.0 Hz, δ_C 15.8; δ_H 1.78, 3H, s, δ_C 20.6; δ_H 1.59, 3H, s, δ_C 23.8), one hydroxymethylene (δ_H 4.18, 3.75; δ_C 65.9), three oxygenated methines (δ_H 3.87, δ_C 78.7; δ_H 4.40, δ_C 76.8; δ_H 5.70, δ_C 65.2), one exocyclic double bond (δ_H 6.24, 5.57, δ_C 120.7; δ_C 135.0), one trisubstituted double bond (δ_H 6.06, δ_C 138.9; δ_C 127.4), one persubstituted double bond (δ_C 141.3 and 130.0), two carbonyls (δ_C 170.0 and 167.1), and one sp^3 oxygenated quaternary carbon (δ_C 82.6). Three double bonds and two carbonyls accounted for five degrees of unsaturation. The remaining three degrees of unsaturation were attributed to a tricyclic ring system in compound **1**.

Comparison of the NMR data of **1** with those of euponin[5] (**1a**), which was also isolated from this genus (*E. japonicum*), suggested that **1** was a tricyclic guaiane-type sesquiterpenoid. In the HMBC spectrum (Figure 1a), H_3-5′ (δ 1.78, 3H, s) correlated with C-1′ (δ 167.1), C-2′ (δ 127.4), and C-3′ (δ 138.9) and H_3-4′ (δ 1.89, 3H, dd, J = 7.2, 1.0 Hz) correlated with C-2′ and C-3′, indicating the presence of a 2′-methyl-2′-butenoxyl moiety. In the NOESY spectrum (Figure 1b), H-3′ showed cross-peaks with both H_3-4′ and H_3-5′, clearly inferring a 2′-methyl-2′*Z*-butenoxyl moiety (angelyloxyl). The downfield shifted proton signal at δ 4.40 was assigned to H-6, and this inferred the presence of a five-membered lactone with an exocyclic double bond as in euponin,[5] and this was supported by HMBC correlations. In the HMBC spectrum, the proton signal at δ 6.24 (H-13a) correlated with C-11 (δ_C 135.0) and C-12 (δ 170.0); H-6 correlated with C-5 (δ 54.6) and C-11; and H-7 at δ 3.13 correlated with the C-6 (δ 76.8). One oxygenated methine was assigned to CH-8 (δ_H 5.70; δ_C 65.2) bearing the 8-angelyloxyl moiety by HMBC. H-8 correlated with C-7 (δ 54.5), C-11, and C-1′, and the downfield shifted proton signal of H-8 supported this connectivity. A $\Delta^{1(10)}$ double bond was assignable on the basis of the HMBC correlations, in which, H_2-2 (δ 2.91, 2.54), H-5 (δ 2.88), H_2-9 (δ 2.98, 2.45), and H_2-14 (δ 4.18, 3.75) showed correlations with both C-1 (δ 141.3) and C-10 (δ 130.0). The oxygenated methine (δ_H 3.87, δ_C 78.7) was attributable to C-3 bearing a hydroxyl judging from the HMBC correlations between H-3 and C-2 (δ 38.0) and between H-3 and C-4. The remaining hydroxyl was placed on the quaternary carbon C-4 by HMBC correlations with H-3, H-5, and H_3-15.

The relative stereochemistry of **1** was fixed by extensive analysis of NOESY correlations (Figure 1b), in which H-5 correlated with H-7, H-2b, H-3, and H_3-15, indicating that H-7, H-2b, H-3, and H_3-15 were in α-orientation since the H-5 of the guaiane-type sesquiterpenoid is defined in the α-configuration. The H-2a was assigned as a *β*-configuration, and it showed a key correlation with H-6, suggesting the *β*-orientation of H-6. The NOESY correlation between H-7α and H-8 indicated that H-8 was α-orientated. A 3D structure (Figure 1c) of **1** was generated by computer

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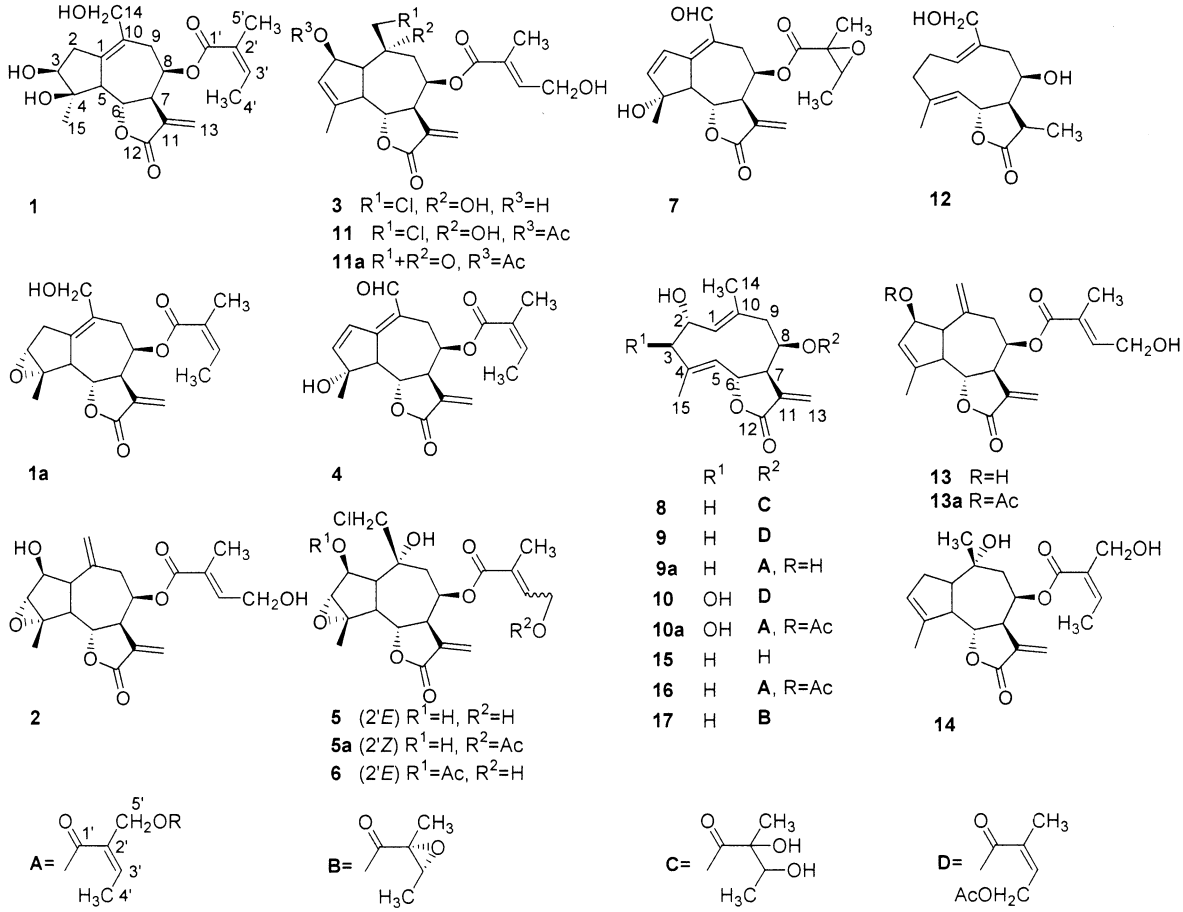
Chart 1



modeling using the molecular modeling program CS Chem 3D Pro Version 6.0, and the MM2 force field calculation was applied for energy minimization. The relative stereochemistry and a favorable conformation of **1** offered by computer modeling were consistent with those of **1** assigned in the NOESY experiment. The structure of eupachinilide A (**1**) was thus unambiguously elucidated as 8*β*-angelyloxy-3*β*,4*β*,14-trihydroxy-5α*H*,6*β**H*,7α*H*-guai-1(10),11(13)-diene-6,12-olide.

Eupachinilide B (**2**) was determined to have a molecular formula $C_{20}H_{24}O_7$ by HREIMS. The carbon signals (Table 2) assigned for the seven-membered ring, five-membered lactone, and the ester moiety were very close to those of eupachifolin C (**13a**)[4] isolated from *E. chinense* var. *simplicifolium*, implying that the partial structure (the aforementioned three subunits) in both compounds was identical. This was consistent with the 2D NMR experiments (HMBC, HMQC, and NOESY; see Figure 2a,b) of **2**. A methine group attributable to CH-3 (δ_C 65.1, δ_H 3.33) and a quaternary carbon assigned for C-4 (δ_C 66.2) indicated the presence of an 3,4-epoxyl and was supported by the HMBC correlations of H_3-15 (δ 1.65, 3H, s) with C-4, C-3, and C-5 (δ 49.5). A carbon signal at δ 75.9 was allocated to the C-2 bearing a hydroxyl as judged by HMBC correlations between H-1 (δ 2.95) and C-2 and between H-3 (δ 3.33) and C-2.

The stereochemistry of **2** was determined via a NOESY experiment (Figure 2b,c) in which H_3-15 correlated with H-6 having been defined in the *β*-orientation, and as a consequence, the epoxyl was α-orientated; H-2 correlating with H-1 was assigned as α-orientated. The H_2-4′ correlating with both H-3′ (δ 6.68) and H_3-5′ clearly revealed the *E* geometry of the ester moiety. The *E* geometry of the ester moiety assigned for **2** was also supported by the relatively downfield shifted proton signal of H-3′. In similar compounds having *E* geometry, H-3′ normally appears at δ 6.60−7.15,[6] while for those with *Z* geometry, the chemical shift of H-3′ usually appears around δ 6.04.[6a] The structure of eupachinilide B (**2**) was therefore identified as 8*β*-(4′-hydroxytiglyloxy)-3α,4α-epoxy-2*β*-hydroxy-1α*H*,5α*H*,6*β**H*,7α*H*-guai-1(10),11(13)-diene-6,12-olide (**2**).

Eupachinilide C (**3**) showed a molecular formula $C_{20}H_{25}ClO_7$ as determined by HREIMS. The EIMS ions at *m*/*z* 396 (2%) and 394 (6%) $[M - H_2O]^+$ supported the occurrence of an atom of chlorine in the molecule. Twenty carbon signals were resolved in its ^{13}C NMR spectrum. A chloromethylene (δ_C 55.9, δ_H 3.87, and δ_H 3.64) was distinguished by its somewhat upfield shifted carbon signal[6b,7] and nearly unchanged proton signals[6−8] compared with those of an oxygenated methylene. A guaiane-type skeleton was elucidated by analysis of its ^1H NMR and ^{13}C NMR data. Comparison of the EIMS, ^1H NMR, and ^{13}C NMR data of **3** with those of a known compound, eupachifolin D[4] (**11**), indicated that **3** was deacetyl eupachifolin D. The H-2 signal (δ 4.54) in **3** was upfield shifted about $\Delta\delta$ 0.84 ppm compared with that of **11**, supporting the structure of **3**. The 2D NMR spectra of **3**, including HMQC, HMBC, and NOESY, were applied to further confirm the structure of eupachinilide C (**3**).

Eupachinilide D (**4**) showed a molecular formula $C_{20}H_{22}O_6$ as determined by HREIMS. Analysis of 1D and 2D NMR spectra (^1H, ^{13}C NMR, HMQC, HMBC, and NOESY) inferred that compound **4** had the same ester moiety, seven-membered ring, and five-membered lactone as that

Table 1. ^{1}H NMR Chemical Shifts (δ) for Compounds **1**−**10**

	1[a] (multi, *J* (Hz))	**2**[a] (multi, *J* (Hz))	**3**[b] (multi, *J* (Hz))	**4**[a] (multi, *J* (Hz))	**5**[c] (multi, *J* (Hz))	**6**[a] (multi, *J* (Hz))
1		2.95 (dd, 9.5, 5.2)	2.50 (t, 6.1)		2.31 (m)	2.64 (br d, 8.8)
2	2.91 (br d, 11.0) 2.54 (br d, 16.4)	4.31 (br d, 5.5)	4.54 (m)	7.10 (d, 5.8)	4.17 (br d, 4.8)	5.29 (br d, 3.2)
3	3.87 (d, 3.8)	3.33 (br s)	5.73 (br s)	6.59 (dd, 5.8, 0.7)	3.23 (s)	3.37 (d, 1.0)
5	2.88 (d, 9.2)	2.32 (dd, 11.3, 8.7)	2.73 (dd, 10.6, 7.3)	3.41 (dd, 11.3, 2.2)	2.47 (dd, 11.5, 8.0)	2.67 (d, 9.1)
6	4.40 (t, 10.5)	4.81 (dd, 11.3, 8.2)	4.68 (dd, 10.6, 8.5)	4.56 (t, 10.8)	4.76 (dd, 11.5, 8.8)	4.69 (dd, 10.7, 8.8)
7	3.13 (dd, 10.5, 1.6)	3.15 (m)	4.01 (m)	3.19 (m)	3.96 (m)	4.00 (m)
8	5.70 (d, 5.9)	5.44 (m)	5.67 (m)	5.91 (br d, 4.8)	5.54 (m)	5.67 (d, 9.1)
9	2.98 (dd, 15.6, 5.9)	2.89 (2H, br d, 7.2)	2.55 (dd, 14.7, 8.3)	3.76 (dd, 16.7, 5.1)	2.47 (dd, 14.3, 8.8)	2.51 (dd, 14.8, 8.9)
	2.45 (d, 15.6)		2.39 (ddd, 14.7, 8.4, 1.5)	2.21 (br d, 6.6)	2.32 (m)	1.96 (dd, 14.8, 7.3)
13	6.24 (d, 3.1) 5.57 (d, 2.8)	6.27 (d, 3.5) 5.48 (d, 3.1)	6.18 (d, 3.6) 5.45 (d, 3.3)	6.30 (d, 3.3) 5.73 (d, 3.0)	6.17 (d, 4.0) 5.38 (d, 3.3)	6.28 (d, 3.7) 5.46 (d, 3.3)
14	4.18 (d, 11.5) 3.75 (d, 11.5)	5.05 (d, 1.4) 5.03 (br s)	3.87 (d, 11.7) 3.64 (d, 11.7)	10.09 (s)	3.65 (d, 11.4) 3.52 (d, 11.4)	3.53 (d, 11.5) 3.49 (d, 11.5)
15	1.59 (3H, s)	1.65 (3H, s)	2.00 (3H, s)	1.57 (3H, s)	1.63 (3H, s)	1.69 (3H, s)
3′	6.06 (qd, 7.2, 1.0)	6.68 (br t, 6.0)	6.69 (br t, 5.9)	6.03 (br q, 7.2)	6.62 (br t, 5.9)	6.71 (br t, 5.9)
4′	1.89 (3H, dd, 7.2, 1.0)	4.29 (2H, dd, 6.0, 0.9)	4.21 (2H, br d, 5.9)	1.85 (3H, br.d, 7.3)	4.19 (dd, 5.9, 1.1)	4.33 (2H, dd, 6.0, 1.1)
5′	1.78 (3H, s)	1.76 (3H, d, 1.1)	1.74 (3H, d, 1.4)	1.72 (3H, br.s)	1.68 (3H, d, 1.1)	1.79 (3H, d, 1.1)
CO*Me*						2.08 (3H, s)

	7[a] (multi, *J* (Hz))	**8**[a] (multi, *J* (Hz))	**9**[a] (multi, *J* (Hz))	**10**[a] (multi, *J* (Hz))	**10a**[a,d] (multi, *J* (Hz))	**13**[a,d] (multi, *J* (Hz))
1		4.97 (d, 10.1)	5.03 (d, 9.9)	5.09 (br d, 9.9)	5.10 (br d, 9.5)	3.42 (dd, 8, 6)
2	7.09 (d, 5.9)	4.76 (dd, 9.7, 5.8)	4.71 (ddd, 9.9, 9.9, 5.9)	4.42 (dd, 9.9, 7.6)	4.45 (dd, 9.5, 8)	5.58 (m)
3	6.61 (dd, 5.8, 0.9)	2.74 (dd, 11.1, 5.8) 2.13 (t, 10.5)	2.70 (dd, 10.0, 5.8) 2.11 (t, 10.3)	4.10 (d, 7.6)	4.12 (d, 8)	5.64 (br s)
5	3.40 (dd, 11.3, 2.2)	5.08 (d, 9.9)	4.98 (d, 10.2)	5.04 (br d, 10.1)	5.04 (br d, 10)	2.72 (m)
6	4.53 (t, 10.8)	5.05 (t, 8.6)	5.06 (dd, 10.0, 8.7)	5.12 (dd, 10.1, 8.5)	5.13 (dd, 10, 9)	4.59 (dd, 11, 8.5)
7	3.17 (m)	2.99 (m)	2.97 (m)	2.96 (m)	2.96 (m)	3.18 (m)
8	5.90 (br d, 4.8)	5.81 (br s)	5.76 (br d, 4.0)	5.77 (br d, 3.3)	5.86 (br dd, 5, 2, <1)	5.56 (m)
9	3.74 (dd, 16.8, 5.2) 2.20 (br d, 6.8)	2.87 (dd, 14.7, 5.0) 2.42 (dd, 14.7, 2.5)	2.82 (dd, 14.4, 5.1) 2.34 (dd, 14.5, 2.3)	2.82 (dd, 14.5, 4.9) 2.34 (dd, 14.5, 2.5)	2.87 (dd, 15, 5) 2.39 (dd, 15, 2)	2.79 (dd, 14, 6.5) 2.53 (14, 8)
13	6.29 (d, 3.3) 5.65 (d, 3.0)	6.36 (d, 3.5) 5.64 (d, 2.9)	6.28 (d, 3.5) 5.58 (d, 3.1)	6.32 (d, 3.6) 5.62 (d, 3.0)	6.34 (d, 3.5) 5.64 (d, 3)	6.26 (d, 3.8) 5.48 (d, 3)
14	10.08 (s)	1.55 (3H, s)	1.50 (3H, s)	1.54 (3H, s)	1.60 (3H, br)	5.08 (br s) 5.03 (br s)
15	1.38 (3H, s)	1.80 (3H, d, 1.1)	1.87 (3H, dd, 3.3, 1.6)	1.89 (3H, m)	1.87 (3H, br)	1.99 (3H, s)
3′	2.96 (q, 5.4)	4.11 (q, 6.8)	6.05 (br t, 5.2)	6.08 (br t, 5.2)	6.56 (br q, 7)	6.67 (m)
4′	1.13 (3H, d, 5.4)	1.50 (3H, d, 6.8)	4.98 (2H, m)	5.02 (m) 4.94 (m)	2.15 (3H, d, 7)	4.32 (2H, br d, 5.8)
5′	1.61 (3H, s)	1.39 (3H, s)	1.75 (3H, d, 1.5)	1.80 (3H, d, 1.1)	4.69 (2H, AB spin)	1.78 (3H, br s)
CO*Me*			2.05 (3H, s)	2.07 (3H, s)	2.01 (3H, s)	1.99 (3H)

[a] Measured in $CDCl_3$. [b] Measured in CD_3OD. [c] Measured in $CDCl_3$+CD_3OD. [d] Literature data.

of compound **1**. An aldehyde group was easily identified by the presence of a proton signal at δ 10.09 and a carbon signal at δ 190.1. The proton signal at δ 10.09 correlating with the C-10 at δ 128.6 in the HMBC (Figure 3a) was assigned to the H-14 of the aldehyde. The HMBC correlations indicated that a connectivity of two double bonds and the aldehyde group constructed a conjugated system, and the persubstituted double bond (δ 160.5, 128.6) and the disubstituted double bond (δ 151.4, 125.8) were assigned to $\Delta^{1(10)}$ and Δ^2, respectively. A quaternary carbon signal at δ 82.6 was assigned to C-4 bearing a hydroxyl, and this was verified by the HMBC correlations of H_3-15 (δ 1.57, 3H, s) with C-3, C-4, and C-5 (δ 61.6). The stereochemistry of **4** was interpreted by NOESY (Figure 3b,c). The structure of **4** was thereby established as 8β-angelyloxy-4α-hydroxy-14-oxo-5α*H*,6β*H*,7α*H*-guai-2,10(14),11(13)-triene-6,12-olide. The complete assignments of ^{1}H NMR and ^{13}C NMR data were achieved by a combination of 1D and 2D NMR spectra (^{1}H, ^{13}C NMR, HMQC, HMBC, and NOESY).

Eupachinilide E (**5**) had the molecular formula $C_{20}H_{25}ClO_8$ as determined by HREIMS. Comparison of its ^{1}H and ^{13}C NMR data (Tables 1 and 2) with those of compound **3** implied the structures of the two compounds were closely related; the only difference was the presence of one epoxy group in **5** rather than the double bond in compound **3**. An oxygenated methine group (δ_C 64.0, δ_H 2.47) and an oxygenated quaternary carbon at δ 65.6 suggested the presence of a 3,4-epoxyl group in **5**. Direct comparison of the ^{1}H NMR data of **5** with those of graminichlorin[6] (**5a**) showed very good similarity of the sesquiterpenoid part, supporting the structural assignment for compound **5**. In comparison with H-3′ (δ 6.03) of graminichlorin,[6] H-3′ (δ 6.62) of **5** was downfield shifted, indicating that the ester moiety of **5** also adopted *E* geometry. Hence, the structure of eupachinilide E (**5**) was elucidated to be 8β-(4′-hydroxy-tiglyloxy)-14-chrorine-3α,4α-epoxy-2β,10α-dihydroxy-1α*H*,5α*H*,6β*H*,7α*H*-guai-11(13)-ene-6,12-olide. The structure of **5** was

Table 2. ^{13}C NMR Data (δ) for Compounds **1**−**10**

	1[a]	**2**[a]	**3**[b]	**4**[a]	**5**[c]	**6**[a]	**7**[a]	**8**[a]	**9**[a]	**10**[a]	**10a**[a,d]	**13**[a,d]
1	141.3	51.5	56.5	160.5	48.3	49.3	160.9	134.6	134.1	126.2	126.16	50.7
2	38.0	75.9	76.0	125.8	70.8	72.9	125.6	69.4	69.1	74.6	74.79	80.3
3	78.7	65.1	130.9	151.4	64.0	60.9	151.7	48.6	48.6	83.5	83.61	126.3
4	82.6	66.2	150.1	82.6	65.6	65.1	82.6	142.6	142.7	143.7	144.23	148.2
5	54.6	49.5	53.3	61.6	49.6	47.4	61.6	129.7	129.2	131.6	132.19	56.0
6	76.8	77.2	84.3	76.2	77.5	76.7	75.7	74.8	75.4	74.9	75.03	80.0
7	54.5	48.2	49.1	53.0	47.3	46.3	52.8	52.9	52.9	52.6	52.64	48.0
8	65.2	68.7	69.1	63.6	67.1	66.6	64.4	74.2	71.7	71.6	71.82	68.0
9	34.3	37.5	37.6	29.1	35.8	36.3	28.7	44.2	43.7	43.9	44.06	39.0
10	130.0	140.5	75.0	128.6	73.1	74.1	127.8	134.3	134.6	136.3	136.62	139.1
11	135.0	133.9	137.2	134.7	134.5	134.0	134.2	135.3	136.2	135.9	135.69	134.0
12	170.0	169.5	172.0	168.6	170.0	169.1	169.0	169.2	169.3	170.8	170.52	169.2
13	120.7	122.7	122.6	121.7	121.6	122.1	121.3	122.4	121.3	121.9	121.57	122.4
14	65.9	120.4	55.9	190.1	54.9	54.9	189.8	20.5	19.6	13.5	13.49	120.1
15	23.8	18.3	18.7	25.3	18.8	18.7	25.3	18.8	18.6	19.7	15.94	17.2
1′	167.1	166.8	168.7	166.4	167.0	166.4	160.9	173.6	165.3	165.3	164.44	166.5
2′	127.4	127.7	129.1	127.1	127.2	127.6	59.4	77.3	127.3	127.3	127.06	127.8
3′	138.9	141.2	143.5	139.0	141.6	141.4	59.7	62.1	140.9	141.0	146.89	141.1
4′	15.8	59.5	60.2	15.9	58.9	59.6	13.7	18.0	62.8	62.9	19.92	59.6
5′	20.6	12.7	13.2	20.4	12.3	12.8	19.1	22.7	19.7	20.1	65.41	12.7
*CO*Me									170.7	169.4	169.56	ND
CO*Me*									20.8	20.8	20.68	21.4

[a] Measured in $CDCl_3$. [b] Measured in CD_3OD. [c] Measured in $CDCl_3$+CD_3OD. [d] Literature data. ND: No data were reported in the literature.

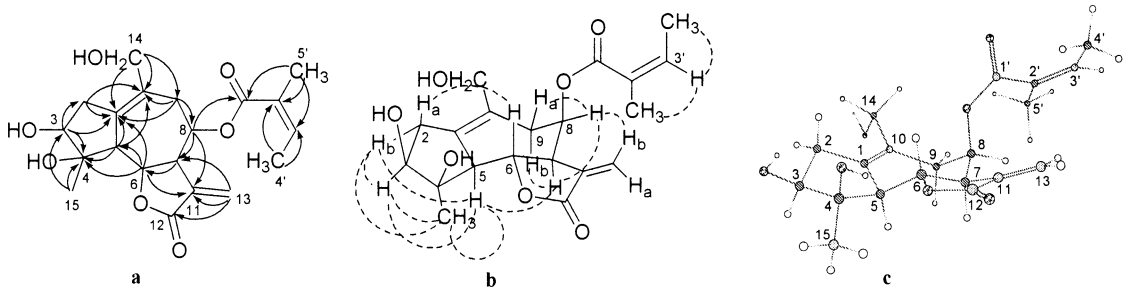


Figure 1. (a) Selected HMBC correlations (H→C) of compound **1**. (b) Key NOE interactions (- - -) of compound **1**. (c) Stereoview of compound **1** generated by computer modeling.

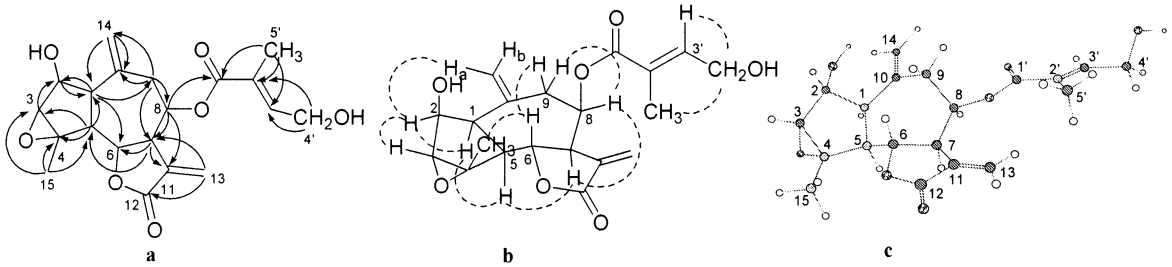


Figure 2. (a) Selected HMBC correlations (H→C) of compound **2**. (b) Key NOE interactions (- - -) of compound **2**. (c) Stereoview of compound **2** generated by computer modeling.

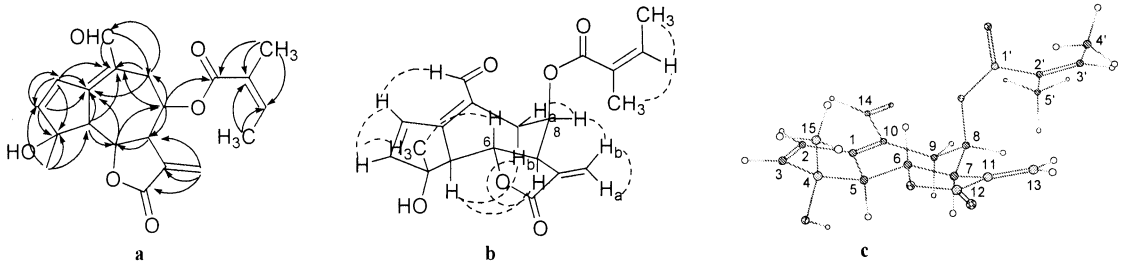


Figure 3. (a) Selected HMBC correlations (H→C) of compound **4**. (b) Key NOE interactions (- - -) of compound **4**. (c) Stereoview of compound **4** generated by computer modeling.

further confirmed by 2D NMR techniques (HMBC, HMQC, and NOESY).

Eupachinilide F (**6**) had a molecular formula $C_{22}H_{27}ClO_9$ as determined by HREIMS. The ^1H and ^{13}C NMR data of eupachinilide F (**6**) were very similar to those of **5**, except for the presence of one more acetyl (δ_C 169.5, 21.2; δ_H 2.08, 3H, s). Compared with **5**, H-2 of **6** was downfield shifted, $\Delta\delta$ 1.12 ppm, indicating that the acetoxyl group was

connected to C-2. The structure of eupachinilide F was thus elucidated to be 8β-(4′-hydroxytiglyloxy)-2β-acetoxy-14-chlorine-3α,4α-epoxy-10α-hydroxy-1α*H*,5α*H*,6β*H*,7αH-guai-11(13)-ene-6,12-olide (**6**).

Eupachinilide G (**7**) showed the molecular formula $C_{20}H_{22}O_7$ as determined by HREIMS. Extensive analysis of the ^{1}H and ^{13}C NMR data of **7** established that the backbone of the sequiterpenoid was identical with that of **4**. Compound **7** had an ester moiety (2′,3′-epoxy-2′-methylbutanonyl) as judged from the ^{1}H and ^{13}C NMR data, which was in good agreement with literature data.[9] Thus, eupachinilide G (**7**) was identified as 8β-(2′,3′-epoxy-2′-methylbutanoxy)-4α-hydroxy-14-oxo-5α*H*,6β*H*,7α*H*-guai-1(10),2,11(13)-triene-6,12-olide.

Eupachinilides H (**8**) and I (**9**) were determined to have molecular formulas $C_{20}H_{28}O_7$ and $C_{22}H_{28}O_7$ by HREIMS, respectively. The ^{1}H and ^{13}C NMR data of **8** and **9** indicated that both compounds were sesqueterpenoids, similar to eupaserrin (**16**), mollisorin B (**17**), and deacetyeupaserrin (**9a**).[9,10]

The differences of these compounds (**8**−**10**, **15**−**17**, and **9a**) were in the ester moieties. For compound **8**, methyl signals at δ 1.50 (d, 3H, J = 6.8 Hz) and 1.39 (3H, s) and a proton signal at δ 4.11 (q, J = 6.8 Hz) indicated the ester moiety was 2′,3′-dihydroxy-2′-methylbutanoxyl. The downfield shifted proton signal of H-8 (δ 5.76) placed the 2′,3′-dihydroxy-2-methylbutanoxyl at C-8. The structure of eupachinilide H (**8**) was elucidated as 8β-(2′,3′-dihydroxy-2′-methybutanoxy)-2α-hydroxy-6β*H*,7α*H*-germacra-1(10)-*E*,4*E*,11(13)-triene-6,12-olide. The ester moiety of eupachinilide I (**9**) was also determined by ^{1}H and ^{13}C NMR data as 4′-acetoxy-2′-methylbutenoxyl with *Z* geometry as judged from the chemical shift of H-3′ at δ 6.05.[6a] The structure of eupachinilide I (**9**) was thus elucidated to be 8β-(4′-acetoxyangelyloxy)-2α-hydroxy-6β*H*,7α*H*-germacra-1(10)-*E*,4*E*,11(13)-triene-6,12-olide.

Eupachinilide J (**10**) had a molecular formula $C_{20}H_{28}O_8$ (HREIMS). The ^{1}H and ^{13}C NMR data indicated that eupachinilide J (**10**) was a germacradiene-type sesqueterpenoid. The 2D NMR experiments, including HMQC, HMBC, and NOESY spectra, indicated that the sesqueterpenoid core of **10** was identical to that of compound **10a** (see Tables 1 and 2),[11] and the only difference between the two compounds was the ester moieties at C-8. The C-8 ester of eupachinilide J (**10**) was assigned as a 4′-acetoxyangelyloxyl moiety by 1D and 2D NMR. Thus, the structure of eupachinilide J (**10**) was determined to be 8β-(4′-acetoxyangelyloxy)-2α,3β-dihydroxy-6β*H*,7α*H*-germacra-1(10)*E*,4*E*,-11(13)-triene-6,12-olide.

The C-15 signal of **10** was observed at δ 19.7 in the current research, and that of **10a** was given as δ 15.94 in the literature.[11] This chemical shift of C-15 at δ 15.94 assigned for compound **10a** in the literature was most likely a typographical error (δ 19.54, not δ 15.94).

Seven known sesqueterpenoids, eupachifolin D (**11**),[4] budlein B (**12**),[12] 8β-(4′-hydroxytiglyloxy)-2β-hydroxy-1α*H*,5α*H*,6β*H*,7α*H*-guai-3,10(14),11(13)-trien-6,12-olide (**13**),[13] 1,10-hydrobahia (**14**),[14] 2α-hydroxyeupatolide (**15**),[9] eupaserrin (**16**),[9,10] and deacetyeupaserrin (**17**),[10] were identified mainly on comparison of NMR spectral data with literature values.

As shown in Tables 1 and 2, the ^{1}H and ^{13}C NMR data of the ester moiety of **2** were very close to those of eupachifolins B (**11a**), C (**13a**), and D (**11**), indicating that the ester moieties in the four compounds adopted the same *E* geometry. The *E* geometry of the 4′-hydroxy-2′-methylbutenoxyl group assigned for **2** was mainly based on the NOESY correlations and was supported by the relatively downfield shifted proton signal of H-3′ due to the *E* geometry; H-3′ normally appears at δ 6.60−7.15,[6] while for the *Z* geometry, the chemical shift of H-3′ usually appears around δ 6.04.[6a] Therefore, the structures of eupachifolins B−D (**11a**, **13a**, and **11**) reported in the literature[4] with *Z* geometry of the 4′-hydroxy-2′-methylbutenoxyl moiety should be revised as *E* geometry. Eupachifolin D (**11**) was isolated from the current plant material.

Table 3. Cytotoxic Activities of Compounds **1**, **5**, **6**, and **9** against Tumor Cell Lines

	IC_{50} (μg/mL)	
	HL-60	BEL-7402
1	10.8	72.2
5	1.30	18.0
6	0.87	3.7
9	0.94	3.6
hydroxycamptothecine[a]	0.024	0.62

[a] Hydroxycamptothecine was used as positive control.

Compounds **1**, **5**, **6**, and **9** were evaluated for their cytotoxic activities according to standard protocols,[15] and hydroxycamptothecine was used as positive control. Compounds **1**, **5**, **6**, and **9** showed cytotoxic activities against HL-60 and BEL-7402 tumor cell lines (see Table 3).

Expermental 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. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker AM-400 spectrometer with TMS as internal standard. EIMS (70 eV) were carried out on a Finnigan MAT 95 instrument. All solvents used were of analytical grade (Shanghai Chemical Plant). Silica gel (200−300 mesh), silica gel H60, and Sephadex LH-20 were used for column chromatography, and precoated silica gel GF_{254} plates (Qingdao Haiyang Chemical Plant, Qingdao, People's Republic of China) were used for TLC. C18 reversed-phase silica gel (250 mesh, Merck) and MCI gel (CHP20P, 75−150μ, Mitsubishi Chemical Industries Ltd.) were also used for column chromatography.

Plant Material. *Eupatorium chinense* L. was collected from Songyang County of Zhejiang Province, the People's Republic China, and was identified by Prof. Z.-T. Wang and Dr. M. 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-2001-1Y).

Extraction and Isolation. The whole plant (2.2 kg) of *E. chinense* L. was ground and percolated with 95% EtOH. After removal of the EtOH under reduced pressure, a dark green residue (194.2 g) remained. The residue was subjected to silica gel column chromatography using a gradient solvent system of petroleum/acetone (10:0 to 0:10; v/v) to give 10 major fractions (1−10). Fraction 4 (3.22 g) was chromatographed sequentially over reversed-phase silica gel (aqueous MeOH, 50%) and silica gel columns ($CHCl_3$/MeOH, 100:1 to 10:1) to yield compounds **4** (80 mg), **6** (60 mg), **16** (30 mg), **9** (40 mg), and **17** (160 mg). Fraction 5 was recrystallized from aqueous MeOH (40%) to afford **15** (690 mg), and the liquid phase was subjected to a reversed-phase silica gel column (aqueous MeOH, 45%) to collect the major fractions. Each major fraction was purified by Sephadex LH-20 column chromatography (MeOH) and then a silica gel column chromatography ($CHCl_3$/MeOH, 10:1, v/v) to afford **3** (7 mg), **7** (5 mg), **10** (40 mg), **11** (50 mg), **12** (6 mg), **13** (30 mg), and **14** (80 mg). Fraction 6 was fractionated by a MCI gel column (aqueous MeOH, 45%) to give several fractions, each of which was further purified by RP-18 silica gel column chromatography (40% MeOH) and

then a silica gel column ($CHCl_3$/MeOH, 7.5:1) to afford **1** (80 mg), **2** (80 mg), **5** (70 mg), and **8** (20 mg), respectively.

Eupachinilide A (1): white powder; $[\alpha]^{20}_D$ −65.8° (*c* 0.58, in CH_3OH); IR (KBr) ν_{max} 3408, 2929, 1767, 1705, 1647, 1458, 1387, 1234, 1155, 1084, 1051, 987, 864, 605 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m/z* $[M]^+$ 378 (1), 278 (4), 260 (91), 242 (17), 227 (13), 217 (26), 199 (23), 186 (100), 173 (18), 143 (11), 91 (10), 83 (93), 55 (57); HREIMS *m/z* 378.1633 (calcd for $C_{20}H_{26}O_7$, 378.1679).

Eupachinilide B (2): white powder; $[\alpha]^{20}_D$ −67.1° (*c* 0.50, CH_3OH); IR (KBr) ν_{max} 3450, 2937, 1755, 1713, 1637, 1385, 1336, 1255, 1140, 1013, 824, 589 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m/z* 376 $[M]^+$ (7), 358 (16), 347 (28), 329 (11), 260 (21), 242 (23), 231 (28), 213 (30), 199 (33), 171 (38), 145 (30), 129 (37), 117 (26), 99 (100), 91 (43), 71 (80); HREIMS *m/z* 376.1526 (calcd for $C_{20}H_{24}O_7$, 376.1522).

Eupachinilide C (3): gum; $[\alpha]^{20}_D$ −66.9° (*c* 0.50, CH_3OH); IR (KBr) ν_{max} 3435, 2924, 1751, 1713, 1653, 1402, 1321, 1259, 1140, 1063, 1013, 818, 739 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m/z* 412 $[M]^+$ (3), 396 $[C_{20}H_{25}{}^{37}ClO_7 - H_2O]^+$ (2), 394 $[M - H_2O]^+$ (6), 376 (2), 361 (3), 345 (13), 328 (25), 278 (16), 229 (30), 199 (30), 99 (100), 87 (89), 69 (86); HREIMS *m/z* 412.1269 (calcd for $C_{20}H_{25}ClO_7$, 412.1289).

Eupachinilide D (4): white powder; $[\alpha]^{20}_D$ −204.0° (*c* 1.53, CH_3OH); IR (KBr) ν_{max} 3447, 2929, 1770, 1716, 1662, 1603, 1385, 1230, 1151, 1078 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m/z* 358 $[M]^+$ (6), 258 (53), 240 (8), 231 (28), 229 (39), 215 (31), 198 (12), 187 (12), 169 (13), 149 (13), 115 (10), 100 (15), 83 (100), 55 (76); HREIMS *m/z* 358.1426 (calcd for $C_{20}H_{22}O_6$, 358.1416).

Eupachinilide E (5): white powder; $[\alpha]^{20}_D$ −59.4° (*c* 0.60, CH_3OH); IR (KBr) ν_{max} 3447, 2935, 1751, 1709, 1653, 1421, 1302, 1259, 1132, 1053, 1009, 825, 741, 501 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m/z* 430 $[C_{20}H_{25}{}^{37}ClO_8]^+$ (1), 428 $[M]^+$ (3), 392 (7), 349 (7), 294 (8), 259 (16), 231 (10), 201 (12), 187 (15), 159 (13), 123 (15), 115 (10), 99 (100), 95 (95), 71 (74); HREIMS *m/z* 428.1231 (calcd for $C_{20}H_{25}ClO_8$, 428.1238).

Eupachinilide F (6): white powder; $[\alpha]^{20}_D$ −52.3° (*c* 0.84, CH_3OH); IR (KBr) ν_{max} 3460, 2937, 1751, 1713, 1373, 1230, 1132, 1020, 824, 741, 511 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS 472 $[C_{22}H_{27}{}^{37}ClO_9]^+$ (2), 470 $[M]^+$ (7), 452 (6), 434 (26), 417 (8), 405 (34), 375 (15), 272 (7), 259 (33), 245 (17), 215 (21), 187 (15), 165 (15), 123 (13), 99 (100), 95 (51), 82 (25), 71 (52); HREIMS *m/z* 470.1329 (calcd for $C_{22}H_{27}ClO_9$, 470.1344).

Eupachinilide G (7): gum; $[\alpha]^{20}_D$ −216.5° (*c* 0.65, CH_3OH); IR (KBr) ν_{max} 3446, 2926, 1768, 1662, 1603, 1450, 1379, 1325, 1265, 1232, 1155, 1080, 1007, 968, 815, 563 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m/z* 374 $[M]^+$ (58), 359 (5), 345 (23), 258 (87), 229 (100), 215 (83), 198 (33), 169 (36), 159 (21), 141 (24), 128 (19), 115 (24), 91 (23), 71 (16), 55 (19); HREIMS *m/z* 374.1359 (calcd for $C_{20}H_{22}O_7$, 374.1366).

Eupachinilide H (8): white powder; $[\alpha]^{20}_D$ +34.7° (*c* 0.54, CH_3OH); IR (KBr) ν_{max} 3581, 3437, 2941, 1747, 1716, 1659, 1645, 1429, 1234, 1138, 1026, 960, 563 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m/z* 380 $[M]^+$ (16), 262 (9), 246 (29), 243 (41), 229 (41), 228 (72), 213 (46), 202 (35), 185 (31), 163 (33), 157 (38), 135 (34), 109 (43), 107 (100), 91 (70), 79 (36), 71 (40), 55 (34); HREIMS *m/z* 380.1858 (calcd for $C_{20}H_{28}O_7$, 380.1835).

Eupachinilide I (9): white powder; $[\alpha]^{20}_D$ +76.1° (*c* 0.65, CH_3OH); IR (KBr) ν_{max} 3466, 2931, 1767, 1716, 1655, 1456, 1367, 1219, 1144, 1024, 966, 814, 554 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m/z* 404 $[M]^+$ (2), 362 (3), 345 (4), 305 (5), 246 (15), 228 (10), 202 (11), 175 (7), 162 (16), 135 (12), 107 (11), 99 (100), 82 (21), 69 (10), 55 (11); HREIMS *m/z* 404.1829 (calcd for $C_{22}H_{28}O_7$, 404.1835).

Eupachinilide J (10): white powder; $[\alpha]^{20}_D$ +40.8° (*c* 0.55, CH_3OH); IR (KBr) ν_{max} 3435, 2931, 1767, 1740, 1713, 1655, 1367, 1220, 1144, 1026, 972, 818, 569 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m/z* 420 $[M]^+$ (1), 361 (2), 281 (3), 262 (8), 245 (7), 234 (11), 218 (16), 201 (10), 176 (8), 163 (8), 135 (8), 99 (100), 82 (41), 69 (15), 55 (8); HREIMS *m/z* 420.1806 (calcd for $C_{20}H_{28}O_8$, 420.1784).

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