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Cassaine diterpenoid dimers isolated from *Erythrophleum succirubrum* with TRAIL-resistance overcoming activity

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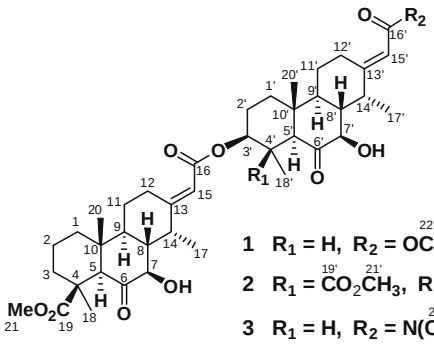
ABSTRACT

Activity-guided fractionation of *Erythrophleum succirubrum* for TRAIL resistance-overcoming activity led to the isolation of four new cassaine diterpenoid dimers, named erythrophlesins A–D (**1**–**4**). Their structures were elucidated by spectral data to show that they have an unsymmetrical dimeric structure through an ester bond between two cassaine diterpenoids. These new compounds were revealed to have a significant reversal effect on TRAIL resistance in human gastric adenocarcinoma (AGS) cells.

1. Introduction

In our screening studies for bioactive natural products targeting cancer-related signaling pathways,[1] we recently investigated a plant material, *Erythrophleum succirubrum*, collected from Thailand in our screening program for TRAIL resistance-overcoming substances.[2] *E. succirubrum* (Leguminosae) is a deciduous tall tree growing in tropical areas. Several diterpenoids, triterpenoids, and alkaloids have been isolated from the plants of the genus *Erythrophleum*.[3,4] Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), a member of the TNF superfamily, is a promising agent for new anticancer therapy, since it triggers apoptosis in a variety of cancer cells but not in many normal cells.[5] TRAIL is a death ligand and is known to bind to death receptors, such as DR5 (death receptor 5 = TRAIL-R2) or DR4 (death receptor 4 = TRAIL-R1), resulting in the activation of caspase-signaling pathways leading to apoptosis; however, it has become a problem that considerable numbers of cancer cells, especially some highly malignant tumors, are resistant to apoptosis induction by TRAIL. A search for compounds capable of abrogating TRAIL resistance has thus become an important strategy for anticancer drug discovery.[6] We recently investigated natural products which exhibited activities related to TRAIL signaling, and identified a cadinane–sesquiterpene dimer with DR5 expression enhancement activity[7] and new isoflavones with TRAIL-mediated apoptosis induction ability.[8] Our screening system of TRAIL resistance-overcoming activity of the extracts was assessed by comparing cell viability in the presence and in the absence of TRAIL against TRAIL-resistant human gastric adenocarcinoma (AGS) cell lines.[9] Bioassay-guided fractionation of the extracts of *E. succirubrum* led to the isolation of four cassaine diterpenoid dimers (**1**–**4**). Here, we report on the isolation, structure identification, and TRAIL resistance-overcoming activity of the isolated compounds.



1 R_1 = H, R_2 = OCH_3
2 R_1 = CO_2CH_3, R_2 = OCH_3
3 R_1 = H, R_2 = $N(CH_3)CH_2CH_2OH$
4 R_1 = CO_2CH_3, R_2 = $N(CH_3)CH_2CH_2OH$

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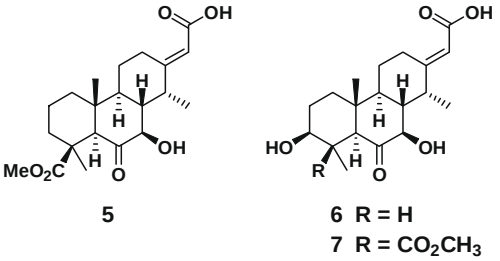
Table 1
^{1}H and ^{13}C NMR data of **1–4** in $CDCl_3$

Position	1		2		3		4	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	1.75 m/1.21 m	38.6	1.74 m/1.18 m	38.7	1.79 m/1.18 m	38.6	1.78 m/1.18 m	38.7
2	1.75 m/1.52 m	19.0	1.74 m/1.53 m	19.0	1.73 m/1.53 m	19.0	1.76 m/1.53 m	19.0
3	2.03 m/1.21 m	38.3	2.04 m/1.18 m	38.3	2.04 m/1.18 m	38.3	2.05 m/1.18 m	38.4
4		42.0		42.0		42.0		42.0
5	2.25 s	64.5	2.24 s	64.5	2.25 s	64.5	2.24 s	64.6
6		208.6		208.5		208.6		208.5
7	3.90 d (10.8)	75.5	3.89 d (10.8)	75.8	3.90 d (10.8)	75.7	3.95 d (10.2)	75.9
8	1.89 m	50.3	1.88 m	50.3	1.89 m	50.3	1.88 m	50.3
9	1.74 m	46.4	1.74 m	46.4	1.73 m	46.4	1.73 m	46.4
10		43.0		43.0		43.0		43.0
11	1.92 m/1.12 m	26.4	1.88 m/1.21 m	26.2	1.90 m/1.21 m	26.2	1.90 m/1.21 m	26.3
12	3.79 br d (14.4)/2.03 m	23.7	3.81 m/2.04 m	23.8	3.79 m/2.04 m	23.7	3.72 m/2.04 m	23.8
13		165.9		166.5		165.9		166.5
14	2.82 m	40.4	2.81 m	40.4	2.84 m	40.4	2.81 m	40.4
15	5.73 br s	113.4	5.66 d (1.2)	113.1	5.73 br s	113.5	5.66 br s	113.2
16		166.6		166.1		166.6		166.1
17	1.22* (3H)	13.8	1.21* (3H)	13.8	1.21* (3H)	13.8	1.22* (3H)	13.8
18	1.22* (3H)	28.6	1.21* (3H)	28.7	1.22 s (3H)	28.7	1.22* (3H)	28.7
19		175.5		175.4		175.5		175.4
20	0.89** (3H)	15.2	0.90 s (3H)	15.2	0.90 s (3H)	15.2	0.90 s (3H)	15.2
21	3.69 s (3H)	51.7	3.70 s (3H)	51.7	3.70 s (3H)	51.7	3.70 s (3H)	51.7
1′	1.85 m/1.41 m	36.5	1.88 m/1.42 td (13.5, 3.0)	36.6	1.84 dt (12.6, 3.0)	36.5	1.88 m/1.42 m	36.6
2′	1.92 m/1.41 m	26.2	2.25 qd (13.5, 3.0)/1.74 m	23.5	1.90 m/1.47 td (13.2, 3.0)	26.4	2.25 m/1.76 m	23.8
3′	4.46 td (10.4, 4.4)	76.6	4.66 dd (12.0, 4.2)	78.2	4.45 td (10.8, 4.8)	76.6	4.67 dd (12.0, 4.8)	78.2
4′	2.14 m	31.0		45.7	2.15**	31.0		45.7
5′	2.14 m	60.6	2.44 s	64.7	2.15**	60.6	2.45 s	64.7
6′		209.5		208.1		209.7		208.2
7′	4.01 d (10.0)	75.7	3.95 d (10.2)	75.6	4.01 d (10.2)	75.6	3.89 br d (10.2)	75.6
8′	1.75 m	52.3	1.88 m	51.0	1.76 m	52.3	1.88 m	51.3
9′	1.74 m	43.6	1.74 m	46.3	1.73 m	43.7	1.73 m	46.5
10′		42.8		43.0		42.9		43.1
11′	1.92 m/1.12 m	26.4	1.88 m/1.21 m	26.2	1.90 m/1.10 qd (12.6, 4.2)	26.4	1.90 m/1.21 m	26.3
12′	3.79 br d (14.4)/2.03 m	23.7	3.72 m/2.04 m	23.8	3.15 br d (13.8)/2.04 m	24.6	3.18 br d (15.0)/2.04 m	24.4
13′		165.2		165.0		157.5		157.3
14′	2.82 m	40.3	2.81 m	40.3	2.84 m	39.9	2.83 m	40.0
15′	5.73 br s	113.3	5.74 d (1.2)	113.4	6.00 br s [5.91 d (1.2)]	114.9	6.00 br s [5.92 br s]	115.0
16′		167.3		167.2		170.1		170.0
17′	1.22* (3H)	13.7	1.21* (3H)	13.7	1.21* (3H)	13.8	1.22* (3H)	13.7
18′	0.89** (3H)	16.2	1.21* (3H)	25.8	0.89 d (6.0) (3H)	16.2	1.22* (3H)	25.8
19′	—	—		172.7	—	—		172.7
20′	0.68 s (3H)	13.5	0.98 s (3H)	14.4	0.68 s (3H)	13.6	0.99 s (3H)	14.5
21′	—	—	3.77 s (3H)	51.9	—	—	3.77 s (3H)	51.9
22′	3.67 s (3H)	51.0	3.68 s (3H)	51.2	3.07 s (3H) [2.99 s]	37.3	3.08 s (3H) [3.00 s]	37.4
23′					3.58 m (2H) [3.48 m]	51.2	3.60 m (2H)	51.3
24′					3.79 m (2H) [3.75 m]	61.9	3.81 t (4.8) (2H)	61.9

*, **: overlapped with other signals within vertical column.
[ ]: signals for minor conformer at amide group.



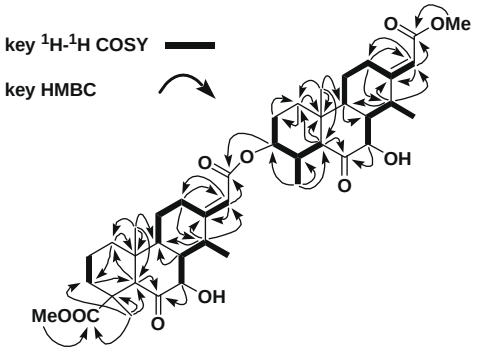


Figure 1. Key ^{1}H–^{1}H COSY and HMBC correlations observed for **1**.

2. Results and discussion

The MeOH extract of the bark of *E. succirubrum*[10] was partitioned successively with hexane, EtOAc, *n*-BuOH, and water. The EtOAc layer, which showed the most potent activity (36% decrease in cell viability in the presence of TRAIL compared with that in the absence of TRAIL at 3.1 μg/mL), was subjected to silica gel column chromatography, followed by ODS column chromatography and ODS-HPLC, to yield four new compounds, erythrophlesins A–D (**1**–**4**).[11]

Erythrophlesin A (**1**),[12] $[\alpha]_D^{20}$ − 98 (*c* 1.0, MeOH), was shown to have a molecular formula, $C_{41}H_{58}O_{10}$, by the HRFABMS data observed at *m/z* 711.4167 (calcd for $C_{41}H_{59}O_{10}$ $[M+H]^+$, 711.4108). The UV spectrum of **1** showed an absorption maximum at 223 nm, indicating the presence of conjugated system(s), while IR absorption bands at 3449 and 1709 cm^{-1} were suggestive of the presence of hydroxyl and carbonyl groups. The ^1H NMR spectrum of **1** (Table 1) revealed signals for six methyl groups (δ_H 1.22 × 3, δ_H 0.89 × 2, and δ_H 0.68 × 1), two methoxy groups (δ_H 3.69 and 3.67), two olefinic protons (δ_H 5.73 × 2), and three oxymethines (δ_H 4.46, 4.01, and 3.90), while the ^{13}C NMR spectrum of **1** (Table 1) showed signals due to two ketones (δ_C 209.5 and 208.6), three ester carbonyl groups (δ_C 175.5, 167.3, and 166.6), two sp^2 methine (δ_C 113.4 and 113.3) and two quaternary sp^2 carbons (δ_C 165.9 and 165.2), three oxymethines (δ_C 76.6, 75.7, and 75.5), three methoxy groups (δ_C 51.7 and 51.0), and six methyls (δ_C 28.6, 16.2, 15.2, 13.8, 13.7, and 13.5). Seven of thirteen unsaturation degrees in compound **1** were thus accounted for, suggesting that compound **1** has six rings. Since most of these ^1H and ^{13}C NMR signals were observed as pairs, it was inferred that compound **1** had a dimeric structure consisting of two tricyclic diterpenoids. Detailed analysis of ^1H–^1H COSY and HMBC correlation data, as shown in Figure 1, indicated that compound **1** was constructed from two cassaine diterpenoids (left and right units) connected through an ester linkage. The presence of a 6-keto-7β-hydroxy group for both monomer units (left and right units) was revealed by observation of the signals due to α-axial hydrogens at C-7 and C-7′ positions at δ_H 3.90 (1H, d, *J* = 10.8 Hz; H-7, left unit) and δ_H 4.01 (1H, d, *J* = 10.0 Hz; H-7′, right unit) along with HMBC correlations from H-7 (δ_H 3.90) to C-6 (δ_C 208.6) for the left unit and from H-7′ (δ_H 4.01) to C-6′ (δ_C 209.5) for the right unit. The ^1H–^1H COSY spectrum showed proton connectivities for H-7/H-8/H-14/H_3-17 for both left and right units. A conjugate ester moiety is located at the C-13/C-15/C-16 position of both units [δ_C 165.9 (C-13), 113.4 (C-15), 166.6 (C-16) for the left unit; δ_C 165.2 (C-13′), 113.3 (C-15′), 167.3 (C-16′) for the right unit], which was revealed from the HMBC correlations for H-14/C-13, H-14/C-15, H_2-12/C-15, H-15/C-12, and H-15/C-16 observed for each unit. The C-16 ester carbonyl of the right unit [δ_C 167.3 (C-16′)] was connected to a methoxy group [C-22′: δ_C 51.0, δ_H 3.67 (3H, s); HMBC correlation, H_3-22′/C-16′], while the C-16 ester carbonyl of the left unit [δ_C 166.6 (C-16)] was connected to an oxymethine [δ_C 76.6, δ_H 4.46 (1H, triplet of doublet, *J* = 10.4 and 4.4 Hz)], which was assignable to the C-3′ position of the right unit (HMBC correlation, H-3′/C-16). The adjacent C-4′ position of the right unit bore a hydrogen (H-4′) and a secondary methyl group (C-18′), which was suggested from the ^1H–^1H COSY correlations for H-3′/H-4′/H_3-18′, while the C-4 position of the left unit bore a methoxycarbonyl (C-19 and C-21) group and a tertiary methyl group (C-18); consistently H-5 of the left unit resonated as a sharp singlet at δ_H 2.25. The C-3 of the left unit was an unoxygenated methylene group (δ_C 38.3, δ_H 2.03 and 1.21; HMBC correlation, H_3-18/C-3). From these results, the carboxylic acid part (left unit) and alcohol part (right unit) of compound **1** were identified as cassminic acid (**5**)[13] and methyl ester of norerythrofordin A (**6**),[14] respectively. **5** was a cassain diterpene (C_{20}) previously isolated from *Erythrophleum guineense*, whereas **6** was a norcassaine (C_{19}) diterpenoid from *Erythrophleum fordii*. The NOESY experiment of **1** showed NOE correlations for H-5/H_3-18, H-5/H-7, H-7/H-9, H-7/H_3-17, H_3-21/H_3-20, and H-14/H-15 for the left unit and H-3′/H_3-18′, H-4′/H_3-20′, H-5′/H_3-18′, H-5′/H-7′, H-7′/H-9′, H-7′/H_3-17′, and H-14′/H-15′ for the right unit. In the right unit, the H-3′ signal was observed as a triplet of doublet (*J* = 10.4 and 4.4 Hz), implying that both H-3′ and H-4′ are axial ($J_{2'axial,3'} = J_{3',4'}$ = 10.4 Hz, $J_{2'equatorial,3'}$ = 4.4 Hz). In left and right units, H-7 and H-7′ signals were observed as doublets (*J* = 10.8 and *J* = 10.0 Hz, respectively), indicating that both H-7 and H-8 are axial ($J_{7,8}$ = 10.8 Hz) and both H-7′ and H-8′ are also axial ($J_{7',8'}$ = 10.0 Hz). Thus, the relative stereochemistry of **1** was deduced to be parallel to those of **5** and **6**, including the assignment of the $\Delta^{13,15}$-double bond of both units as *Z* (NOE correlations, H-14/H-15 and H-14′/H-15′). Compound **1** was converted into its (*R*)- and (*S*)-MTPA esters (**1r** and **1s**), which were esterified at C-7 and C-7′ hydroxyl groups. On the basis of the modified Mosher's method,[15] the $\Delta\delta$ values of H-8 and H_3-17 (left unit) were +0.114 and +0.147 ppm, respectively, and those of H-8′ and H_3-17′ (right unit) were +0.114 and +0.145 ppm, respectively, implying that the absolute configurations of both C-7 and C-7′ were *R*.[16] From these results, the structure of erythrophlesin A was concluded as **1**.

Erythrophlesin B (**2**),[12] $[\alpha]_D^{20}$ − 111 (*c* 1.0, MeOH), had a molecular formula, $C_{43}H_{60}O_{12}$, as established by HRFABMS data (*m/z* 769.4140, calcd for $C_{43}H_{61}O_{12}$ $[M+H]^+$, 769.4163). Comparison of the ^1H and ^{13}C NMR spectra of **2** with those of **1** revealed that these compounds were similar and, particularly, the carboxyl acid part (left unit) was identical to that of **1** [= cassminic acid (**5**)]. The major difference of **2** from **1** was the appearance of one additional methoxy signal [δ_H 3.77 (3H, s), δ_C 51.9] and an additional ester carbonyl signal (δ_C 172.7), which implied the presence of an additional methoxycarbonyl group in **2**, which is consistent with the difference of the molecular formula ($C_2H_2O_2$) between **1** and **2**.

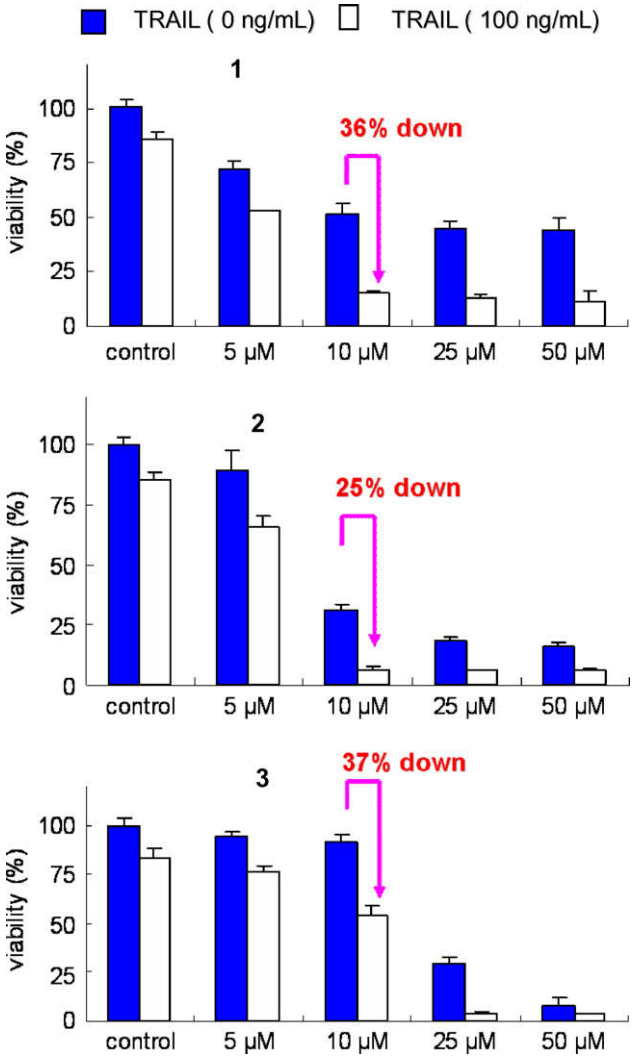


Figure 2. Effect of compounds **1**–**3** on the cell viability of AGS cells in the presence and in the absence of TRAIL.

The additional ester carbonyl carbon (δ_C 172.7) was correlated with a double doublet signal at δ_H 4.66 (1H, dd, J = 12.0 and 4.2 Hz) and a sharp singlet at δ_H 2.44 (1H, s) in the HMBC spectrum of **2**. The double doublet (δ_H 4.66) was also correlated with another ester carbonyl (δ_C 166.1), which was assignable to the C-16 carboxyl group in the left unit (HMBC, H-15/C-16). Thus, the double doublet (δ_H 4.66) was assigned to H-3′ of the right unit. The H-3′ of **1** was observed as a triplet of doublet, while that of **2** was a double doublet, suggesting the absence of a hydrogen atom at C-4. The observation of the sharp singlet at δ_H 2.44 also corroborated the absence of H-4, since this singlet was assignable to H-5′ from its HMBC correlation with C-6′ (δ_C 208.1), C-4′ (δ_C 45.7), C-10′ (δ_C 43.0), C-18′ (δ_C 25.8), and C-20′ (δ_C 14.4). From these observations, the additional methoxycarbonyl group was reasonably attached at C-4′of the right unit. Thus, the structure of the alcohol part (right unit) of **2** was revealed to be identical to the methyl ester of erythrofordin A (**7**),[10] a cassaine diterpenoid (C_{20}) previously isolated from *E. fordii*.

The molecular formula of erythrophlesin C (**3**),[12] $[\alpha]_D^{20}$ − 73 (*c* 1.0, MeOH), was revealed as $C_{43}H_{63}O_{10}N$ on the basis of HRFABMS data (*m/z* 754.4591, calcd for $C_{43}H_{64}O_{10}N$ $[M+H]^+$, 754.4530). Different from compound **1** or **2**, compound **3** contained a nitrogen atom. Detailed comparison of the spectral data of compound **3** with those of **1** suggested that erythrophlesin C (**3**), similar to erythrophlesin A (**1**), consisted of one cassaine (C_{20}) and one norcassaine (C_{19}) diterpenoid, and the methyl ester group (C-16′ position) in the right unit of **1** was replaced with an amide group in **3**. The ^1H and ^{13}C NMR spectra of **3** showed signals due to an *N*-methyl group [δ_H 3.07 (3H, s), δ_C 37.3] and 2-hydroxyethyl group [δ_H 3.58 (2H) and 3.79 (2H); δ_C 51.2 and 61.9], thus suggesting that a (2-hydroxylethyl)methylamine was attached at C-16′ position through an amide bond in the right unit of **3**. ^1H NMR signals due to H-15′, H_3-22′, H_2-23′, and H_2-24′ were observed as paired signals, which were ascribed to a minor conformer around the amide bond; this observation further supported the presence of the amide group for **3**. Erythrophlesin D (**4**),[12] $[\alpha]_D^{20}$ − 55 (*c* 0.5, MeOH), had a molecular formula, $C_{45}H_{65}O_{10}N$, as established by HRFABMS data (*m/z* 812.4554, calcd for $C_{45}H_{66}O_{12}N$ $[M+H]^+$, 812.4585). The ^1H and ^{13}C NMR spectral data of **4** revealed that erythrophlesin D (**4**) consisted of two cassaine (C_{20}) diterpenoids, having a methoxycarbonyl group at C-4′ of the right unit, similar to erythrophlesin B (**2**). Compound **4** also possessed an *N*-(2-hydroxyethyl)methylamide group at C-16′ position in the right unit, similar to erythrophlesin C (**3**).

Diterpenoid dimers connected through an ester bond are still rarely known previously as natural products, although a kaurane diterpene dimer was previously isolated from *Parinari campestris*.[17,18] Erythrophlesins A–C (**1**–**3**) isolated here were evaluated for TRAIL resistance-overcoming activity in AGS cells through cell viability tests using FMCA methods,[9,19] and the assay results are summarized in Figure 2. In this experiment, luteolin was used as a positive control, which produced about 70% more inhibition along with TRAIL than with TRAIL alone.[2,20] As a result, these compounds proved to exhibit a significant TRAIL resistance-overcoming activity at micromolar concentrations, implying that these compounds (**1**–**3**) had a synergistic effect in combination with TRAIL against AGS cells. In particular, compound **3** at 10 μM showed a 37% decrease in cell viability in the presence of TRAIL (100 ng/mL) compared with that in the absence of TRAIL, and, at the same concentration, **3** alone showed still high cell viability (92%). Resistance of cancer cells to TRAIL may occur at different points in the TRAIL-mediated apoptotic pathways including, for example, increase of apoptosis inhibitors, or decrease of apoptosis inducers or death receptors. Studies clarifying the mechanism of TRAIL resistance-overcoming activity of these diterpenoid dimers are underway. Here we report the isolation of four new cassaine diterpenoid dimers from *E. succirubrum* as having synergistic activity in sensitizing TRAIL-resistant AGS cells, thereby suggesting their possible use in combination with TRAIL against human gastric adenocarcinoma.

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References and notes

1. (a) Hosoya, T.; Arai, M. A.; Koyano, T.; Kowithayakorn, T.; Ishibashi, M. *ChemBioChem.* **2008**, *9*, 1082–1092; (b) Aida, W.; Ohtsuki, T.; Li, X.; Ishibashi, M. *Tetrahedron* **2009**, *65*, 369–373; (c) Li, X.; Ohtsuki, T.; Koyano, T.; Kowithayakorn, T.; Ishibashi, M. *Chem. Asian J.* **2009**, *4*, 540–547.
2. Ahmed, F.; Ohtsuki, T.; Aida, W.; Ishibashi, M. *J. Nat. Prod.* **2008**, *71*, 1963–1966.
3. Qu, J.; Hu, Y. C.; Yu, S. S.; Chen, X. G.; Li, Y. *Planta Med.* **2006**, *72*, 442–449.
4. Verotta, L.; Aburjai, T.; Rogers, C. B.; Dorigo, P.; Maragno, I.; Fraccarollo, D.; Santostasi, G.; Gaion, R. M.; Floreani, M.; Carpenedo, F. *Planta Med.* **1995**, *61*, 271–274.
5. Johnstone, R. W.; Frew, A. J.; Smyth, M. J. *Nat. Rev. Cancer* **2008**, *8*, 782–798.
6. Ishibashi, M.; Ohtsuki, T. *Med. Res. Rev.* **2008**, *28*, 688–714.
7. Ohtsuki, T.; Tamaki, M.; Toume, K.; Ishibashi, M. *Bioorg. Med. Chem.* **2008**, *16*, 1756–1763.
8. (a) Kikuchi, H.; Ohtsuki, T.; Koyano, T.; Kowithayakorn, T.; Sakai, T.; Ishibashi, M. *J. Nat. Prod.* **2007**, *70*, 1910–1914; (b) Kikuchi, H.; Ohtsuki, T.; Koyano, T.; Kowithayakorn, T.; Sakai, T.; Ishibashi, M. *Bioorg. Med. Chem.* **2009**, *17*, 1181–1186.
9. *Fluorometric microculture cytotoxicity assay (FMCA)*: AGS cells were seeded in a 96-well culture plate (6 × 103 cells per well) in 200 μL RPMI medium containing 10% FBS. Cells were incubated at 37 °C in a 5% CO_2 incubator for 24 h, and then test samples with or without TRAIL (100 ng/mL) at different doses were added to each well. After 24 h incubation, the cells were washed with PBS, and 200 μL PBS containing fluorescein diacetate (10 μg/mL) was added to each well. The plates were then incubated at 37 °C for 1 h, and fluorescence was measured in a 96-well scanning spectrofluorometer at 538 nm, following excitation at 485 nm.
10. *Erythrophleum succirubrum* was collected in Khon Kaen, Thailand, and identified by T. Kowithayakorn. Voucher specimens have been deposited in our laboratory.
11. The MeOH extract (19.3 g) of air-dried leaves suspended in 10% aqueous MeOH (400 mL) was partitioned with hexane (400 mL × 3), EtOAc (400 mL × 3), and *n*-BuOH (400 mL × 3). The EtOAc-soluble fraction (2.9 g) was subjected to silica gel column chromatography (3.62 × 22 cm) eluted with 0–100% MeOH in $CHCl_3$. The fraction (0.75 g) eluted with 30–50% MeOH in $CHCl_3$ was further separated by ODS column chromatography (2.8 × 40 cm) eluted with 30–100% MeOH in H_2O, followed by reversed-phase HPLC with 75% CH_3CN (Develosil C30-UG-5, 10 × 250 mm; flow rate, 2.0 mL/min; UV detection at 230 nm) to give compounds **1** (11.5 mg, t_R = 44 min), **2** (2.1 mg, t_R = 37 min), **3** (7.3 mg, t_R = 12 min), and **4** (0.9 mg, t_R = 11 min).
12. *Erythrophlesin A* (**1**): amorphous solid; $[\alpha]_D^{20}$ − 98 (*c* 1.0, MeOH); UV (MeOH) λ_{max} 223 nm (ε 32000); IR (ATR) ν_{max} 3449, 1709, and 1645 cm^{-1}; FABMS *m/z* 711 $[M+H]^+$, 733 $[M+Na]^+$, and 749 $[M+K]^+$; HRFABMS *m/z* 711.4167 (calcd for $C_{41}H_{59}O_{10}$ $[M+H]^+$, 711.4108); ^1H and ^{13}C NMR (Table 1). *Erythrophlesin B* (**2**): amorphous solid; $[\alpha]_D^{20}$ − 111 (*c* 1.0, MeOH); UV (MeOH) λ_{max} 222 nm (ε 34000); IR (ATR) ν_{max} 3463, 1717, and 1646 cm^{-1}; FABMS *m/z* 769 $[M+H]^+$ and 791 $[M+Na]^+$; HRFABMS *m/z* 769.4140 (calcd for $C_{43}H_{61}O_{120}$ $[M+H]^+$, 769.4163); ^1H and ^{13}C NMR (Table 1). *Erythrophlesin C* (**3**): amorphous solid; $[\alpha]_D^{20}$ − 73 (*c* 1.0, MeOH); IR (ATR) ν_{max} 3448 and 1714 cm^{-1}; FABMS *m/z* 754 $[M+H]^+$, 776 $[M+Na]^+$, and 792 $[M+K]^+$; HRFABMS *m/z* 754.4591 (calcd for $C_{43}H_{64}O_{10}N$ $[M+H]^+$, 754.4530); ^1H and ^{13}C NMR (Table 1). Erythrophlesin D (**4**): amorphous solid; $[\alpha]_D^{20}$ − 55 (*c* 0.5, MeOH); IR (ATR) ν_{max} 3545 and 1716 cm^{-1}; FABMS *m/z* 812 $[M+H]^+$ and 834 $[M+Na]^+$; HRFABMS *m/z* 812.4554 (calcd for $C_{45}H_{66}O_{12}N$ $[M+H]^+$, 812.4585); ^1H and ^{13}C NMR (Table 1).
13. Blessington, B.; Mathieson, D. W.; Karim, A. *J. Chem. Soc. (C)* **1970**, 1703–1704.
14. Tsao, C.-C.; Shen, Y.-C.; Su, C.-R.; Li, C.-Y.; Liou, M.-J.; Dung, N.-X.; Wu, T.-S. *Bioorg. Med. Chem.* **2008**, *16*, 9867–9870.
15. Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4902–4906.
16. The Δδ values of H-5 (left unit) and H-5′ (right unit) were nearly zero, but were actually slightly positive (+0.018), which were inconsistent with the rules of the modified Moshers' method.[15] We supposed that the presence of ketone groups (C-6 and C-6′) might have had a contradicting effect on the rule, and we concluded the absolute configuration by using Δδ value data of H-8 and H_3-17 (left unit) as well as those of H-8′ and H_3-17′ (right unit). ^1H NMR chemical shifts (δ_H in $CDCl_3$) of **1r**/**1s**: H-5, 2.266/2.284; H-5′, 2.217/2.235; H-8 and H-8′, 2.116/2.230; H_3-17, 0.990/1.137; H_3-17′, 0.996/1.141.

17. Braca, A.; Abdel-Razik, A. F.; Mendez, J.; Morelli, I. *Fitoterapia* **2005**, *76*, 614–619.
18. For another kaurane diterpenoid dimer through an ester bond from *Annona glabra*: Yang, N.-Y.; Tian, L.-J.; Meng, Z.-M.; Han, Y. *Chinese Chem. Lett.* **2003**, *14*, 58–61.
19. Larsson, R.; Kristensen, J.; Sandberg, C.; Nygren, P. *Int. J. Cancer* **1992**, *50*, 177–185.
20. Horinaka, M.; Yoshida, T.; Shiraishi, T.; Nakata, S.; Wakada, M.; Nakanishi, R.; Nishino, H.; Sakai, T. *Biochem. Biophys. Res. Commun.* **2005**, *333*, 833–838.