

Terpenoids from *Salvia trijuga*

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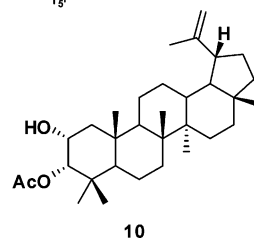
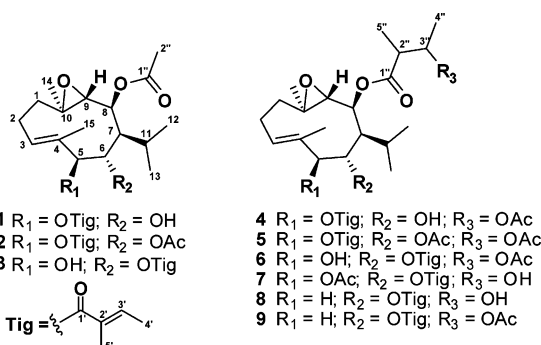
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Nine new germacrane sesquiterpenes, trijugins A–I (1–9), a new lupane triterpenoid, 3 $\alpha$ -*O*-acetyl-20(29)-lupen-2 $\alpha$ -ol (10), and 24 known terpenoids were isolated from *Salvia trijuga*. The structure of compound 1 was confirmed by single-crystal X-ray diffraction. Compounds 1–10 and 32 were evaluated for their cytotoxicity against five human tumor cell lines. Compounds 9 and 32 exhibited moderate toxicity effects against several cell lines.

*Salvia*, consisting of about 900 species, is the largest genus in the family Labiatae and widely distributed in various regions of the world, namely, the Mediterranean area, South Africa, Central and South America, and Southeast Asia.<sup>1</sup> Plants of the genus *Salvia* have attracted much attention owing to a variety of medicinal properties and biological activities, such as antibacterial, antioxidant, antitumor, cardioactive, antidiabetic, and antiinflammatory activities.<sup>2–4</sup> Many species of this genus are being used as traditional drugs in China.<sup>5</sup> *Salvia trijuga*, usually called “Xiao-Hong-Shen” by local inhabitants of the Yunnan Province in China, has been used as a surrogate for *Salvia miltiorrhiza* (Danshen) to treat cardiovascular diseases.<sup>6</sup> Previous reports showed that a number of compounds, mainly diterpenoids, had been isolated from the root of this plant.<sup>7</sup> In our continuing investigation on the phytochemistry of the genus *Salvia*, nine new germacrane sesquiterpenes, trijugins A–I (1–9), a new lupane triterpenoid, 3 $\alpha$ -*O*-acetyl-20(29)-lupen-2 $\alpha$ -ol (10), and 24 known terpenoids have been isolated from the acetone extract of the whole plant of *S. trijuga*. This is the first report of sesquiterpenoids from this plant. In this paper, we describe the isolation, structural elucidation, and cytotoxicity of these new compounds.

## Results and Discussion

The acetone extract of the whole plant of *S. trijuga* was partitioned between H<sub>2</sub>O and EtOAc. The EtOAc portion were subjected to MCI, silica gel, RP-18, Sephadex LH-20, and semi-preparative HPLC chromatography to afford nine new germacrane sesquiterpenes, trijugins A–I (1–9), a new lupane triterpenoid, 3 $\alpha$ -*O*-acetyl-20(29)-lupen-2 $\alpha$ -ol (10), and 24 known terpenoids (11–34). The structures of the known compounds were established by comparing their observed and reported physical data to those reported in the literature and by TLC comparison with authentic samples. They were identified as 2-isopropyl-8-methylphenanthrene-3,4-dione (11),<sup>8</sup> tanshinone I (12), tanshinone IIA (13), cryptotanshinone (14), dihydrotanshinone I (15),<sup>9</sup> methylenetanshinone (16),<sup>10</sup> 1,2-dihydrotanshinone (17),<sup>10</sup> danshenol A (18),<sup>11</sup> danshenol C (19),<sup>12</sup> tanshinol B (20),<sup>13</sup> tanshinone IIB (21),<sup>14</sup> danshexinkun A (22),<sup>15</sup> methylenedihydrotanshinone (23),<sup>16</sup> trijuganone B (24),<sup>7a</sup> prioketolactone (25),<sup>17</sup> lupeol (26),<sup>18</sup> 20(29)-lupene-2 $\alpha$ ,3 $\alpha$ -diol (27),<sup>19</sup> 20(29)-lupene-2 $\alpha$ ,3 $\beta$ -diol (28),<sup>20</sup> glochilodiol (29),<sup>21</sup> maslinic acid (30),<sup>22</sup> 2 $\alpha$ ,3 $\alpha$ -dihydroxyolean-12-en-28-oic acid



(31),<sup>23</sup> hyptadienic acid (32),<sup>24</sup> oleanolic acid (33), and ursolic acid (34), respectively.

Compound 1 was obtained as colorless crystals. Its molecular formula, C<sub>22</sub>H<sub>34</sub>O<sub>6</sub>, was deduced from its HRESIMS ([M + Na]<sup>+</sup> *m/z* 417.2259; calcd 417.2253), indicating six degrees of unsaturation. The IR spectrum of 1 showed absorptions of hydroxy (3561 cm<sup>-1</sup>), carbonyl (1721 and 1703 cm<sup>-1</sup>), and olefinic (1649 and 1631 cm<sup>-1</sup>) functionalities. The <sup>13</sup>C NMR spectrum (Table 3) showed seven methyl, two methylene, eight methine (two olefinic and four oxygenated), three quaternary (two olefinic and one oxygenated), and two ester carbonyl carbons. Further analysis of the 1D and 2D NMR data of 1 displayed some characteristic signals that could be readily assigned to an isopropyl unit [ $\delta_C$  46.8 (C-7), 24.9 (C-11), 21.1 (C-12), and 23.4 (C-13)], a tiglate moiety [ $\delta_C$  167.7 (C-1'), 137.8 (C-2'), 128.4 (C-3'), 14.4 (C-4'), and 12.1 (C-5')],<sup>25</sup> an *O*-acetyl group [ $\delta_C$  170.0 (C-1'') and 21.0 (C-2'')], and a trisubstituted double bond [ $\delta_C$  129.5 (C-3) and 133.4 (C-4)]. On comparison of its spectroscopic data with those of known sesquiterpenes,<sup>25,26</sup> compound 1 appeared to be a sesquiterpenoid with the germacrane skeleton containing an epoxy unit, a tiglate moiety, and an *O*-acetyl group. The epoxy ring was located at C-9 ( $\delta_C$  66.5) and C-10 ( $\delta_C$  59.3) by inspection of the <sup>1</sup>H–<sup>1</sup>H COSY, HMBC correlations, and the chemical shifts. The HMBC correlations of H-5 ( $\delta_H$  4.79) with C-1' ( $\delta_C$  167.7) and of H-8 ( $\delta_H$  4.98) with C-1'' ( $\delta_C$  170.0) implied that the tiglate group and the *O*-acetyl unit were attached to C-5 and C-8, respectively, thereby establishing that the hydroxy group was present at C-6.

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**Table 1.**  $^1\text{H}$  NMR Data of Compounds **1–5** in  $\text{CDCl}_3$  ( $\delta_{\text{H}}$ ,  $J$  in Hz)<sup>a</sup>

| no.        | 1 <sup>b</sup>    | 2 <sup>b</sup>       | 3 <sup>b</sup> | 4 <sup>c</sup>    | 5 <sup>b</sup>   |
|------------|-------------------|----------------------|----------------|-------------------|------------------|
| 1 $\alpha$ | 2.16, overlap     | 2.14, m              | 2.14, br s     | 2.11, br d (15.6) | 2.15, m          |
| 1 $\beta$  | 1.18, overlap     | 1.23, overlap        | 1.24, overlap  | 1.12, overlap     | 1.24, overlap    |
| 2 $\alpha$ | 2.17, overlap     | 2.25, br s           | 2.30, br s     | 2.18, m           | 2.26, br s       |
| 2 $\beta$  | 2.40, m           | 2.40, br s           | 2.45, br s     | 2.38, m           | 2.40, br s       |
| 3          | 5.41, d (12.0)    | 5.66, dd (12.0, 2.0) | 5.71, br s     | 5.37, br d (11.2) | 5.69, d (11.5)   |
| 5          | 4.79, d (10.0)    | 4.97, d (8.0)        | 4.06, d (10.0) | 4.77, d (9.6)     | 4.98, br d (8.0) |
| 6          | 4.36, br d (10.0) | 5.78, br d (8.5)     | 5.55, br s     | 4.28, br d (9.6)  | 5.83, br d (7.5) |
| 7          | 1.34, br d (10.0) | 1.40, br s           | 1.39, br s     | 1.31, br d (9.6)  | 1.42, br s       |
| 8          | 4.98, br d (7.0)  | 4.85, br d (6.5)     | 4.88, d (5.5)  | 4.93, br d (6.8)  | 4.91, br d (6.5) |
| 9          | 2.86, d (6.5)     | 3.00, br s           | 3.00, br s     | 2.76, br s        | 2.96, br s       |
| 11         | 1.95, overlap     | 1.74, overlap        | 1.80, overlap  | 1.90, overlap     | 1.76, overlap    |
| 12         | 0.92, d (6.5)     | 0.90, d (6.5)        | 0.92, d (6.5)  | 0.89, d (6.8)     | 0.90, d (6.5)    |
| 13         | 1.08, d (6.5)     | 1.15, d (6.5)        | 1.14, d (6.5)  | 1.06, d (6.8)     | 1.16, d (6.5)    |
| 14         | 1.18, s           | 1.16, s              | 1.18, s        | 1.16, s           | 1.17, s          |
| 15         | 1.95, s           | 1.92, s              | 1.87, s        | 1.93, s           | 1.92, s          |
| 3'         | 6.91, q (7.0)     | 6.81, m (7.0)        | 6.91, q (7.0)  | 6.93, q (7.2)     | 6.83, q (6.5)    |
| 4'         | 1.80, d (7.0)     | 1.79, d (7.0)        | 1.82, d (7.0)  | 1.79, d (7.2)     | 1.80, d (7.5)    |
| 5'         | 1.84, s           | 1.83, s              | 1.87, s        | 1.84, s           | 1.84, s          |
| 2''        | 2.11, s           | 1.94, s              | 1.92, s        | 2.72, m           | 2.53, m          |
| 3''        |                   |                      |                | 5.19, m           | 5.03, m          |
| 4''        |                   |                      |                | 1.21, d (6.4)     | 1.17, d (6.5)    |
| 5''        |                   |                      |                | 1.18, d (7.2)     | 1.06, d (7.5)    |
| 5/6-OAc    |                   | 1.94, s              |                |                   | 1.97, s          |
| 3''-OAc    |                   |                      |                | 2.02, s           | 1.94, s          |

<sup>a</sup> Assignments are based on 1D and 2D NMR experiments. <sup>b</sup> 500 MHz. <sup>c</sup> 400 MHz.

**Table 2.**  $^1\text{H}$  NMR Data of Compounds **6–9** in  $\text{CDCl}_3$  ( $\delta_{\text{H}}$ ,  $J$  in Hz)<sup>a</sup>

| no.        | 6 <sup>c</sup>   | 7 <sup>c</sup>       | 8 <sup>b</sup>    | 9 <sup>b</sup>    |
|------------|------------------|----------------------|-------------------|-------------------|
| 1 $\alpha$ | 2.11, m          | 2.12, m              | 2.12, overlap     | 2.09, br d (11.5) |
| 1 $\beta$  | 1.22, overlap    | 1.21, overlap        | 1.22, overlap     | 1.19, overlap     |
| 2 $\alpha$ | 2.28, br s       | 2.26, overlap        | 2.29, 2H, overlap | 2.27, 2H, br s    |
| 2 $\beta$  | 2.41, br s       | 2.39, m              |                   |                   |
| 3          | 5.70, br s       | 5.66, dd (10.0, 1.6) | 5.36, br d (9.0)  | 5.34, br d (9.0)  |
| 5          | 4.02, d (9.6)    | 4.96, d (9.2)        | 1.25, overlap     | 1.23, overlap     |
| 6          | 5.56, br s       | 5.80, br d (8.4)     | 5.50, br s        | 5.45, br s        |
| 7          | 1.34, br s       | 1.41, br d (7.6)     | 1.51, br s        | 1.49, br s        |
| 8          | 4.90, br d (5.2) | 4.87, br d (6.4)     | 4.94, br d (7.0)  | 4.95, br d (7.0)  |
| 9          | 2.92, br s       | 3.00, br s           | 2.87, br s        | 2.80, br s        |
| 11         | 1.78, overlap    | 1.74, overlap        | 1.91, m           | 1.89, m           |
| 12         | 0.88, d (6.4)    | 0.87, d (6.4)        | 0.94, d (6.5)     | 0.93, d (6.5)     |
| 13         | 1.13, d (6.4)    | 1.15, d (6.4)        | 1.17, d (6.5)     | 1.16, d (6.5)     |
| 14         | 1.15, s          | 1.17, s              | 1.21, s           | 1.19, s           |
| 15         | 1.84, s          | 1.92, s              | 1.79, s           | 1.77, s           |
| 3'         | 6.89, q (7.2)    | 6.82, m (7.2)        | 6.86, m (7.0)     | 6.83, q (7.0)     |
| 4'         | 1.79, d (7.2)    | 1.79, d (6.8)        | 1.81, d (7.0)     | 1.80, d (7.0)     |
| 5'         | 1.84, s          | 1.82, s              | 1.85, s           | 1.83, s           |
| 2''        | 2.51, m          | 2.29, m              | 2.29, m           | 2.55, m           |
| 3''        | 4.98, br s       | 3.78, m              | 3.80, m           | 4.99, m           |
| 4''        | 1.14, d (6.0)    | 1.10, d (6.4)        | 1.13, d (6.0)     | 1.15, d (6.5)     |
| 5''        | 1.03, d (7.2)    | 1.08, d (7.2)        | 1.10, d (7.5)     | 1.04, d (7.0)     |
| 5/6-OAc    |                  |                      |                   |                   |
| 3''-OAc    | 1.95, s          | 1.92, s              |                   | 1.97, s           |

<sup>a</sup> Assignments are based on 1D and 2D NMR experiments. <sup>b</sup> 500 MHz. <sup>c</sup> 400 MHz.

The relative configuration of compound **1** was determined via ROESY experiment (Figure 1). Assuming H-7 to be  $\alpha$ -oriented, as in most natural germacranes isolated from higher plants,<sup>25,26</sup> the correlation displayed by H-7 with H-5 indicated that the tiglate group was  $\beta$ -oriented. However, the ROESY spectrum could not provide sufficient information to establish the orientations of H-6, H-8, H-9, and the endocyclic double bond at C-3. Finally, a single-crystal X-ray diffraction study unambiguously established the relative configuration and structure of **1** (Figure 2). Thus, compound **1** was elucidated as 9,10-epoxy-5 $\beta$ -*O*-tigloyl-7 $\alpha$ H-8 $\beta$ -*O*-acetylgermacra-3(4)*E*-en-6 $\alpha$ -ol and named trijugin A.

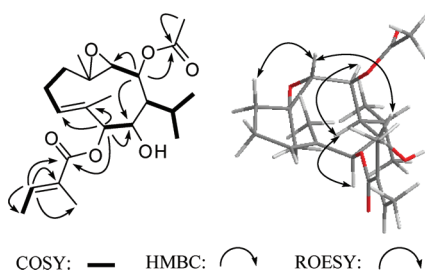
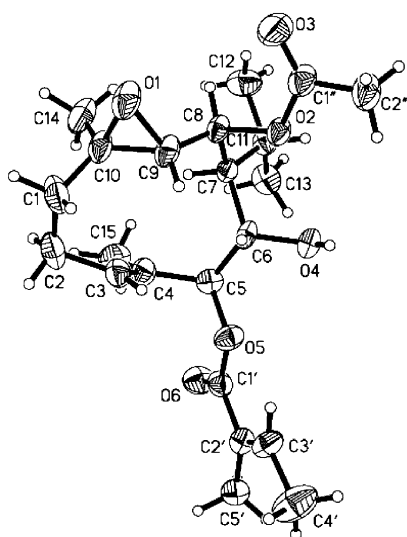
The NMR data of trijugin B (**2**) and trijugin C (**3**) showed that their structures were closely related to **1**. In comparison with **1**, the H-6 resonance in **2** was shifted downfield by  $\Delta\delta$  1.42 ppm and was attributed to the presence of an *O*-acetyl group at C-6. The difference between **3** and **1** involved the positions of the hydroxy and the tiglate group. Analysis of the HMBC correlations of **3**

indicated that the tiglate group was assigned at C-6, while the hydroxy group was located at C-5. The relative configurations of **2** and **3** were also identical to those of **1**, as H-7 $\alpha$  correlated with H-5 and H-8, whereas H-9 correlated with H-6. Therefore, compound **2** was deduced as 9,10-epoxy-5 $\beta$ -*O*-tigloyl-6 $\alpha$ ,8 $\beta$ -di-*O*-acetyl-7 $\alpha$ H-germacra-3(4)*E*-ene, while the structure 9,10-epoxy-6 $\alpha$ -*O*-tigloyl-7 $\alpha$ H-8 $\beta$ -*O*-acetylgermacra-3(4)*E*-en-5 $\beta$ -ol was proposed for compound **3**.

Compound **4** had the molecular formula  $\text{C}_{27}\text{H}_{42}\text{O}_8$  based on HRESIMS ( $[\text{M} + \text{Na}]^+ m/z$  517.2795; calcd 517.2777). Comparison of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of **4** with those of **1** (Tables 1 and 3) indicated that the two compounds were related, except for the existence of an *O*-isovaleryl group in **4**, as deduced from the  $^1\text{H}$ – $^1\text{H}$  COSY and HMBC correlations. The HMBC correlations from H-8 to C-1'' and from H-3'' to C-1'' and C-6'' implied that the *O*-isovaleryl group was assigned at C-8 and the *O*-acetyl group at C-3''. The  $\beta$ -orientation of the *O*-isovaleryl group was deduced from

**Table 3.**  $^{13}\text{C}$  NMR Data of Compounds **1–9** in  $\text{CDCl}_3$  ( $\delta_{\text{C}}$ )<sup>a</sup>

| no.     | 1       | 2       | 3       | 4       | 5       | 6       | 7       | 8       | 9       |
|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| 1       | 38.0 t  | 37.8 t  | 38.0 t  | 38.0 t  | 37.8 t  | 37.9 t  | 37.7 t  | 38.3 t  | 38.2 t  |
| 2       | 24.3 t  | 24.4 t  | 24.4 t  | 24.3 t  | 24.4 t  | 24.3 t  | 24.4 t  | 24.3 t  | 24.3 t  |
| 3       | 129.5 d | 131.4 d | 129.9 d | 129.2 d | 131.6 d | 130.0 d | 131.6 d | 128.8 d | 128.9 d |
| 4       | 133.4 s | 132.2 s | 134.8 s | 133.6 s | 131.9 s | 134.7 s | 132.0 s | 137.3 s | 137.1 s |
| 5       | 80.2 d  | 77.7 d  | 77.0 d  | 80.2 d  | 77.9 d  | 77.2 d  | 77.7 d  | 28.8 t  | 29.3 t  |
| 6       | 73.8 d  | 72.2 d  | 76.6 d  | 73.4 d  | 72.0 d  | 76.0 d  | 72.0 d  | 72.1 d  | 71.5 d  |
| 7       | 46.8 d  | 46.9 d  | 47.5 d  | 47.0 d  | 46.7 d  | 47.3 d  | 46.6 d  | 48.1 d  | 47.7 d  |
| 8       | 73.9 d  | 73.2 d  | 73.5 d  | 74.3 d  | 73.4 d  | 73.6 d  | 73.7 d  | 74.2 d  | 73.9 d  |
| 9       | 66.5 d  | 66.0 d  | 66.1 d  | 66.3 d  | 65.8 d  | 65.8 d  | 65.7 d  | 66.3 d  | 66.2 d  |
| 10      | 59.3 s  | 59.0 s  | 59.2 s  | 59.2 s  | 59.0 s  | 59.1 s  | 59.2 s  | 59.4 s  | 59.2 s  |
| 11      | 24.9 d  | 25.8 d  | 25.9 d  | 24.9 d  | 25.8 d  | 25.8 d  | 25.7 d  | 26.1 d  | 26.1 d  |
| 12      | 21.1 q  | 21.1 q  | 21.2 q  | 21.1 q  | 21.0 q  | 21.1 q  | 21.0 q  | 21.3 q  | 21.4 q  |
| 13      | 23.4 q  | 23.0 q  | 23.1 q  | 23.5 q  | 23.2 q  | 23.2 q  | 23.1 q  | 23.2 q  | 23.3 q  |
| 14      | 16.5 q  | 16.6 q  | 16.6 q  | 16.5 q  | 16.5 q  | 16.5 q  | 16.5 q  | 16.7 q  | 16.6 q  |
| 15      | 19.5 q  | 19.4 q  | 19.9 q  | 19.5 q  | 19.5 q  | 19.9 q  | 19.4 q  | 20.5 q  | 20.9 q  |
| 1'      | 167.7 s | 166.3 s | 168.3 s | 167.7 s | 166.3 s | 168.1 s | 166.7 s | 167.0 s | 166.9 s |
| 2'      | 128.4 s | 128.5 s | 128.5 s | 128.5 s | 128.5 s | 128.5 s | 128.4 s | 128.8 s | 128.9 s |
| 3'      | 137.8 d | 137.1 d | 138.1 d | 137.7 d | 137.3 d | 138.0 d | 137.8 d | 137.3 d | 137.1 d |
| 4'      | 14.4 q  | 14.4 q  | 14.5 q  | 14.4 q  | 14.4 q  | 14.5 q  | 14.5 q  | 14.4 q  | 14.4 q  |
| 5'      | 12.1 q  | 12.0 q  | 12.1 q  | 12.1 q  | 12.0 q  | 12.0 q  | 12.0 q  | 11.9 q  | 11.9 q  |
| 1''     | 170.0 s | 169.8 s | 169.9 s | 172.4 s | 172.5 s | 172.5 s | 174.6 s | 174.5 s | 172.4 s |
| 2''     | 21.0 q  | 20.8 q  | 20.7 q  | 45.4 d  | 45.2 d  | 45.2 d  | 47.3 d  | 47.2 d  | 45.1 d  |
| 3''     |         |         |         | 71.6 d  | 71.5 d  | 71.5 d  | 68.8 d  | 69.0 d  | 71.5 d  |
| 4''     |         |         |         | 16.8 q  | 20.8 q  | 17.0 q  | 20.5 q  | 20.5 q  | 17.0 q  |
| 5''     |         |         |         | 12.8 q  | 12.7 q  | 12.7 q  | 13.7 q  | 13.6 q  | 12.7 q  |
| 5/6-OAc |         | 170.4 s |         |         | 169.9 s |         | 170.5 s |         |         |
| 3''-OAc |         | 20.6 q  |         | 170.6 s | 21.1 q  | 170.0 s | 20.8 q  |         |         |
|         |         |         |         |         | 21.1 q  | 20.9 q  | 20.9 q  |         | 20.9 q  |

<sup>a</sup> Assignments are based on 1D and 2D NMR experiments.**Figure 1.** Key 2D NMR correlations of **1**.**Figure 2.** ORTEP drawing of the crystal structure of **1**.

the coupling constant ( $J = 6.8$  Hz) and ROESY correlation of H-8 with H<sub>3</sub>-14. The relative configurations of the other positions were also identical to those of **1**. Accordingly, compound **4** was

determined as 9,10-epoxy-5 $\beta$ -*O*-tigloyl-7 $\alpha$ H-8 $\beta$ -*O*-(3''-acetoxy-2''-methylbutyryl)germacra-3(4)*E*-en-6 $\alpha$ -ol, with the trivial name trijugin D.

Comparison of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of **5–9** with those of **4** showed that their structures were closely related. Compound **5** had one more *O*-acetyl group compared with **4**, which was located at C-6, as elucidated from the HMBC correlation of  $\delta_{\text{H}}$  5.83 (H-6) with the acetyl carbonyl carbon ( $\delta_{\text{C}}$  169.9). Hence, compound **5** was defined as 9,10-epoxy-5 $\beta$ -*O*-tigloyl-6 $\alpha$ -*O*-acetyl-7 $\alpha$ H-8 $\beta$ -*O*-(3''-acetoxy-2''-methylbutyryl)germacra-3(4)*E*-ene. Compound **6** was found to be an isomer of **4**. The only difference was the different location of the tiglate group, located at C-6 in **6**, as validated by the HMBC correlation of  $\delta_{\text{H}}$  5.56 (H-6) with  $\delta_{\text{C}}$  168.1 (C-1'). Consequently, compound **6** was characterized as 9,10-epoxy-6 $\alpha$ -*O*-tigloyl-7 $\alpha$ H-8 $\beta$ -*O*-(3''-acetoxy-2''-methylbutyryl)germacra-3(4)*E*-en-5 $\beta$ -ol. Compound **7** had the same molecular formula ( $\text{C}_{27}\text{H}_{42}\text{O}_8$ ) as **6**. The only difference was the different positions of the *O*-acetyl group. The HMBC correlation from  $\delta_{\text{H}}$  4.96 (H-5) to the acetyl carbonyl carbon ( $\delta_{\text{C}}$  170.5) suggested that the *O*-acetyl was connected to C-5. Therefore, compound **7** was established as 9,10-epoxy-5 $\beta$ -*O*-acetyl-6 $\alpha$ -*O*-tigloyl-7 $\alpha$ H-8 $\beta$ -*O*-(3''-hydroxy-2''-methylbutyryl)germacra-3(4)*E*-ene.

Compound **8** differed structurally from **7** only at C-5. The *O*-acetyl group at C-5 in **7** was absent in **8**, as deduced from the  $^{13}\text{C}$  NMR and HMBC spectra. The structure of **8** was therefore elucidated as 9,10-epoxy-6 $\alpha$ -*O*-tigloyl-7 $\alpha$ H-8 $\beta$ -*O*-(3''-hydroxy-2''-methylbutyryl)germacra-3(4)*E*-ene. Compared to the NMR data and MS spectrum of **8**, compound **9** had one more *O*-acetyl group, which was connected to C-3'', as deduced from the HMBC correlations of  $\delta_{\text{H}}$  4.99 (H-3'') with the acetyl carbonyl carbon ( $\delta_{\text{C}}$  170.0). Thus, compound **9** was deduced as 9,10-epoxy-6 $\alpha$ -*O*-tigloyl-7 $\alpha$ H-8 $\beta$ -*O*-(3''-acetoxy-2''-methylbutyryl)germacra-3(4)*E*-ene and named trijugin I.

Compound **10**, white amorphous powder, had the molecular formula  $\text{C}_{32}\text{H}_{52}\text{O}_3$ , as established by HREIMS ( $M^+$   $m/z$  484.3902; calcd 484.3916). The IR spectrum displayed the presence of hydroxy ( $3448\text{ cm}^{-1}$ ), carbonyl ( $1743\text{ cm}^{-1}$ ), and olefinic ( $1640$  and  $882\text{ cm}^{-1}$ ) functionalities. The  $^{13}\text{C}$  NMR spectrum (Table 4)

**Table 4.**  $^{13}\text{C}$  NMR and  $^1\text{H}$  NMR Data of Compound **10**.  $\delta$  in ppm,  $J$  in Hz<sup>a</sup>

| no. | $\delta_{\text{C}}$ | $\delta_{\text{H}}$                   | no. | $\delta_{\text{C}}$ | $\delta_{\text{H}}$            |
|-----|---------------------|---------------------------------------|-----|---------------------|--------------------------------|
| 1   | 42.3 t              | 1.76, dd (15.5, 5.5)<br>1.09, overlap | 17  | 42.9 s              |                                |
| 2   | 65.8 d              | 4.08, td (15.0, 5.0)                  | 18  | 48.2 d              | 1.35, overlap                  |
| 3   | 80.6 d              | 4.87, d (3.0)                         | 19  | 47.9 d              | 2.37, td (13.5, 7.5)           |
| 4   | 38.2 s              |                                       | 20  | 150.7 s             |                                |
| 5   | 49.6 d              | 1.09, overlap                         | 21  | 29.8 t              | 1.91, m<br>1.32, overlap       |
| 6   | 17.8 t              | 1.40, overlap<br>1.35, overlap        | 22  | 39.9 t              | 1.38, overlap<br>1.20, overlap |
| 7   | 33.9 t              | 1.40, overlap                         | 23  | 27.9 q              | 0.86, s                        |
| 8   | 40.9 s              |                                       | 24  | 21.6 q              | 0.91, s                        |
| 9   | 50.1 d              | 1.41, overlap                         | 25  | 17.0 q              | 0.88, s                        |
| 10  | 38.5 s              |                                       | 26  | 16.0 q              | 1.02, s                        |
| 11  | 21.1 t              | 1.44, overlap<br>1.26, overlap        | 27  | 14.7 q              | 0.98, s                        |
| 12  | 24.9 t              | 1.68, overlap<br>1.08, overlap        | 28  | 17.9 q              | 0.78, s                        |
| 13  | 37.9 d              | 1.65, overlap                         | 29  | 109.3 t             | 4.69, s<br>4.57, s             |
| 14  | 42.9 s              |                                       | 30  | 19.2 q              | 1.67, s                        |
| 15  | 27.4 t              | 1.69, overlap<br>1.00, overlap        | OAc | 172.0 s<br>20.8 q   |                                |
| 16  | 35.5 t              | 1.48, overlap<br>1.38, overlap        |     |                     | 2.14, s                        |

<sup>a</sup> 500 MHz for  $\delta_{\text{H}}$ , 125 MHz for  $\delta_{\text{C}}$ , in  $\text{CDCl}_3$ .

**Table 5.** Cytotoxicity of Compounds **9** and **32** against Tumor Cell Lines with  $\text{IC}_{50}$  ( $\mu\text{M}$ ) Values<sup>a</sup>

| compound               | HL-60 | SMMC-7721 | A-549 | MCF-7 | SW480 |
|------------------------|-------|-----------|-------|-------|-------|
| <b>9</b>               | 17.05 | 34.05     | >40   | >40   | 24.88 |
| <b>32</b>              | 15.48 | 21.01     | 24.68 | >40   | 10.86 |
| cisplatin <sup>b</sup> | 0.75  | 12.97     | 15.23 | 20.17 | 11.94 |

<sup>a</sup> Cell lines: HL-60 acute leukemia; SMMC-7721 liver cancer; A-549 lung cancer; MCF-7 breast cancer; SW480 colon cancer. <sup>b</sup> Positive control.

exhibited signals for 32 carbons, including seven quaternary (one carbonyl and one olefinic), seven methine (two oxygenated), 10 methylene (one olefinic), and eight methyl carbons. Comparison of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of **10** with those of the known compound lup-20(29)-ene-2 $\alpha$ ,3 $\alpha$ -diol (**27**) showed their similarity.<sup>19</sup> The only difference was the replacement of a hydroxy at C-3 of **27** by an *O*-acetyl in **10**, as indicated by HMBC correlations of  $\delta_{\text{H}}$  4.87 (H-3) with the acetyl carbonyl carbon ( $\delta_{\text{C}}$  172.0). Finally, the presence of a ROESY correlation of H-2 with H-3 and the small coupling constant of H-3 ( $J = 3.0$  Hz) verified that the hydroxy group at C-2 and the *O*-acetyl group at C-3 were similarly oriented, which were also in accord with those of lup-20(29)-ene-2 $\alpha$ ,3 $\alpha$ -diol (**27**).<sup>19</sup> Accordingly, the structure of **10** was determined as 3 $\alpha$ -*O*-acetyl-20(29)-lupen-2 $\alpha$ -ol.

It is interesting to note that two pairs of regioisomers, **1/3** and **4/6**, were obtained from the same plant. Compounds **1** and **3** were detected in the acetone extract of the dried whole plants of *S. trijuga* obtained under mild conditions (Figure S39, Supporting Information), indicating that they must be natural products rather than artifacts from the isolation procedure. Since the trans-esterification process is feasible in plants,<sup>27</sup> we cannot exclude the possibility of intramolecular trans-esterification between **1** and **3** and between **4** and **6**.

Compounds **1–10** and **32** were tested for their toxicity effects in the human tumor cell lines HL-60, SMMC-7721, A-549, MCF-7, and SW480 (Table 5). Among these compounds, compound **32** showed moderate toxicity against HL-60, SMMC-7721, A-549, and SW480, while compound **9** exhibited moderate toxicity against HL-60, SMMC-7721, and SW480.

## Experimental Section

**General Experimental Procedures.** Melting points were obtained on an X-4 micro melting point apparatus. Optical rotations were measured on a JASCO-20C digital polarimeter. IR spectra were obtained on a Tensor 27 spectrometer with KBr pellets. UV spectra were recorded using a Shimadzu UV-2401A spectrophotometer. 1D and 2D NMR spectra were performed on a Bruker AM-400 or DRX-500 spectrometer with TMS as an internal standard. Mass spectra were recorded on a VG Auto Spec-3000 or API-Qstar-Pulsar instrument. Semipreparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C18 (9.4 mm  $\times$  25 cm) column. Column chromatography (CC) was performed using silica gel (100–200 and 200–300 mesh, Qingdao Marine Chemical Co. Ltd., Qingdao, People's Republic of China), Lichroprep RP-18 gel (40–63  $\mu\text{m}$ , Merck, Darmstadt, Germany), MCI gel (75–150  $\mu\text{m}$ ; Mitsubishi Chemical Corporation, Japan), and Sephadex LH-20 (Amersham Pharmacia Biotech, Sweden). All solvents were distilled prior to use.

**Plant Material.** Plants of *S. trijuga* (whole plant) were collected in the Habaxueshan of Yunnan Province, People's Republic of China, in July 2003. The sample was identified by Prof. Xi-Wen Li, and a voucher specimen (200301) was deposited with the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

**Extraction and Isolation.** The dried and powdered whole plants of *S. trijuga* (19.5 kg) were extracted with  $\text{Me}_2\text{CO}$  (3  $\times$  75 L, 3  $\times$  24 h) at room temperature. The extract was evaporated to dryness under reduced pressure. The residue was suspended in  $\text{H}_2\text{O}$  (3 L) and partitioned with EtOAc (3  $\times$  2 L) to afford an EtOAc extract (580 g). The EtOAc extract was decolorized on MCI gel (eluted with 90% MeOH) and subjected to a silica gel (100–200 mesh) CC eluted with a gradient of petroleum ether– $\text{Me}_2\text{CO}$  (1:0  $\rightarrow$  0:1) to obtain five fractions (A–E). Fraction B (73.5 g) was further purified over silica gel CC eluted with petroleum ether–EtOAc (1:0  $\rightarrow$  0:1) to provide four subfractions (B<sub>1</sub>–B<sub>4</sub>). Subfraction B<sub>1</sub> was further chromatographed over repeated silica gel CC combined with Sephadex LH-20 eluted with  $\text{CHCl}_3$ –MeOH (1:1) to afford **9** (30 mg), **5** (35 mg), **2** (432 mg), **11** (5 mg), and **12** (13 mg). Subfraction B<sub>2</sub> was further chromatographed over MCI (90% MeOH– $\text{H}_2\text{O}$ , then 100% MeOH) to yield fractions B<sub>2.1</sub>–B<sub>2.3</sub>. Compound **1** (320 mg) was crystallized in  $\text{CHCl}_3$  from subfraction B<sub>2.1</sub>. Subfraction B<sub>2.2</sub> was chromatographed over silica gel CC eluted with petroleum ether– $\text{CHCl}_3$ –EtOAc (8.5:1:0.5) and then separated by semipreparative HPLC (75% MeOH– $\text{H}_2\text{O}$ ) to give **8** (18 mg), **16** (7 mg), and **17** (7 mg). Compound **13** (5 g) was obtained by recrystallization in MeOH from subfraction B<sub>2.3</sub>. Subfraction B<sub>3</sub> was subjected to an RP-18 gel eluted with MeOH– $\text{H}_2\text{O}$  (1:1  $\rightarrow$  1:0) followed by repeated silica gel CC (petroleum ether– $\text{CHCl}_3$ –EtOAc, 8:1:1) to afford **3** (64 mg), **4** (56 mg), **14** (3 g), **15** (25 mg), and **26** (80 mg). Subfraction B<sub>4</sub> was applied to an RP-18 gel eluted with MeOH– $\text{H}_2\text{O}$  (1:1  $\rightarrow$  1:0), followed by chromatography over repeated silica gel CC, and finally purified by semipreparative HPLC (70% MeOH– $\text{H}_2\text{O}$ ) to yield **6** (356 mg), **7** (115 mg), **23** (5 mg), **24** (5 mg), and **25** (6 mg). Fraction C (63.5 g) was chromatographed on MCI gel (7:3  $\rightarrow$  1:0 MeOH– $\text{H}_2\text{O}$ ) to give subfractions C<sub>1</sub>–C<sub>3</sub>. Subfraction C<sub>1</sub> was further subjected to repeated silica gel CC eluted with petroleum ether–acetone (9:1  $\rightarrow$  0:1) and then purified by semipreparative HPLC (65% MeOH– $\text{H}_2\text{O}$ ) to obtain **18** (20 mg), **19** (10 mg), **20** (20 mg), **21** (8 mg), and **22** (50 mg). Compounds **10** (20 mg), **27** (30 mg), **28** (15 mg), and **29** (15 mg) were isolated from subfraction C<sub>2</sub> by repeated chromatography including silica gel CC, RP-18, and Sephadex LH-20. Compound **33** (10 g) was crystallized from subfraction C<sub>3</sub> directly. Fraction D (45.0 g) was submitted to repeated chromatography and purified by Sephadex LH-20 to afford **30** (30 mg), **31** (46 mg), **32** (35 mg), and **34** (235 mg).

The dried and powdered whole plants of *S. trijuga* (5 g) were extracted with acetone (100 mL) at room temperature for 30 min to give a crude extract. The MeOH-soluble portion was subjected to HPLC analysis (Zorbax SB-C18, 4.6  $\times$  250 mm, 3.5  $\mu\text{m}$ ;  $\text{CH}_3\text{OH}$ – $\text{H}_2\text{O}$  (65:35), 1 mL/min; 30  $^\circ\text{C}$ ; 238 nm).

**Trijugin A (1):** colorless crystals ( $\text{CHCl}_3$ ); mp 123–125  $^\circ\text{C}$ ;  $[\alpha]_{\text{D}}^{26.6} -75.6$  (c 0.31,  $\text{CHCl}_3$ ); UV ( $\text{CHCl}_3$ )  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 240 (5.18); IR (KBr)  $\nu_{\text{max}}$  3561, 2970, 2871, 1721, 1703, 1649, 1631, 1388, 1263, and 1077  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 1 and 3; positive ESIMS 417  $[\text{M} + \text{Na}]^+$ ; positive HRESIMS  $m/z$  417.2259  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{22}\text{H}_{34}\text{O}_6\text{Na}$ , 417.2253).

**Trijugin B (2):** colorless powder;  $[\alpha]_D^{25}$   $-123.6$  ( $c$  0.21,  $\text{CHCl}_3$ ); UV ( $\text{CHCl}_3$ )  $\lambda_{\text{max}}$  ( $\log \epsilon$ ) 239 (5.16); IR (KBr)  $\nu_{\text{max}}$  2980, 2933, 1742, 1711, 1653, 1372, 1260, 1134, and 1077  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 1 and 3; positive FABMS 437  $[\text{M} + \text{H}]^+$ ; positive HRFABMS  $m/z$  437.2518  $[\text{M} + \text{H}]^+$  (calcd for  $\text{C}_{24}\text{H}_{37}\text{O}_7$ , 437.2539).

**Trijugin C (3):** colorless crystals ( $\text{CHCl}_3$ ); mp 139–141  $^\circ\text{C}$ ;  $[\alpha]_D^{15}$   $-19.0$  ( $c$  0.08,  $\text{CHCl}_3$ ); UV ( $\text{CHCl}_3$ )  $\lambda_{\text{max}}$  ( $\log \epsilon$ ) 240 (4.94); IR (KBr)  $\nu_{\text{max}}$  3452, 2925, 2854, 1742, 1720, 1649, 1385, 1268, and 1234  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 1 and 3; negative ESIMS 429  $[\text{M} + \text{Cl}]^-$ ; negative HRESIMS  $m/z$  429.2045  $[\text{M} + \text{Cl}]^-$  (calcd for  $\text{C}_{22}\text{H}_{34}\text{O}_6\text{Cl}$ , 429.2043).

**Trijugin D (4):** colorless powder;  $[\alpha]_D^{15.5}$   $-44.3$  ( $c$  0.16,  $\text{CHCl}_3$ ); UV ( $\text{CHCl}_3$ )  $\lambda_{\text{max}}$  ( $\log \epsilon$ ) 239 (5.34); IR (KBr)  $\nu_{\text{max}}$  3458, 2934, 1739, 1713, 1650, 1385, 1260, and 1077  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 1 and 3; positive ESIMS 517  $[\text{M} + \text{Na}]^+$ ; positive HRESIMS  $m/z$  517.2795  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{27}\text{H}_{42}\text{O}_8\text{Na}$ , 517.2777).

**Trijugin E (5):** colorless gum;  $[\alpha]_D^{25}$   $-178.6$  ( $c$  0.14,  $\text{CHCl}_3$ ); UV ( $\text{CHCl}_3$ )  $\lambda_{\text{max}}$  ( $\log \epsilon$ ) 239 (5.24); IR (KBr)  $\nu_{\text{max}}$  2935, 2874, 1742, 1651, 1376, 1240, and 1071  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 1 and 3; positive ESIMS 559  $[\text{M} + \text{Na}]^+$ ; positive HRESIMS  $m/z$  559.2894  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{29}\text{H}_{44}\text{O}_9\text{Na}$ , 559.2883).

**Trijugin F (6):** colorless crystals ( $\text{CHCl}_3$ ); mp 108–110  $^\circ\text{C}$ ;  $[\alpha]_D^{17.8}$   $-52.3$  ( $c$  0.10,  $\text{CHCl}_3$ ); UV ( $\text{CHCl}_3$ )  $\lambda_{\text{max}}$  ( $\log \epsilon$ ) 239 (5.28) nm; IR (KBr)  $\nu_{\text{max}}$  3421, 2932, 2868, 1739, 1723, 1650, 1379, 1259, and 1070  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 2 and 3; positive ESIMS 517  $[\text{M} + \text{Na}]^+$ ; positive HRESIMS  $m/z$  517.2777  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{27}\text{H}_{42}\text{O}_8\text{Na}$ , 517.2777).

**Trijugin G (7):** colorless powder;  $[\alpha]_D^{17.7}$   $-83.3$  ( $c$  0.12,  $\text{CHCl}_3$ ); UV ( $\text{CHCl}_3$ )  $\lambda_{\text{max}}$  ( $\log \epsilon$ ) 239 (5.22) nm; IR (KBr)  $\nu_{\text{max}}$  3468, 2978, 2874, 1736, 1716, 1694, 1652, 1382, 1236, and 1132  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 2 and 3; positive ESIMS 517  $[\text{M} + \text{Na}]^+$ ; positive HRESIMS  $m/z$  517.2782  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{27}\text{H}_{42}\text{O}_8\text{Na}$ , 517.2777).

**Trijugin H (8):** colorless gum;  $[\alpha]_D^{22.8}$   $-42.1$  ( $c$  0.08,  $\text{CHCl}_3$ ); UV ( $\text{CHCl}_3$ )  $\lambda_{\text{max}}$  ( $\log \epsilon$ ) 239 (5.46); IR (KBr)  $\nu_{\text{max}}$  3487, 2975, 2926, 2863, 1733, 1687, 1650, 1274, and 1149  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 2 and 3; positive ESIMS 459  $[\text{M} + \text{Na}]^+$ ; positive HRESIMS  $m/z$  459.2712  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{25}\text{H}_{40}\text{O}_6\text{Na}$ , 459.2722).

**Trijugin I (9):** colorless gum;  $[\alpha]_D^{22.8}$   $-36.9$  ( $c$  0.38,  $\text{CHCl}_3$ ); UV ( $\text{CHCl}_3$ )  $\lambda_{\text{max}}$  ( $\log \epsilon$ ) 239 (5.45); IR (KBr)  $\nu_{\text{max}}$  2983, 2930, 1742, 1711, 1651, 1385, 1258, and 1077  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 2 and 3; positive ESIMS 501  $[\text{M} + \text{Na}]^+$ ; positive HRESIMS  $m/z$  501.2826  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{27}\text{H}_{42}\text{O}_7\text{Na}$ , 501.2828).

**3 $\alpha$ -O-Acetyl-20(29)-lupen-2 $\alpha$ -ol (10):** white, amorphous powder;  $[\alpha]_D^{15}$   $-1.59$  ( $c$  0.14,  $\text{CHCl}_3$ ); IR (KBr)  $\nu_{\text{max}}$  3448, 2942, 2858, 1743, 1640, 1379, 1246, and 882  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Table 4; EIMS 484  $[\text{M}]^+$ ; HREIMS  $m/z$  484.3902  $[\text{M}]^+$  (calcd for  $\text{C}_{32}\text{H}_{52}\text{O}_3$ , 484.3916).

**X-ray Single-Crystal Structure Determination of Trijugin A (1).**  $\text{C}_{22}\text{H}_{34}\text{O}_6$ ,  $M = 394.49$ ; orthorhombic, space group  $P2_1$ ;  $a = 8.9800(18)$   $\text{\AA}$ ,  $b = 27.932(6)$   $\text{\AA}$ ,  $c = 9.2638(19)$   $\text{\AA}$ ,  $\alpha = 90.00^\circ$ ,  $\beta = 90.00^\circ$ ,  $\gamma = 90.00^\circ$ ,  $V = 2323.6(8)$   $\text{\AA}^3$ ,  $Z = 5$ ,  $d = 1.179$   $\text{g/cm}^3$ , crystal dimensions  $0.20 \times 0.14 \times 0.11$  mm were used for measurement on a SHELXL-97 with a graphite monochromator, Mo  $K\alpha$  radiation. The total number of reflections measured was 15 232, of which 9959 were observed,  $I > 2\sigma(I)$ . Final indices:  $R_1 = 0.0715$ ,  $wR_2 = 0.1576$ . The crystal structure of **1** was solved by direct method SHELXS-97 (Sheldrick, 1990) and expanded using difference Fourier technique, refined by the program SHELXL-97 (Sheldrick, 1997) and the full-matrix least-squares calculations. Crystallographic data for the structure of **1** have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 762467). Copies of these data can be obtained free of charge via [www.ccdc.cam.ac.uk/conts/retrieving.html](http://www.ccdc.cam.ac.uk/conts/retrieving.html) (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, U.K.; fax: (+44) 1223-336-033; or e-mail: [desposit@ccdc.cam.ac.uk](mailto:desposit@ccdc.cam.ac.uk)).

**Cytotoxicity Assay.** The cytotoxicity of compounds **1–10** and **32** against HL-60, SMMC-7721, A-549, MCF-7, and SW480 cell lines was assessed using the MTT method.<sup>28</sup> Cells were plated in 96-well plates 12 h before treatment, and continuously exposed to different concentrations of compounds. After 48 h, 20  $\mu\text{L}$  of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution was added to each well, which were incubated for a further 4 h. Then 20% SDS (100  $\mu\text{L}$ ) was added to each well. After 12 h at room temperature, the

OD value of each well was recorded at 595 nm. The  $\text{IC}_{50}$  value of each compound was calculated by the Reed and Muench method.<sup>29</sup>

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**Supporting Information Available:** This material is available free of charge via the Internet at <http://pubs.acs.org>.

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