

# Cytotoxic isoprenylated xanthenes from *Cudrania tricuspidata*

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**Abstract**—Eight new isoprenylated xanthenes, cudratricusxanthenes A–H (**1**–**8**), were isolated from the roots of *Cudrania tricuspidata*, together with ten known compounds, cudraxanthenes H (**9**) and M (**10**), xanthone V<sub>1a</sub> (**11**), toxyloxanthone C (**12**), macluraxanthone B (**13**), 1-hydroxy-3, 6, 7-trimethoxyxanthone (**14**), cycloartocarpesin (**15**), artocarpesin (**16**), cudraflavone B (**17**), and kaempferol (**18**). Their structures were characterized by spectroscopic methods. Xanthenes **5**, **7**, **10**, and **12** showed inhibitory effects on four kinds of human digestive apparatus tumor cell lines (HCT-116, SMMC-7721, SGC-7901, and BGC-823) with IC<sub>50</sub> values of 1.6–11.8 µg/mL. Xanthenes **2**, **4**, and **11** displayed significant cytotoxicity against HCT-116, SMMC-7721, and SGC-7901 (IC<sub>50</sub> = 1.3–9.8 µg/mL). Flavonoids **15**–**17** were almost inactive.

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## 1. Introduction

*Cudrania* species (Moraceae), a rich source of prenylated xanthenes and flavonoids, have been investigated phytochemically and biologically.<sup>1–5</sup> Recently, we reported the structures, cytotoxicity, and antifungal activity of some prenylated phenolic compounds (benzophenones, xanthenes, and flavonoids) from the roots of *C. cochinchinensis*.<sup>6</sup> As part of our continuing research on this genus, further investigations of *Cudrania tricuspidata* (Carr.) Bur., a deciduous shrub or tree distributed over China, Korea, and Japan, were carried out. Its roots are applied in clinic for the treatment of digestive apparatus tumor, especially gastric carcinoma,<sup>7,8</sup> and are also used as Chinese folk medicine ‘Chuan-po-shi’ together with the roots of *C. cochinchinensis* (Lour) against gonorrhea, rheumatism, jaundice, boils, scabies, bruising, and dysmenorrhea.<sup>9</sup> The pharmacological study showed that the crude extract from the roots of *C. tricuspidata* could inhibit the growth of NKM cell line.<sup>10</sup> However, the anti-tumor principles are unknown

although some prenylated xanthenes and flavonoids were isolated from the root bark of this plant previously.<sup>1–3</sup>

Our primary bioassay showed that the chloroform-soluble fraction from an ethanol extract of the roots of *C. tricuspidata* exhibited cytotoxic activity against human gastric carcinoma cell lines (SGC-7901 and BGC-823) in vitro. Further separation of this fraction afforded eight new isoprenylated xanthenes (cudratricusxanthenes A–H, **1**–**8**) and ten known compounds **9**–**18**. Thirteen isolates were screened for their inhibitory effects on four kinds of human digestive apparatus tumor cell lines, including human colon carcinoma (HCT-116), hepatocellular carcinoma (SMMC-7721), and gastric carcinoma (SGC-7901 and BGC-823). We herein present the structure elucidation of **1**–**8** and the cytotoxicity evaluation.

## 2. Results and discussion

### 2.1. Structural elucidation of new compounds **1**–**8**

Cudratricusxanthone A (**1**), yellow prisms, showed a [M+Na]<sup>+</sup> peak at *m/z* 419.1472 in the HRESIMS,

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corresponding to a molecular formula of  $C_{23}H_{24}O_6$ . The IR spectrum showed the presence of hydroxyl groups ( $3386\text{ cm}^{-1}$ ), a conjugated carbonyl group ( $1647\text{ cm}^{-1}$ ), and benzene rings ( $1614$ ,  $1556$ , and  $1501\text{ cm}^{-1}$ ). The UV spectrum was similar to those of **1**, **3**, **6**, **7**-tetraoxygenated xanthenes.<sup>1a,1d</sup> The  $^1\text{H}$  NMR spectrum contained signals of four hydroxyl groups [ $\delta$  13.78 (1H, s), 9.32 (2H, br s), and 7.99 (1H, br s)], two aromatic singlets [ $\delta$  6.85 and 6.23 (each 1H, s)], a 3,3-dimethylallyl (prenyl) group [ $\delta$  5.31 (1H, m), 4.17 (2H, br d,  $J=6.8$  Hz), and 1.83, 1.63 (each 3H, br s)], and a 1, 1-dimethylallyl group [ $\delta$  6.35 (1H, dd,  $J=10.6$ ,  $17.4$  Hz), 5.00 (1H, dd,  $J=1.2$ ,  $17.4$  Hz), 4.89 (1H, dd,  $J=1.2$ ,  $10.6$  Hz), and 1.65 (6H, s)]. The obviously downfield methylene proton signal at  $\delta_{\text{H}}$  4.17 was explained reasonably by the prenyl group at C-8, *peri* to the carbonyl group.<sup>11</sup> The  $^{13}\text{C}$  NMR spectrum revealed the presence of 23 carbons (Table 1), including one carbonyl group, two aromatic rings with six oxygenated carbons, and two  $\text{C}_5$  groups, corresponding to a diprenylated and tetrahydroxylated xanthone. The position of six substituents on the xanthone skeleton was determined on the basis of HMQC and HMBC spectral analysis. In the HMBC spectrum, the hydrogen-bonded hydroxyl group at  $\delta_{\text{H}}$  13.78 (OH-1) correlated with C-1 ( $\delta_{\text{C}}$  162.9), C-2 ( $\delta_{\text{C}}$  99.9), and C-9a ( $\delta_{\text{C}}$  104.9). The aromatic proton at  $\delta_{\text{H}}$  6.23 (H-2) coupled with C-1, C-3 ( $\delta_{\text{C}}$  164.0), C-4 ( $\delta_{\text{C}}$  111.3), and C-9a. The olefinic proton at  $\delta_{\text{H}}$  6.35 (H-14) and the methyl groups at  $\delta_{\text{H}}$  1.65 (H<sub>3</sub>-12, 13) showed cross-peaks with C-4. These results established a partial structure of **1** as 1,3-dihydroxy-4-(1, 1-dimethyl-2-propenyl) xanthone. In addition, the following HMBC correlations were also observed: the aromatic singlet at  $\delta_{\text{H}}$  6.85 (H-5) with C-4b ( $\delta_{\text{C}}$  152.9), C-6 ( $\delta_{\text{C}}$  153.7), and C-8a ( $\delta_{\text{C}}$  112.0); the broad doublet at  $\delta_{\text{H}}$  4.17 (H<sub>2</sub>-16) with C-7 ( $\delta_{\text{C}}$  142.1) and C-8a. Accordingly, the two

hydroxyl groups and the prenyl group were located at C-6, C-7, and C-8, respectively. Thus, the structure of cudraticusxanthone A was identified as **1** (Fig. 1).

Cudraticusxanthone B (**2**), yellow needles, had a molecular formula of  $C_{23}H_{24}O_6$  deduced from HREIMS. The  $^1\text{H}$  NMR spectrum provided signals for four hydroxyl groups, two prenyl groups, and two aromatic singlets. A comparison of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of **2** and **1** (Table 1) revealed that **2** had a prenyl group at C-4 instead of a **1**, 1-dimethylallyl group in **1**. Confirmed by the HMQC and HMBC spectra, the structure of cudraticusxanthone B was concluded to be **2** (Fig. 1).

Cudraticusxanthone C (**3**), yellow prisms,  $C_{23}H_{26}O_7$  (HRESIMS),  $[\alpha]_{\text{D}} -48.4^\circ$ , was also regarded as a 1, 3, 6, 7-tetraoxygenated xanthone derivative by its UV and IR data. The  $^1\text{H}$  NMR spectrum showed evidence for three hydroxyl groups [ $\delta$  13.86, 9.21, and 8.58 (each 1H, s)], two aromatic protons [ $\delta$  6.90 and 6.10 (each 1H, s)], and a 2, 3, 3-trimethyl-2, 3-dihydrofuran ring [ $\delta$  4.54 (1H, q,  $J=6.5$  Hz), 1.56, 1.29 (each 3H, s), and 1.39 (3H, d,  $J=6.5$  Hz)]. Moreover, the presence of a 3-hydroxy-3-methylbutyl group was suggested by the following spectral data:  $\delta_{\text{H}}$  4.30 (1H, br s, OH), 3.47 (2H, t,  $J=7.2$  Hz), 1.89 (2H, t,  $J=7.2$  Hz), and 1.30 (6H, s);  $\delta_{\text{C}}$  22.5 (C-16), 44.1 (C-17), 71.4 (C-18), and 29.7 (C-19, 20) (Table 1). By detailed analysis of HMBC spectrum (Fig. 2), the two aromatic singlets at  $\delta_{\text{H}}$  6.90 and 6.10 were assigned to H-5 and H-2, and three hydroxyl groups at  $\delta_{\text{H}}$  13.86, 9.21, and 8.58 were located at C-1, C-6, and C-7, respectively. The HMBC cross-peaks of H<sub>3</sub>-12, 13 ( $\delta_{\text{H}}$  1.56 and 1.29) with C-4 ( $\delta_{\text{C}}$  112.5) indicated that the 2, 3-dihydro-2, 3, 3-trimethylfuran ring was fused at C-3 and C-4. The long-range correlations of H<sub>2</sub>-16 ( $\delta_{\text{H}}$  3.47) with C-7 ( $\delta_{\text{C}}$  141.5), C-8 ( $\delta_{\text{C}}$  131.5), and C-8a ( $\delta_{\text{C}}$  111.8) showed that the 3-hydroxy-3-methylbutyl group was attached to C-8. Consequently, cudraticusxanthone C was elucidated as **3** (Fig. 1).

Cudraticusxanthone D (**4**) was isolated as yellow needles. HRESIMS established the molecular formula as  $C_{23}H_{22}O_6$ . UV and IR spectra showed that **4** should have a xanthone skeleton. The  $^1\text{H}$  NMR spectrum exhibited signals of three hydroxyl groups [ $\delta$  13.61 (1H, s), and 9.31, 8.77 (each 1H, br s)], two aromatic singlets [ $\delta$  7.56 and 7.02 (each 1H, s)], a prenyl group [ $\delta$  5.23 (1H, m), 3.32 (2H, br d,  $J=7.4$  Hz), 1.80 (3H, br s), and 1.65 (3H, br d,  $J=0.6$  Hz)], and a 2, 2-dimethylpyran ring [ $\delta$  6.86, 5.73 (each 1H, d,  $J=10.0$  Hz), and 1.48 (6H, s)]. In the HMBC experiment, the following  $^2J$  and  $^3J$  couplings appeared (Fig. 3): the hydroxyl group at  $\delta_{\text{H}}$  13.61 (OH-1) with C-1 ( $\delta_{\text{C}}$  160.9), C-2 ( $\delta_{\text{C}}$  111.7), and C-9a ( $\delta_{\text{C}}$  103.3); the methylene protons at  $\delta_{\text{H}}$  3.32 (H<sub>2</sub>-11) with C-1, C-2, and C-3 ( $\delta_{\text{C}}$  158.4); one *cis*-olefinic proton at  $\delta_{\text{H}}$  6.86 (H-16) with C-3, C-4 ( $\delta_{\text{C}}$  101.4), and C-4a ( $\delta_{\text{C}}$  151.0); another *cis*-olefinic proton at  $\delta_{\text{H}}$  5.73 (H-17) with C-4. These facts clarified the structure of the A ring, on which an angular 2, 2-dimethylpyran ring was attached at C-3 and C-4. Because no *ortho*- or *meta*-coupled aromatic proton signals were observed, the two aromatic singlets at  $\delta_{\text{H}}$  7.56 and 7.02 were assigned to H-8 and H-5, and the two remaining hydroxyl groups

**Table 1.**  $^{13}\text{C}$  NMR data for compounds **1–8** (acetone- $d_6$ , 100 or 125 MHz,  $\delta$  in ppm)

| Carbon | <b>1</b> <sup>a</sup> | <b>2</b> <sup>a</sup> | <b>3</b>          | <b>4</b> <sup>a</sup> | <b>5</b> <sup>a</sup> | <b>6</b>          | <b>7</b> | <b>8</b> |
|--------|-----------------------|-----------------------|-------------------|-----------------------|-----------------------|-------------------|----------|----------|
| 1      | 162.9                 | 162.4                 | 165.4             | 160.9                 | 159.2                 | 157.7             | 162.6    | 163.8    |
| 2      | 99.9                  | 98.1                  | 93.7              | 111.7                 | 110.7                 | 117.0             | 95.7     | 117.6    |
| 3      | 164.0                 | 162.4                 | 166.2             | 158.4                 | 160.4                 | 164.6             | 163.7    | 160.3    |
| 4      | 111.3                 | 106.0                 | 112.5             | 101.4                 | 106.6                 | 103.2             | 108.2    | 102.5    |
| 4a     | 156.4                 | 155.1                 | 153.2             | 151.0                 | 153.9                 | 156.1             | 155.0    | 151.6    |
| 4b     | 152.9                 | 152.4                 | 153.75            | 152.6                 | 152.7                 | 153.0             | 152.9    | 147.1    |
| 5      | 101.3                 | 101.3                 | 101.4             | 103.7                 | 103.6                 | 103.9             | 103.7    | 133.4    |
| 6      | 153.7                 | 153.7                 | 153.67            | 154.3                 | 154.1                 | 154.4             | 154.3    | 153.0    |
| 7      | 142.1                 | 141.6                 | 141.5             | 144.1                 | 143.9                 | 144.4             | 144.0    | 114.1    |
| 8      | 129.2                 | 129.2                 | 131.5             | 109.3                 | 109.4                 | 109.6             | 109.4    | 118.2    |
| 8a     | 112.0                 | 111.9                 | 111.8             | 113.8                 | 113.7                 | 114.1             | 113.7    | 115.1    |
| 9      | 184.0                 | 183.4                 | 183.2             | 180.8                 | 180.9                 | 181.5             | 181.0    | 182.1    |
| 9a     | 104.9                 | 104.0                 | 104.5             | 103.3                 | 103.4                 | 104.6             | 103.4    | 103.6    |
| 11     | 42.0                  | 22.1                  | 44.4              | 21.7                  | 22.2                  | 44.8              | 66.5     | 42.0     |
| 12     | 30.0                  | 123.5                 | 21.6 <sup>b</sup> | 123.2                 | 123.2                 | 21.3 <sup>b</sup> | 120.3    | 29.9     |
| 13     | 30.0                  | 131.4                 | 26.1 <sup>b</sup> | 131.5                 | 132.4                 | 26.0 <sup>b</sup> | 138.9    | 29.9     |
| 14     | 151.9                 | 18.0                  | 91.5              | 18.0                  | 18.0                  | 91.7              | 18.3     | 151.8    |
| 15     | 108.3                 | 25.9                  | 14.5              | 25.9                  | 25.9                  | 15.1              | 25.8     | 108.0    |
| 16     | 26.6                  | 26.3                  | 22.5              | 116.0                 | 22.5                  | 22.9              | 22.3     | 116.8    |
| 17     | 124.8                 | 124.5                 | 44.1              | 127.8                 | 123.2                 | 123.3             | 123.4    | 127.8    |
| 18     | 131.7                 | 131.3                 | 71.4              | 78.7                  | 132.3                 | 132.4             | 131.6    | 79.4     |
| 19     | 26.4                  | 26.0                  | 29.7              | 28.3                  | 18.1                  | 18.3              | 18.0     | 28.3     |
| 20     | 18.7                  | 18.3                  | 29.7              | 28.3                  | 25.9                  | 26.2              | 25.9     | 28.3     |

<sup>a</sup> Spectra were recorded at 100 MHz.

<sup>b</sup> The assignment may be exchangeable.

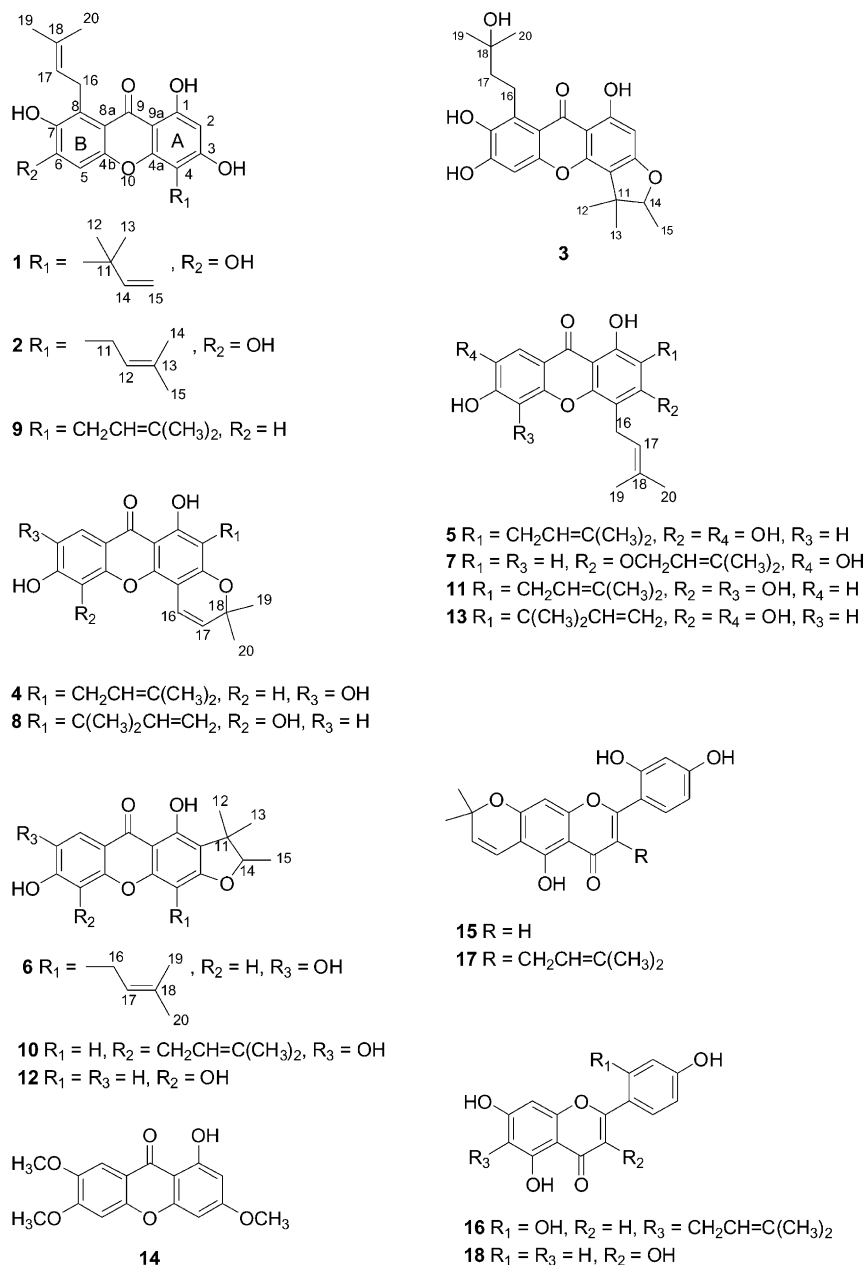


Figure 1. Structures of compounds 1–18.

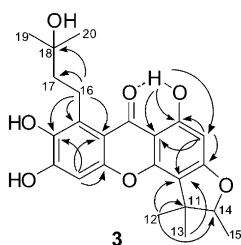


Figure 2. Key HMBC correlations of compound 3.

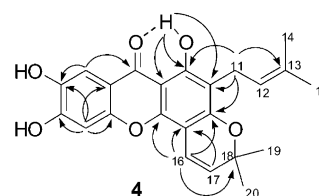


Figure 3. Key HMBC correlations of compound 4.

were located at C-6 and C-7, respectively. Thus, the structure of cudratricusxanthone D was determined as **4** (Fig. 1).

Cudratricusxanthone E (**5**), yellow needles, was deduced to have a molecular formula of  $C_{23}H_{24}O_6$  by HRE-SIMS. A spectral comparison of **5** and **4** indicated that the 2, 2-dimethylpyran ring in **4** changed into a prenyl chain and a hydroxyl group in **5**, which was confirmed by the HMBC cross-peaks of H<sub>2</sub>-16 ( $\delta_H$  3.57) with C-3 ( $\delta_C$  160.4), C-4 ( $\delta_C$  106.6), C-4a ( $\delta_C$  153.9), C-17 ( $\delta_C$  123.2), and C-18 ( $\delta_C$  132.3). Therefore, the structure of cudratricusxanthone E was demonstrated to be **5** (Fig. 1).

Cudratricusxanthone F (**6**), pale yellow prisms,  $[\alpha]_D -6.4^\circ$ , showed a molecular ion at  $m/z$  396.1583 in the HREIMS, suggesting the molecular formula of  $C_{23}H_{24}O_6$ . It was also a 1,6,7-trihydroxy-4-(3-methyl-2-butenyl) xanthone derivative the same as **5**. Their structural difference was the presence of a 2, 3, 3-trimethyl-2, 3-dihydrofuran ring attached at C-2 and C-3 in **6** rather than the prenyl and hydroxyl groups in **5**, which was supported by the NMR data of **6** [ $\delta_H$  4.53 (1H, q,  $J=6.5$  Hz), 1.49, 1.24 (each 3H, s), and 1.45 (3H, d,  $J=6.5$  Hz);  $\delta_C$  44.8 (C-11), 21.3 (C-12), 26.0 (C-13), 91.7 (C-14), and 15.1 (C-15)] (Table 1) and the long-range correlations in the HMBC spectrum [two methyl protons at  $\delta_H$  1.49 and 1.24 (H<sub>3</sub>-12, 13) with C-2 ( $\delta_C$  117.0), C-11, and C-14]. Therefore, the structure of cudratricusxanthone F was assigned as **6** (Fig. 1).

Cudratricusxanthone G (**7**), yellow needles, was assigned a molecular formula of  $C_{23}H_{24}O_6$  by HRE-SIMS. IR and UV spectra indicated that **7** was an analogue of 1, 3, 6, 7-tetraoxygenated xanthone. In the  $^1H$  NMR spectrum, signals of three hydroxyl groups [ $\delta$  13.21 (1H, s), and 9.30, 8.74 (each 1H, br s)], three aromatic singlets [ $\delta$  7.55, 6.98, and 6.41 (each 1H, s)], and a prenyl group [ $\delta$  5.23 (1H, br t,  $J=7.2$  Hz), 3.46 (2H, br d,  $J=7.2$  Hz), and 1.86, 1.64 (each 3H, br s)] were exhibited. A noticeable downfield broad doublet at  $\delta_H$  4.70 (2H, br d,  $J=6.5$  Hz) and signals at  $\delta_H$  5.54 (1H, br t,  $J=6.5$  Hz) and 1.80, 1.81 (each 3H, br s) proposed the presence of an *O*-prenyl group, which were in agreement with the relevant signals [ $\delta_C$  66.5 (C-11), 120.3 (C-12), 138.9 (C-13), 18.3 (C-14), and 25.8 (C-15)] in the  $^{13}C$  NMR spectrum (Table 1). Key HMBC correlations [H<sub>2</sub>-11 ( $\delta_H$  4.70) with C-3 ( $\delta_C$  163.7); and H<sub>2</sub>-16 ( $\delta_H$  3.46) with C-3, C-4 ( $\delta_C$  108.2), and C-4a ( $\delta_C$  155.0)] and some NOESY cross-peaks [H<sub>2</sub>-11 with H-2 ( $\delta_H$  6.41) and H<sub>2</sub>-16; and H<sub>2</sub>-16 with H-5 ( $\delta_H$  6.98)] showed that the *O*-prenyl group and the prenyl group were located at C-3 and C-4, respectively. The complete assignments of the proton and carbon signals were achieved by HMQC and HMBC spectra. Thus, the structure of cudratricusxanthone G was elucidated as **7** (Fig. 1).

Cudratricusxanthone H (**8**), yellow prisms, produced a molecular ion at  $m/z$  394.1423 in the HREIMS, corresponding to a molecular formula of  $C_{23}H_{22}O_6$ . Its UV spectrum showed the characteristic absorptions of a 1, 3, 5, 6-tetraoxygenated xanthone chromophore con-

jugated with a 2H-pyran ring system.<sup>12</sup> In the  $^1H$  NMR spectrum, the signals at  $\delta$  7.05 (1H, d,  $J=10.0$  Hz), 5.69 (1H, d,  $J=10.0$  Hz), and 1.47 (6H, s) indicated the presence of a 2, 2-dimethylpyran ring system. The  $^1H$  NMR spectrum also contained signals of a hydrogen-bonded hydroxyl group [ $\delta$  14.42 (1H, s)], two *ortho*-coupled aromatic protons [ $\delta$  7.65 (1H, d,  $J=8.6$  Hz) and 6.99 (1H, d,  $J=8.6$  Hz)], and a 1, 1-dimethylallyl group [ $\delta$  6.32 (1H, dd,  $J=10.3, 17.4$  Hz), 4.91 (1H, br d,  $J=17.4$  Hz), 4.80 (1H, br d,  $J=10.3$  Hz), and 1.61 (6H, s)]. The downfield proton signal of OH-1 ( $\delta_H$  14.42) indicated that the 1, 1-dimethylallyl group was possibly located at C-2.<sup>6a</sup> This possibility was proved by the HMBC correlations of H-14 ( $\delta_H$  6.32) and H<sub>3</sub>-12, 13 ( $\delta_H$  1.61) with C-2 ( $\delta_C$  117.6). The 2, 2-dimethylpyran ring was connected to C-3 and C-4 based on the observed long-range correlations: H-16 ( $\delta_H$  7.05) with C-3 ( $\delta_C$  160.3), C-4 ( $\delta_C$  102.5), and C-4a ( $\delta_C$  151.6); H-17 ( $\delta_H$  5.69) with C-4. In addition, the doublets at  $\delta_H$  7.65 and 6.99 were assigned to H-8 and H-7, respectively, as evidenced by an HMBC correlation between H-8 and C-9 ( $\delta_C$  182.1). Thus, the structure of cudratricusxanthone H was identified as **8** (Fig. 1).

## 2.2. Structural identification of known compounds 9–18

Ten known compounds (six xanthones and four flavonoids) were cudraxanthones H (**9**)<sup>1c</sup> and M (**10**),<sup>1d</sup> xanthone V<sub>1a</sub> (**11**),<sup>13</sup> toxyloxanthone C (**12**),<sup>14</sup> macluraxanthone B (**13**),<sup>15</sup> 1-hydroxy-3, 6, 7-trimethoxyxanthone (**14**),<sup>16</sup> cycloartocarpesin (**15**),<sup>17</sup> artocarpesin (**16**),<sup>18</sup> cudraflavone B (**17**),<sup>2a</sup> and kaempferol (**18**).<sup>19</sup> The structures were identified by comparison of their spectral data ( $^1H$ ,  $^{13}C$  NMR, MS, and  $[\alpha]_D$ ) with those reported.

From the present investigation, it is known that most xanthones in *C. tricuspidata* have di-isoprenoid-substituted 1, 3, 6, 7-tetraoxygenated pattern (**1–7**, **10**, and **13**), similar to the xanthones isolated from this plant before.<sup>1</sup> However, xanthones in *C. cochinchinensis* are mostly 1, 3, 5, 6-tetraoxygenated with two isoprenoid groups.<sup>6a</sup> On the other hand, benzophenones, found in *C. cochinchinensis*,<sup>6a</sup> have not appeared in *C. tricuspidata*.

## 2.3. Cytotoxicity evaluation and structure–activity relationship discussion

Compounds **1–5**, **7**, **9–12**, and **15–17** were screened for cytotoxicity against HCT-116, SMMC-7721, SGC-7901, and BGC-823 cell lines (Table 2). In this test, vincristine was used as positive control. Xanthones **5**, **7**, **10**, and **12** showed significant inhibitory effects on four kinds of cell lines with IC<sub>50</sub> values of 1.6–5.4, 1.6–3.4, 2.6–9.5, and 2.8–11.8  $\mu$ g/mL, respectively. Xanthones **2**, **4**, and **11** displayed prominent cytotoxicity against HCT-116, SMMC-7721, and SGC-7901 (IC<sub>50</sub> values = 3.9–6.9, 4.1–9.8, 1.3–6.2  $\mu$ g/mL, respectively) and insignificant activity against BGC-823 (IC<sub>50</sub> values > 30  $\mu$ g/mL). Xanthone **9** exhibited higher cytotoxicity against SGC-7901 (IC<sub>50</sub> = 1.8  $\mu$ g/mL) than against BGC-823 (IC<sub>50</sub> = 9.2  $\mu$ g/mL) and SMMC-7721 (IC<sub>50</sub> = 11.7  $\mu$ g/mL), and no

**Table 2.** IC<sub>50</sub> values (μg/mL) of compounds against human tumor cell lines

| Compd             | HCT-116         | SMMC-7721 | SGC-7901 | BGC-823 |
|-------------------|-----------------|-----------|----------|---------|
| <b>Xanthones</b>  |                 |           |          |         |
| <b>1</b>          | ND <sup>a</sup> | ND        | ND       | 15.2    |
| <b>2</b>          | 3.9             | 6.9       | 4.3      | ND      |
| <b>3</b>          | 12.2            | 8.9       | ND       | ND      |
| <b>4</b>          | 4.1             | 4.2       | 9.8      | ND      |
| <b>5</b>          | 4.7             | 4.2       | 5.4      | 1.6     |
| <b>7</b>          | 1.8             | 2.7       | 3.4      | 1.6     |
| <b>9</b>          | ND              | 11.7      | 1.8      | 9.2     |
| <b>10</b>         | 3.4             | 5.1       | 9.5      | 2.6     |
| <b>11</b>         | 1.3             | 6.2       | 3.4      | ND      |
| <b>12</b>         | 2.8             | 8.8       | 11.8     | 5.2     |
| <b>Flavonoids</b> |                 |           |          |         |
| <b>15</b>         | ND              | ND        | ND       | ND      |
| <b>16</b>         | ND              | ND        | ND       | ND      |
| <b>17</b>         | ND              | ND        | ND       | 7.2     |
| Vincristine       | 0.0089          | 0.034     | 0.0029   | 19      |

<sup>a</sup> ND: not determined (IC<sub>50</sub> values > 30 μg/mL not considered to be significant and not calculated).

cytotoxicity against HCT-116. Xanthone **3** were potent against HCT-116 (IC<sub>50</sub> = 12.2 μg/mL) and SMMC-7721 (IC<sub>50</sub> = 8.9 μg/mL), and inactive against SGC-7901 and BGC-823. Xanthone **1** only exerted weak activity against BGC-823 (IC<sub>50</sub> = 15.2 μg/mL). Except for the cytotoxicity of **17** toward BGC-823 (IC<sub>50</sub> = 7.2 μg/mL), the prenylated flavonoids (**15–17**) were inactive.

An analysis of the cytotoxic results and the structure characteristics of the tested compounds led to a hypothesis of structure–activity relationship as follows: (1) The cytotoxicity is due to the presence of xanthone skeleton because flavonoids (**15–17**) are almost non-cytotoxic. (2) Most active xanthones (**2, 4, 5, 7, 9, 11**, and **12**) have one or two hydrophobic groups (isoprenoid unit) at one domain (ring A) and one or two hydrophilic groups (hydroxyl) at another domain (ring B). (3) The substituent at C-8 seemed to have little or no effect on the resultant cytotoxicity. (4) Xanthones (**1** and **3**) with a 1,1-dimethylallyl group at C-4 or a 2,3,3-trimethylfuran ring at C-3, 4 (a cyclization between a 1,1-dimethylallyl group and a hydroxyl group) become less active. However, further studies are necessary to confirm this SAR hypothesis.

### 3. Experimental

#### 3.1. General

Melting points were measured on a XT-4 micro-melting point apparatus and are uncorrected. Optical rotations were run on a Perkin–Elmer digital polarimeter 341. UV spectra were obtained from a Shimadzu UV-2401PC spectrophotometer. IR spectral data were recorded on a Nicolet Avatar 360 spectrometer with KBr pellets. NMR spectra were operated at Bruker DRX-300, 400, and 500 instruments. Chemical shifts were reported with TMS as internal standard. EIMS were recorded on Agilent 5973N and HP 5989A mass spectrometers. HREIMS were obtained on Concept 1H series and

Finnigan MAT 95 instruments. HRESIMS were carried out on a AB QSTAR Pulsar mass spectrometer. Column chromatography was performed on silica gel (200–300 mesh, Yantai, People's Republic of China), silica gel H (10–40 μm, Yantai, People's Republic of China), and MCI gel CHP-20P (75–150 μm, Mitsubishi Chemical Corporation, Japan). TLC analysis was run on GF<sub>254</sub> precoated silica gel plates (10–40 μm, Yantai, People's Republic of China).

#### 3.2. Plant material

The roots of *C. tricuspidata* (Carr.) Bur. were collected in Dali, Yunnan, People's Republic of China, in November 2001, and air-dried. The plant material was identified by Prof. Han-Dong Sun (Kunming Institute of Botany), and a voucher specimen (TCM 0111Hou) was deposited in the Herbarium of Department of Pharmacognosy, School of Pharmacy, Fudan University.

#### 3.3. Extraction and isolation

The dried and powdered roots (13.65 kg) were extracted with EtOH for four times in a percolator and filtered. The filtrate was evaporated in vacuo to give a dark brown residue (1.1 kg), which was suspended in water and partitioned successively with petroleum ether, CHCl<sub>3</sub>, EtOAc, and *n*-BuOH. The CHCl<sub>3</sub> extract showed potent cytotoxicity against SGC-7901 and BGC-823 cell lines in vitro, and this extract (427 g) was thus subjected to silica gel column chromatography eluted with petroleum ether–acetone (20:1, 12:1, 10:1, 9:1, 8:2, 7:3, 6:4, and 5:5) to yield fractions 1–30 and afford compounds **2** (400 mg), **15** (475 mg), and **16** (970 mg). Fraction 16 (8 g) was chromatographed on silica gel (CHCl<sub>3</sub>–EtOAc, 40:1) to give fractions 16a–16 h. Fraction 16c was fractionated on silica gel developed with petroleum ether–EtOAc (5:1) to give compound **9** (40 mg). Fraction 16f was chromatographed repeatedly over silica gel with petroleum ether–CHCl<sub>3</sub>–isopropanol (40:10:1) and petroleum ether–CHCl<sub>3</sub>–MeOH (40:10:1) to afford compound **10** (120 mg). Fraction 17 (15 g) was passed through silica gel column (CHCl<sub>3</sub>–Et<sub>2</sub>O, 15:1) to give fractions 17a–17e. Fraction 17a was subjected to further silica gel column chromatography (CHCl<sub>3</sub>–Et<sub>2</sub>O, 15:1) to yield compounds **8** (167 mg), **13** (2.74 g), and **14** (20 mg). Fraction 17e was purified by column chromatography over MCI gel CHP-20P (MeOH–H<sub>2</sub>O, 9:1) to afford compound **6** (710 mg). Fraction 19 (11 g) was eluted with C<sub>6</sub>H<sub>6</sub>–EtOAc (7:1) over silica gel to afford fractions 19a–19f and compound **4** (220 mg). Fraction 19a was separated over silica gel (CHCl<sub>3</sub>–isopropanol, 30:1), followed by passage over MCI gel CHP-20P (MeOH–H<sub>2</sub>O, 9:1) to provide compound **17** (66 mg). Fraction 19c was isolated over MCI gel CHP-20P (MeOH–H<sub>2</sub>O, 9:1), followed by silica gel (CHCl<sub>3</sub>–EtOAc, 10:1) to give compound **12** (73 mg). Fraction 19d was fractionated by column chromatography on silica gel (petroleum ether–Et<sub>2</sub>O, 2:1), followed by MCI gel CHP-20P (MeOH–H<sub>2</sub>O, 8:2) to provide compound **7** (48 mg). Further purification of fraction 19e on silica gel (petroleum ether–isopropanol, 22:1) yielded compound

**3** (12 mg). Fraction 20 (8.1 g) was passed through silica gel column ( $\text{CHCl}_3$ –EtOAc, 40:1) to afford fractions 20a–20c. Further separation of fraction 20b over silica gel ( $\text{C}_6\text{H}_6$ –EtOAc, 10:1, and  $\text{CHCl}_3$ –Et<sub>2</sub>O, 20:1) gave compound **1** (79 mg). Fraction 20c was purified by column chromatography over silica gel ( $\text{C}_6\text{H}_6$ –EtOAc, 9:1) and MCI gel CHP-20P (MeOH–H<sub>2</sub>O, 8:2) to give compounds **3** (21 mg), **5** (30 mg), and **11** (60 mg). Fraction 24 (13.1 g) was eluted with petroleum ether–EtOAc (2:1) and  $\text{CHCl}_3$ –MeOH (20:1) over silica gel to yield compounds **16** (665 mg) and **18** (41 mg).

**3.3.1. Cudraticusxanthone A (1).** Yellow prisms ( $\text{CHCl}_3$ ); mp 145 °C; UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ): 223 (4.55), 244 (4.64), 260 (4.70), 316 (4.48), 365 (4.19); IR (KBr)  $\nu_{\text{max}}$  3386, 2961, 1647, 1614, 1556, 1501, 1415, 1340, 1279, 1168, 1125, 1068, 939, 835, 571  $\text{cm}^{-1}$ ; <sup>1</sup>H NMR (acetone-*d*<sub>6</sub>, 400 MHz)  $\delta$  13.78 (1H, s, OH-1), 9.32 (2H, br s, OH), 7.99 (1H, s, OH), 6.85 (1H, s, H-5), 6.35 (1H, dd, *J* = 10.6, 17.4 Hz, H-14), 6.23 (1H, s, H-2), 5.31 (1H, m, H-17), 5.00 (1H, dd, *J* = 1.2, 17.4 Hz, H-15a), 4.89 (1H, dd, *J* = 1.2, 10.6 Hz, H-15b), 4.17 (2H, br d, *J* = 6.8 Hz, H<sub>2</sub>-16), 1.83 (3H, br s, H<sub>3</sub>-20), 1.65 (6H, s, H<sub>3</sub>-12, H<sub>3</sub>-13), 1.63 (3H, br s, H<sub>3</sub>-19); <sup>13</sup>C NMR data, see Table 1; EIMS *m/z* (%): 396 [*M*]<sup>+</sup> (25), 381 (21), 353 (57), 325 (24), 245 (48), 69 (57), 57 (66), 46 (100); HRESIMS *m/z* 419.1472 [*M* + Na]<sup>+</sup> (calcd for C<sub>23</sub>H<sub>24</sub>O<sub>6</sub>Na, 419.1470).

**3.3.2. Cudraticusxanthone B (2).** Yellow needles (MeOH); mp 204–205 °C; UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ): 241 (4.35), 259 (4.43), 314 (4.20), 370 (3.93); IR (KBr)  $\nu_{\text{max}}$  3372, 2910, 1640, 1615, 1558, 1497, 1429, 1274, 1170  $\text{cm}^{-1}$ ; <sup>1</sup>H NMR (acetone-*d*<sub>6</sub>, 400 MHz)  $\delta$  13.52 (1H, s, OH-1), 9.48 (2H, br s, OH), 7.68 (1H, br s, OH), 6.90 (1H, s, H-5), 6.26 (1H, s, H-2), 5.31 (1H, m, H-17), 5.26 (1H, m, H-12), 4.18 (2H, br d, *J* = 6.8 Hz, H<sub>2</sub>-16), 3.45 (2H, br d, *J* = 7.3 Hz, H<sub>2</sub>-11), 1.85 (3H, br s, H<sub>3</sub>-14), 1.82 (3H, br s, H<sub>3</sub>-20), 1.634 (3H, br s, H<sub>3</sub>-15), 1.628 (3H, br s, H<sub>3</sub>-19); <sup>13</sup>C NMR data, see Table 1; EIMS *m/z* (%): 396 [*M*]<sup>+</sup> (70), 381 (18), 367 (7), 353 (100), 341 (15), 325 (18), 311 (7), 297 (62), 285 (32), 257 (11); HREIMS *m/z* 396.1565 [*M*]<sup>+</sup> (calcd for C<sub>23</sub>H<sub>24</sub>O<sub>6</sub>, 396.1573).

**3.3.3. Cudraticusxanthone C (3).** Yellow prisms ( $\text{CHCl}_3$ ); mp 189 °C; [ $\alpha$ ]<sub>D</sub><sup>20</sup> –48.4° (*c* 0.60, acetone); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ): 224 (4.68), 240 (4.69), 259 (4.67), 276 (sh) (4.43), 316 (4.47), 365 (4.18); IR (KBr)  $\nu_{\text{max}}$  3532, 3373, 2967, 1651, 1620, 1560, 1505, 1482, 1375, 1286, 1157, 1060, 822, 565  $\text{cm}^{-1}$ ; <sup>1</sup>H NMR (acetone-*d*<sub>6</sub>, 500 MHz)  $\delta$  13.86 (1H, s, OH-1), 9.21 (1H, s, OH-6), 8.58 (1H, s, OH-7), 6.90 (1H, s, H-5), 6.10 (1H, s, H-2), 4.54 (1H, q, *J* = 6.5 Hz, H-14), 4.30 (1H, br s, OH-18), 3.47 (2H, t, *J* = 7.2 Hz, H<sub>2</sub>-16), 1.89 (2H, t, *J* = 7.2 Hz, H<sub>2</sub>-17), 1.56, 1.29 (each 3H, s, H<sub>3</sub>-12, H<sub>3</sub>-13), 1.39 (3H, d, *J* = 6.5 Hz, H<sub>3</sub>-15), 1.30 (6H, s, H<sub>3</sub>-19, H<sub>3</sub>-20); <sup>13</sup>C NMR data, see Table 1; EIMS *m/z* (%): 414 [*M*]<sup>+</sup> (20), 396 (45), 381 (100), 353 (86), 325 (69); HRESIMS *m/z* 437.1581 [*M* + Na]<sup>+</sup> (calcd for C<sub>23</sub>H<sub>26</sub>O<sub>7</sub>Na, 437.1576).

**3.3.4. Cudraticusxanthone D (4).** Yellow needles ( $\text{CHCl}_3$ ); mp 234 °C; UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ): 224 (4.66), 258 (4.76), 286 (4.74), 325 (4.43), 385 (4.20); IR

(KBr)  $\nu_{\text{max}}$  3491, 3295, 2969, 1630, 1595, 1517, 1474, 1438, 1354, 1133, 1007, 771  $\text{cm}^{-1}$ ; <sup>1</sup>H NMR (acetone-*d*<sub>6</sub>, 400 MHz)  $\delta$  13.61 (1H, s, OH-1), 9.31 (1H, br s, OH), 8.77 (1H, br s, OH), 7.56 (1H, s, H-8), 7.02 (1H, s, H-5), 6.86 (1H, d, *J* = 10.0 Hz, H-16), 5.73 (1H, d, *J* = 10.0 Hz, H-17), 5.23 (1H, m, H-12), 3.32 (2H, br d, *J* = 7.4 Hz, H<sub>2</sub>-11), 1.80 (3H, br s, H<sub>3</sub>-14), 1.65 (3H, br d, *J* = 0.6 Hz, H<sub>3</sub>-15), 1.48 (6H, s, H<sub>3</sub>-19, H<sub>3</sub>-20); <sup>13</sup>C NMR data, see Table 1; EIMS *m/z* (%): 394 [*M*]<sup>+</sup> (45), 379 (100), 351 (33), 339 (38), 323 (26), 311 (7), 256 (8), 191 (13), 162 (9); HRESIMS *m/z* 417.1312 [*M* + Na]<sup>+</sup> (calcd for C<sub>23</sub>H<sub>22</sub>O<sub>6</sub>Na, 417.1314).

**3.3.5. Cudraticusxanthone E (5).** Yellow needles ( $\text{CHCl}_3$ ); mp 203–204 °C; UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ): 235 (4.86), 262 (4.87), 321 (4.64), 373 (4.43); IR (KBr)  $\nu_{\text{max}}$  3380, 3156, 2969, 2914, 1642, 1615, 1567, 1488, 1434, 1290, 1202, 1163, 802  $\text{cm}^{-1}$ ; <sup>1</sup>H NMR (acetone-*d*<sub>6</sub>, 400 MHz)  $\delta$  13.48 (1H, s, OH-1), 8.97, 8.23 (3H, br s, OH-3, 6, 7), 7.57 (1H, s, H-8), 6.98 (1H, s, H-5), 5.24 (2H, m, H-12, H-17), 3.57 (2H, br d, *J* = 7.2 Hz, H<sub>2</sub>-16), 3.44 (2H, br d, *J* = 7.0 Hz, H<sub>2</sub>-11), 1.89 (3H, br s, H<sub>3</sub>-19), 1.79 (3H, br s, H<sub>3</sub>-14), 1.66 (6H, br s, H<sub>3</sub>-15, H<sub>3</sub>-20); <sup>13</sup>C NMR data, see Table 1; EIMS *m/z* (%): 396 [*M*]<sup>+</sup> (4), 325 (6), 311 (14), 297 (24), 153 (15), 115 (10), 55 (38), 43 (61), 41 (100); HRESIMS *m/z* 419.1481 [*M* + Na]<sup>+</sup> (calcd for C<sub>23</sub>H<sub>24</sub>O<sub>6</sub>Na, 419.1470).

**3.3.6. Cudraticusxanthone F (6).** Pale yellow prisms ( $\text{CHCl}_3$ ); mp 208 °C; [ $\alpha$ ]<sub>D</sub><sup>20</sup> –6.4° (*c* 1.00, acetone); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ): 244 (4.30), 261 (4.32), 322 (4.10), 369 (3.94); IR (KBr)  $\nu_{\text{max}}$  3518, 3272, 2965, 1633, 1610, 1565, 1520, 1476, 1290, 1069, 998  $\text{cm}^{-1}$ ; <sup>1</sup>H NMR (acetone-*d*<sub>6</sub>, 500 MHz)  $\delta$  13.41 (1H, s, OH-1), 9.00 (2H, br s, OH-6, 7), 7.55 (1H, s, H-8), 6.98 (1H, s, H-5), 5.27 (1H, br t, *J* = 7.2 Hz, H-17), 4.53 (1H, q, *J* = 6.5 Hz, H-14), 3.40 (2H, br d, *J* = 7.2 Hz, H<sub>2</sub>-16), 1.86 (3H, br s, H<sub>3</sub>-19), 1.66 (3H, br s, H<sub>3</sub>-20), 1.49, 1.24 (each 3H, s, H<sub>3</sub>-12, H<sub>3</sub>-13), 1.45 (3H, d, *J* = 6.5 Hz, H<sub>3</sub>-15); <sup>13</sup>C NMR data, see Table 1; EIMS *m/z* (%): 396 [*M*]<sup>+</sup> (46), 381 (100), 353 (6), 325 (10), 313 (43), 285 (8), 183 (4); HREIMS *m/z* 396.1583 [*M*]<sup>+</sup> (calcd for C<sub>23</sub>H<sub>24</sub>O<sub>6</sub>, 396.1573).

**3.3.7. Cudraticusxanthone G (7).** Yellow needles ( $\text{CHCl}_3$ ); mp 206–207 °C; UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ): 215 (4.44), 235 (4.64), 259 (4.80), 312 (4.47), 375 (4.35); IR (KBr)  $\nu_{\text{max}}$  3474, 3229, 2960, 2916, 1642, 1599, 1473, 1423, 1289, 1257, 1175, 1078, 802  $\text{cm}^{-1}$ ; <sup>1</sup>H NMR (acetone-*d*<sub>6</sub>, 500 MHz)  $\delta$  13.21 (1H, s, OH-1), 9.30 (1H, br s, OH), 8.74 (1H, br s, OH), 7.55 (1H, s, H-8), 6.98 (1H, s, H-5), 6.41 (1H, s, H-2), 5.54 (1H, br t, *J* = 6.5 Hz, H-12), 5.23 (1H, br t, *J* = 7.2 Hz, H-17), 4.70 (2H, br d, *J* = 6.5 Hz, H<sub>2</sub>-11), 3.46 (2H, br d, *J* = 7.2 Hz, H<sub>2</sub>-16), 1.86 (3H, br s, H<sub>3</sub>-19), 1.80 (3H, br s, H<sub>3</sub>-14), 1.81 (3H, br s, H<sub>3</sub>-15), 1.64 (3H, br s, H<sub>3</sub>-20); <sup>13</sup>C NMR data, see Table 1; EIMS *m/z* (%): 396 [*M*]<sup>+</sup> (38), 381 (2), 328 (54), 313 (100), 285 (23), 273 (37), 260 (69), 153 (6), 69 (25); HRESIMS *m/z* 419.1464 [*M* + Na]<sup>+</sup> (calcd for C<sub>23</sub>H<sub>24</sub>O<sub>6</sub>Na, 419.1470).

**3.3.8. Cudraticusxanthone H (8).** Yellow prisms ( $\text{CHCl}_3$ ); mp 175 °C; UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ): 209 (4.33), 218 (4.37), 258 (4.80), 272 (4.70), 335 (4.36), 380

(sh) (3.90); IR (KBr)  $\nu_{\max}$  3460, 3213, 2976, 1618, 1600, 1562, 1524, 1438, 1252, 1183, 1152, 1000  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (acetone- $d_6$ , 500 MHz)  $\delta$  14.42 (1H, s, OH-1), 8.88 (2H, br s, OH), 7.65 (1H, d,  $J=8.6$  Hz, H-8), 7.05 (1H, d,  $J=10.0$  Hz, H-16), 6.99 (1H, d,  $J=8.6$  Hz, H-7), 6.32 (1H, dd,  $J=10.3, 17.4$  Hz, H-14), 5.69 (1H, d,  $J=10.0$  Hz, H-17), 4.91 (1H, br d,  $J=17.4$  Hz, H-15a), 4.80 (1H, br d,  $J=10.3$  Hz, H-15b), 1.61 (6H, s, H<sub>3</sub>-12, H<sub>3</sub>-13), 1.47 (6H, s, H<sub>3</sub>-19, H<sub>3</sub>-20);  $^{13}\text{C}$  NMR data, see Table 1; EIMS  $m/z$  (%): 394 [M]<sup>+</sup> (32), 379 (100), 365 (4), 351 (14), 339 (9), 311 (4), 182 (4), 162 (5), 84 (1); HREIMS  $m/z$  394.1423 [M]<sup>+</sup> (calcd for C<sub>23</sub>H<sub>22</sub>O<sub>6</sub>, 394.1416).

### 3.4. Cytotoxicity assay

Compounds were assayed for cytotoxic activity against human tumor cell lines using a reported procedure,<sup>20</sup> but with some modifications. Tumor cells were incubated for 72 h at 37°C in the presence of various concentrations of the drugs (0.31–20  $\mu\text{g/mL}$ ) from DMSO-diluted stock. The OD of each well was measured on an ELISA reader (DG3022) at the wavelength of 550 nm. Vincristine was used as the positive reference substance with concentrations of  $10^{-3}$ – $10^2$   $\mu\text{g/mL}$ . The human tumor cell lines consisted of colon carcinoma (HCT-116), hepatocellular carcinoma (SMMC-7721), and gastric carcinoma (SGC-7901 and BGC-823).

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### References and notes

- (a) Fujimoto, T.; Hano, Y.; Nomura, T. *Planta Med.* **1984**, *50*, 218. (b) Hano, Y.; Matsumoto, Y.; Sun, J. Y.; Nomura, T. *Planta Med.* **1990**, *56*, 399. (c) Hano, Y.; Matsumoto, Y.; Sun, J. Y.; Nomura, T. *Planta Med.* **1990**, *56*, 478. (d) Hano, Y.; Matsumoto, Y.; Shinohara, K.; Sun, J. Y.; Nomura, T. *Planta Med.* **1991**, *57*, 172.
- (a) Fujimoto, T.; Hano, Y.; Nomura, T.; Uzawa, J. *Planta Med.* **1984**, *50*, 161. (b) Hano, Y.; Matsumoto, Y.; Shinohara, K.; Sun, J. Y.; Nomura, T. *Heterocycles* **1990**, *31*, 1339.
- Fujimoto, T.; Nomura, T. *Planta Med.* **1985**, *51*, 190.
- (a) Chang, C. H.; Lin, C. C.; Hattori, M.; Namba, T. *Phytochemistry* **1989**, *28*, 595. (b) Chang, C. H.; Lin, C. C.; Kawata, Y.; Hattori, M.; Namba, T. *Phytochemistry* **1989**, *28*, 2823. (c) Chang, C. H.; Lin, C. C.; Kadota, S.; Hattori, M.; Namba, T. *Phytochemistry* **1995**, *40*, 945.
- Chang, C. H.; Lin, C. C.; Hattori, M.; Namba, T. *Journal of Ethnopharmacology* **1994**, *44*, 79.
- (a) Hou, A. J.; Fukai, T.; Shimazaki, M.; Sakagami, H.; Sun, H. D.; Nomura, T. *J. Nat. Prod.* **2001**, *64*, 65. (b) Fukai, T.; Yonekawa, M.; Hou, A. J.; Nomura, T.; Sun, H. D.; Uno, J. *J. Nat. Prod.* **2003**, *66*, 1118.
- Ding, G. M.; Hua, H. Q.; Zhou, C. X. In *Clinical Application of Antitumor Traditional Chinese Medicines*; Zhang, M. Q., Gong, H. M., Eds.; People's Medical Publishing House: Beijing, 1998; p 286 (Chinese).
- Cao, G.S. *Research on Chinese Traditional Patent Medicines* **1980**, *1*, 34 (Chinese).
- Jiangsu New Medical College. *Dictionary of Traditional Chinese Medicines*, Shanghai Science and Technology Publisher: Shanghai, 2000, pp 1731–1732 (Chinese).
- Xu, Y. T.; Zhang, K. W.; Li, Y.; Zhang, Y. H.; Song, J. Q.; Wang, H. R. *Acta Chinese Medicine and Pharmacology* **1998**, *5*, 47 (Chinese).
- Waterman, P. G.; Hussain, R. A. *Phytochemistry* **1982**, *21*, 2099.
- Delle Monache, G.; Botta, B.; de Mello, J. F.; de Barros Coelho, J. S. *J. Nat. Prod.* **1984**, *47*, 620.
- Botta, B.; Delle Monache, G.; Delle Monache, F.; Marini Bettolo, G. B.; Menichini, F. *Phytochemistry* **1986**, *25*, 1217.
- Deshpande, V. H.; Rama Rao, A. V.; Mala Varadan; Venkataraman, K. *Indian J. Chem.* **1973**, *11*, 518.
- Groweiss, A.; Cardellina, J. H., II; Boyd, M. R. *J. Nat. Prod.* **2000**, *63*, 1537.
- Ikeya, Y.; Sugama, K.; Okada, M.; Mitsushashi, H. *Phytochemistry* **1991**, *30*, 2061.
- Parthasarathy, P. C.; Radhakrishnan, P. V.; Rathi, S. S.; Venkataraman, K. *Indian J. Chem.* **1969**, *7*, 101.
- Lee, S. J.; Wood, A. R.; Maier, C. G.-A.; Dixon, R. A.; Mabry, T. J. *Phytochemistry* **1998**, *49*, 2573.
- Tan, M. H.; Zhang, Z. R.; Qin, H. Y.; Liu, J. H. *Chin. Tradit. Herb. Drugs* **1990**, *21*, 246 (Chinese).
- Hou, A. J.; Li, M. L.; Jiang, B.; Lin, Z. W.; Ji, S. Y.; Zhou, Y. P.; Sun, H. D. *J. Nat. Prod.* **2000**, *63*, 599.