

## Bioactive Xanthenes from the Stems of *Cratoxylum formosum* ssp. *pruniflorum*

Ying-hui Duan,<sup>†</sup> Yi Dai,<sup>‡,§</sup> Guang-hui Wang,<sup>‡</sup> Xue Zhang,<sup>†</sup> Hai-feng Chen,<sup>‡</sup> Jie-bo Chen,<sup>∇</sup> Xin-sheng Yao,<sup>\*,†,‡,§</sup> and Xiao-kun Zhang<sup>\*,‡,∇</sup>

College of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang 110016, People's Republic of China, Institute of Traditional Chinese Medicine and Natural Products, College of Pharmacy, Jinan University, Guangzhou 510632, People's Republic of China, Guangdong Province Key Laboratory of Pharmacodynamic Constituents of TCM and New Drugs Research, Jinan University, Guangzhou 510632, People's Republic of China, Institute for Biomedical Research, Xiamen University, Xiamen 361005, People's Republic of China, and Burnham Institute for Medical Research, Cancer Center, La Jolla, California 92037.

Received March 15, 2010

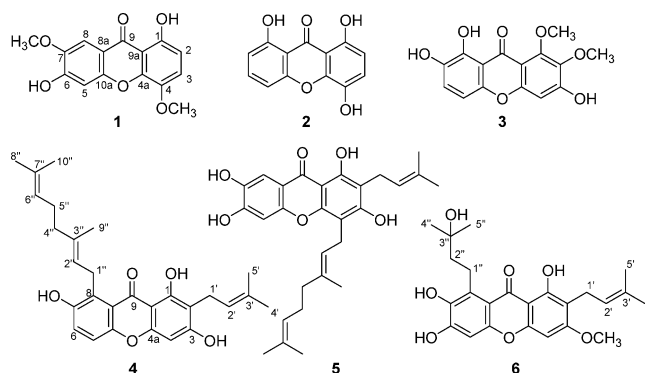
Six new compounds, pruniflorones M–R (1–6), together with 19 known compounds (7–25) were isolated from the stems of *Cratoxylum formosum* ssp. *pruniflorum*. The structures of the new compounds were established on the basis of extensive spectroscopic data interpretation. In addition, their RXR $\alpha$  transcriptional activities were evaluated using an in vitro assay.

Retinoid X receptors (RXRs) are members of the nuclear receptor superfamily, which function as transcriptional factors to positively or negatively regulate gene expression.<sup>1</sup> RXRs play an important role in many diverse physiologic processes, including embryogenesis, calcium homeostasis, and lipid and glucose metabolism. This is due to their ability to heterodimerize with a number of nuclear receptors, such as retinoic acid receptor, vitamin D<sub>3</sub> receptor, thyroid hormone receptors, peroxisome proliferator-activated receptors, and a number of orphan receptors.<sup>2–4</sup> Therefore, there has been tremendous interest in identifying agents that regulate RXR activities. *Cratoxylum formosum* ssp. *pruniflorum* belongs to the family Clusiaceae, which is distributed widely in several Southeast Asian countries.<sup>5</sup> This plant is known locally as “Kuding Tea” in southwest mainland China and has been used as a folk medicine for the treatment of fever, coughs, ulcers, and diarrhea.<sup>6</sup> Previous chemical investigations on this species have revealed a series of xanthenes and anthraquinones.<sup>7,8</sup> Some of these compounds possess various bioactivities, such as antimalarial,<sup>9</sup> antibacterial,<sup>7,8</sup> and cytotoxic effects.<sup>7,9</sup> The present investigation on the stems of *C. formosum* ssp. *pruniflorum* led to the isolation of six new xanthenes, pruniflorones M–R (1–6), and 19 known xanthenes (7–25). The compounds isolated were investigated for their effects on RXR $\alpha$  transcriptional activities using an in vitro bioassay.

### Results and Discussion

A 60% EtOH extract of the stems of *C. formosum* ssp. *pruniflorum* was subjected to column chromatography to yield six new compounds (1–6). All of these gave characteristic UV absorption bands in the range 226–269 and 312–379 nm, typical of a xanthone chromophore.<sup>7,10</sup> Their IR spectra also showed characteristic conjugated carbonyl and hydroxy groups in the range 1618–1650 and 3200–3435 cm<sup>-1</sup>, respectively.

Compound **1** was obtained as a yellow powder. Its molecular formula was established as C<sub>15</sub>H<sub>12</sub>O<sub>6</sub> by HRESIMS. The <sup>1</sup>H NMR data of **1** (Table 1) showed signals for *ortho*-coupled aromatic protons at  $\delta$  7.40 (1H, d, *J* = 8.8 Hz, H-3) and 6.69 (1H, d, *J* = 8.8 Hz, H-2), two aromatic protons at  $\delta$  7.47 (1H, s, H-8) and 6.97



(1H, s, H-5), two methoxy groups at  $\delta$  3.90 (3H, s) and 3.88 (3H, s), and a hydrogen-bonded hydroxy group at  $\delta$  12.06 (1H, s, OH-1). The *ortho*-coupled aromatic protons at  $\delta$  6.69 and 7.40 were assigned to H-2 and H-3, respectively, due to the HMBC correlation between the hydrogen-bonded hydroxy group at  $\delta$  12.06 (OH-1) and an aromatic carbon at  $\delta$  108.0 (C-2) (Figure 1). The singlet signal at  $\delta$  7.47 was assigned to H-8, according to the significant deshielding shift arising from the anisotropic effect of the carbonyl group.<sup>11,12</sup> The H-8 assignment was confirmed by the HMBC correlation between H-8 and C-9 (180.0). In the ROESY spectrum, the methoxy groups at  $\delta$  3.88 (3H, s) and 3.90 (3H, s) correlated with H-3 and H-8, respectively, suggesting these two methoxy groups to be located at C-4 (139.6) and C-7 (146.5). The two oxygenated aromatic carbon signals at  $\delta$  152.3 and 155.6 were then assigned to C-6 and C-10a, due to their HMBC correlations with H-8. Furthermore, the remaining aromatic proton at  $\delta$  6.97 was located at C-5 (102.8), according to the HMBC correlations of H-5/C-7, C-8a (111.7), C-6, and C-10a. Thus, compound **1** was proposed as 1,6-dihydroxy-4,7-dimethoxyxanthone, and the trivial name pruniflorone M was assigned to this substance.

Compound **2**, a brownish, amorphous powder, gave a molecular formula of C<sub>13</sub>H<sub>8</sub>O<sub>5</sub> by HREIMS. The <sup>1</sup>H NMR spectrum of **2** (Table 1) revealed the presence of three hydroxy groups [ $\delta$  11.78, 11.00, and 9.73 (1H each, s, OH-8, OH-1, and OH-4)] and *ortho*-coupled aromatic protons [ $\delta$  7.31, 6.67 (1H each, d, *J* = 8.8 Hz, H-3 and H-2)], in addition to a 1,2,3-trisubstituted benzene ring [ $\delta$  7.75 (1H, t, *J* = 8.4 Hz, H-6), 7.06 (1H, d, *J* = 8.4 Hz, H-5), and 6.83 (1H, d, *J* = 8.4 Hz, H-7)]. In the <sup>13</sup>C NMR spectrum, the carbonyl carbon was observed at  $\delta$  185.6, a significant downfield shift of about 5 ppm, compared to that of compound **1**, which indicated that **2** is a 1,8-dihydroxyxanthone derivative.<sup>13</sup> The *ortho*-coupled aromatic protons at  $\delta$  6.67 and 7.31 were assigned to H-2

\* To whom correspondence should be addressed. Tel: +86-20-85225849. Fax: +86-20-85221559. E-mail: tyaoxs@jnu.edu.cn (X.S.Y.); xzhang@burnham.org (X.K.Z.).

<sup>†</sup> Shenyang Pharmaceutical University.

<sup>‡</sup> College of Pharmacy, Jinan University.

<sup>§</sup> Guangdong Province Key Laboratory of Pharmacodynamic Constituents of TCM and New Drugs Research, Jinan University.

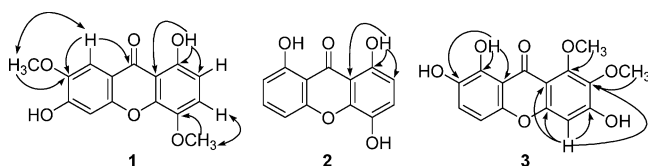
<sup>∇</sup> Xiamen University.

<sup>∇</sup> Burnham Institute for Medical Research.

**Table 1.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR Data for Compounds 1–3 (400 MHz for  $^1\text{H}$  NMR)

position	compound 1 <sup>a</sup>		compound 2 <sup>a</sup>		compound 3 <sup>b</sup>	
	$\delta_{\text{C}}$ , mult	$\delta_{\text{H}}$ ( $J$ in Hz)	$\delta_{\text{C}}$ , mult	$\delta_{\text{H}}$ ( $J$ in Hz)	$\delta_{\text{C}}$ , mult	$\delta_{\text{H}}$ ( $J$ in Hz)
1	153.2, C		151.8, C		154.4, C	
2	108.0, CH	6.69, d, (8.8)	109.4, CH	6.67, d (8.8)	139.5, C	
3	119.4, CH	7.40, d (8.8)	124.3, CH	7.31, d (8.8)	155.8, <sup>c</sup> C	
4	139.6, C		137.4, C		99.9, CH	6.73, s
4a	144.8, C		143.6, C		158.8, <sup>c</sup> C	
5	102.8, CH	6.97, s	107.4, CH	7.06, d (8.4)	105.9, CH	6.79, d (8.8)
6	152.3, <sup>c</sup> C		138.0, CH	7.75, t (8.4)	123.3, CH	7.24, d (8.8)
7	146.5, C		110.4, CH	6.83, d (8.4)	141.0, C	
8	104.5, CH	7.47, s	160.4, C		148.9, C	
8a	111.7, C		107.5, C		109.5, C	
9	180.0, C		185.6, C		182.3, C	
9a	108.1, C		107.9, C		108.9, C	
10a	155.6, <sup>c</sup> C		155.8, C		149.1, C	
OCH <sub>3</sub> -1					62.1, CH <sub>3</sub>	3.97, s
OCH <sub>3</sub> -2					61.6, CH <sub>3</sub>	3.90, s
OCH <sub>3</sub> -4	56.7, CH <sub>3</sub>	3.88, s				
OCH <sub>3</sub> -7	55.9, CH <sub>3</sub>	3.90, s				
OH-1		12.06, s		11.00, s		
OH-4				9.73, s		
OH-8				11.78, s		13.26, s

<sup>a</sup> Measured in DMSO-*d*<sub>6</sub>. <sup>b</sup> Measured in acetone-*d*<sub>6</sub>. <sup>c</sup> Signals may be interchanged in each column.

**Figure 1.** Key HMBC (→) and ROESY (↔) correlations of 1–3.

and H-3, respectively, on the basis of the HMBC correlation between the hydrogen-bonded hydroxy group at  $\delta$  11.00 (OH-1) and an aromatic carbon at  $\delta$  109.4 (C-2) (Figure 1). Therefore, compound 2 (pruniflorone N) was assigned as 1,4,8-trihydroxyxanthone.

Compound 3, obtained as a yellow, amorphous powder, gave a molecular formula of  $\text{C}_{15}\text{H}_{12}\text{O}_7$  by analysis of the HRESIMS data. The  $^1\text{H}$  NMR data of 3 (Table 1) showed one hydrogen-bonded hydroxy signal at  $\delta$  13.26 (1H, s, OH-8), two *ortho*-coupled aromatic signals at  $\delta$  7.24 and 6.79 (1H, each, d,  $J = 8.8$  Hz, H-6 and H-5), and an aromatic signal at  $\delta$  6.73 (1H, s, H-4), in addition to two methoxy signals at  $\delta$  3.97 and 3.90 (3H each, s). The aromatic carbon signals at  $\delta$  148.9, 141.0, and 109.5 were assigned to C-8, C-7, and C-8a, due to their HMBC correlations with the hydroxy group at  $\delta$  13.26 (OH-8) (Figure 1). The *ortho*-coupled aromatic protons at  $\delta$  7.24 and 6.79 were then assigned to H-6 and H-5, on the basis of the HMBC correlations of H-6/C-8 and C-7. The aromatic carbon signal at  $\delta$  149.1 was assigned to C-10a, as a result of its HMBC correlation with H-6. The remaining six aromatic carbon signals at  $\delta$  158.8, 155.8, 154.4, 139.5, 108.9, and 99.9 were assigned to the other benzene ring of 3. The quaternary carbon signal at  $\delta$  154.4, which correlated with the methoxy group at  $\delta$  3.97 in the HMBC spectrum, was attached to the benzene ring *para* to the aromatic proton at  $\delta$  6.73, which gave the HMBC correlations with the quaternary carbon signals at  $\delta$  158.8, 155.8, 139.5, and 108.9. The aromatic proton at  $\delta$  6.73 was assigned to H-4 when the chemical shift was taken into account.<sup>11,12</sup> Furthermore, the methoxy carbon signal appeared at  $\delta$  61.6, suggesting that both of the *ortho*-positions of this methoxy group are substituted.<sup>5,13</sup> Therefore, the carbon signal at 139.5, with a HMBC correlation with the methoxy group at  $\delta$  61.6/3.90, was assigned to C-2. Thus, compound 3 (pruniflorone O) was elucidated as 1,2-dimethoxy-3,7,8-trihydroxyxanthone.

Compound 4 was obtained as a reddish-brown gum with a molecular formula of  $\text{C}_{28}\text{H}_{32}\text{O}_5$ , on the basis of its HRESIMS data.

The  $^1\text{H}$  NMR data of 4 (Table 2) exhibited signals of a hydrogen-bonded hydroxy group at  $\delta$  13.65 (1H, s, OH-1), two *ortho*-coupled aromatic signals at  $\delta$  7.13 and 7.08 (1H each, d,  $J = 8.8$  Hz, H-6 and H-5), and an aromatic signal at  $\delta$  6.22 (1H, s, H-4). Furthermore, the proton signals at  $\delta$  3.41 (2H, d,  $J = 7.0$  Hz, H-1'), 5.29 (1H, t,  $J = 7.0$  Hz, H-2'), 1.74 (3H, s, H-4'), and 1.83 (3H, s, H-5') suggested the presence of a prenyl moiety in the structure of 4.<sup>12</sup> In addition, the presence of a geranyl side chain was indicated as a series of proton signals observed at  $\delta$  4.24 (2H, d,  $J = 6.6$  Hz, H-1''), 5.27 (1H, t,  $J = 6.6$  Hz, H-2''), 2.06 (2H, m, H-4''), 2.09 (2H, m, H-5''), 5.04 (1H, t,  $J = 6.4$  Hz, H-6''), 1.64 (3H, s, H-8''), 1.86 (3H, s, H-9''), and 1.57 (3H, s, H-10''). The prenyl moiety was placed at C-2 on the basis of the HMBC correlations of H-1'/C-1 (160.4), C-2 (108.9), and C-3 (161.9). The geranyl unit was located at the *peri*-position to the carbonyl group (C-8), according to the downfield shift of H-1'' at  $\delta$  4.24.<sup>10,14</sup> This was confirmed by the HMBC correlations of H-1''/C-7 (150.9), C-8 (127.3), and C-8a (118.3) (Figure 2). Therefore, the structure of 4 (pruniflorone P) was determined as 1,3,7-trihydroxy-2-prenyl-8-geranyl-xanthone.

Compound 5, a pale yellow, amorphous powder, gave a molecular formula of  $\text{C}_{28}\text{H}_{32}\text{O}_6$ , as established by HRESIMS. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of 5 (Table 2) were similar to those of cudraticusxanthone E (19),<sup>15</sup> except that a geranyl group in 5 replaced the prenyl group in the latter compound. The geranyl group was located at C-4 (106.5) using the HMBC correlations of H-1'/C-3 (160.2), C-4, and C-4a (154.0) (Figure 2). Thus, the structure of 5 (pruniflorone Q) was elucidated as 1,3,6,7-tetrahydroxy-2-prenyl-4-geranyl-xanthone.

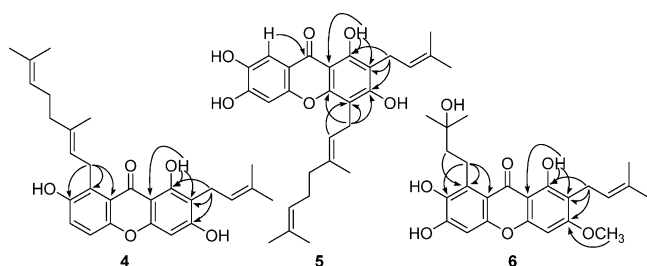
Compound 6 was afforded as a yellow, amorphous powder, with the molecular formula  $\text{C}_{24}\text{H}_{28}\text{O}_7$  established by HRESIMS. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of 6 (Table 2) were similar to those of dulcisxanthone B (17),<sup>16</sup> except for the appearance of a 3-hydroxy-3-methylbutyl moiety at  $\delta$  3.48 (2H, t,  $J = 7.2$  Hz, H-1''), 1.88 (2H, t,  $J = 7.2$  Hz, H-2''), and 1.30 (6H, s, H-4'' and H-5'') instead of the prenyl group presented in dulcisxanthone B. Similarly, the linkage of the 3-hydroxy-3-methylbutyl group was located at C-8 by the HMBC correlations of H-1''/C-7 (141.4), C-8 (131.1), and C-8a (112.0) (Figure 2). Thus, the structure of 6 (pruniflorone R) was determined as 1,6,7-trihydroxy-2-prenyl-3-methoxy-8-(3-hydroxy-3-methylbutyl)xanthone.

Several known compounds were identified as 1,7-dihydroxy-8-methoxyxanthone (7),<sup>17</sup> 1,6-dihydroxy-7,8-dimethoxyxanthone (8),<sup>18</sup> 1,7-dihydroxyxanthone (9),<sup>19</sup> 1,6-dihydroxy-5-methoxyxanthone (10),<sup>20</sup> 1,4,7-trihydroxy-8-methoxyxanthone (11),<sup>5</sup> 1,4,7-

**Table 2.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR Data for Compounds 4–6 (400 MHz for  $^1\text{H}$  NMR)

position	compound 4 <sup>a</sup>		compound 5 <sup>b</sup>		compound 6 <sup>b</sup>	
	$\delta_{\text{C}}$ , mult	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{C}}$ , mult	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{C}}$ , mult	$\delta_{\text{H}}$ (J in Hz)
1	160.4, C		158.8, C		160.5, C	
2	108.9, C		110.5, C		111.5, C	
3	161.9, C		160.2, C		164.4, C	
4	93.0, CH	6.22, s	106.5, C		89.3, CH	6.46, s
4a	155.0, C		154.0, C		156.2, C	
5	116.4, CH	7.08, d (8.8)	103.4, CH	6.97, s	101.1, CH	6.80, s
6	123.9, CH	7.13, d (8.8)	152.6, <sup>c</sup> C		153.4, <sup>c</sup> C	
7	150.9, C		143.8, C		141.4, C	
8	127.3, C		109.2, CH	7.56, s	131.1, C	
8a	118.3, C		113.7, C		112.0, C	
9	183.2, C		180.8, C		183.1, C	
9a	103.8, C		103.3, C		104.3, C	
10a	151.7, C		153.9, <sup>c</sup> C		154.0, <sup>c</sup> C	
1'	21.4, CH <sub>2</sub>	3.41, d (7.0)	22.1, CH <sub>2</sub>	3.43, d (7.0)	21.9, CH <sub>2</sub>	3.31, d (7.2)
2'	121.6, CH	5.29, t (7.0)	123.2, CH	5.24, t (7.0)	123.4, CH	5.21, t (7.2)
3'	134.4, C		132.3, C		131.4, C	
4'	25.6, CH <sub>3</sub>	1.74, s	25.8, CH <sub>3</sub>	1.66, s	25.8, CH <sub>3</sub>	1.64, s
5'	17.8, CH <sub>3</sub>	1.83, s	17.9, CH <sub>3</sub>	1.79, s	17.8, CH <sub>3</sub>	1.78, s
1''	25.7, CH <sub>2</sub>	4.24, d (6.6)	22.4, CH <sub>2</sub>	3.57, d (7.0)	22.5, CH <sub>2</sub>	3.48, t (7.2)
2''	121.7, CH	5.27, t (6.6)	123.2, CH	5.24, t (7.0)	44.1, CH <sub>2</sub>	1.88, t (7.2)
3''	138.1, C		135.9, C		71.2, C	
4''	39.7, CH <sub>2</sub>	2.06, m	40.3, CH <sub>2</sub>	1.99, m	29.6, CH <sub>3</sub>	1.30, s
5''	26.4, CH <sub>2</sub>	2.09, m	27.2, CH <sub>2</sub>	2.06, m	29.6, CH <sub>3</sub>	1.30, s
6''	123.9, CH	5.04, t (6.4)	124.9, CH	5.02, t (6.6)		
7''	131.8, C		131.6, C			
8''	25.5, CH <sub>3</sub>	1.64, s	25.7, CH <sub>3</sub>	1.52, s		
9''	16.3, CH <sub>3</sub>	1.86, s	16.4, CH <sub>3</sub>	1.90, s		
10''	17.6, CH <sub>3</sub>	1.57, s	17.6, CH <sub>3</sub>	1.50, s		
OCH <sub>3</sub> -3					56.4, CH <sub>3</sub>	3.96, s
OH-1		13.65, s		13.47, s		13.75, s

<sup>a</sup> Measured in CDCl<sub>3</sub>. <sup>b</sup> Measured in acetone-*d*<sub>6</sub>. <sup>c</sup> Signals may be interchanged in each column.

**Figure 2.** Key HMBC (→) correlations of 4–6.

trihydroxyxanthone (**12**),<sup>5</sup> 1,3,6-trihydroxy-5-methoxyxanthone (**13**),<sup>21</sup> 1,5-dihydroxy-6-methoxyxanthone (**14**),<sup>22</sup> 1,7-dihydroxy-4-methoxyxanthone (**15**),<sup>17</sup> 1,3,6-trihydroxy-7-methoxyxanthone (**16**),<sup>23</sup> dulcisxanthone B (**17**),<sup>16</sup> 1,3,7-trihydroxy-2,4-diprenylxanthone (**18**),<sup>24</sup> cudraticusxanthone E (**19**),<sup>15</sup> 1,3,5-trihydroxy-4-geranyl-xanthone (**20**),<sup>25</sup>  $\gamma$ -mangostin (**21**),<sup>26</sup> cochinchinone A (**22**),<sup>12</sup> 1,3,7-trihydroxy-2-prenylxanthone (**23**),<sup>27</sup> cochinchinone B (**24**),<sup>12</sup> and  $\alpha$ -mangostin (**25**),<sup>26</sup> by comparison of their physical and spectroscopic data with those reported previously. Compounds **15**, **22**, and **24** were isolated from the stems of *C. formosum* ssp. *pruniflorum* for the first time. In addition, compounds **8**, **9**, **16**, **19**, **20**, and **23** have not been isolated from the genus *Cratoxylum* previously.

The isolated compounds (**2**–**25**) were evaluated for their effects on RXR $\alpha$  transcriptional activity using a reporter gene assay. CV-1 cells were transiently transfected with the TREpal-tk-CAT reporter, which is known to be activated by RXR $\alpha$  homodimers, and the RXR $\alpha$  expression vector. Cells were then treated with RXR $\alpha$  ligand 9-*cis*-RA in the presence of the indicated compounds, and the CAT reporter activities were determined. Consistent with previous results,<sup>28</sup> treatment of cells with 9-*cis*-RA strongly induced the reporter transcription, which was inhibited by co-treatment with BI1003, a known RXR $\alpha$  antagonist.<sup>29</sup> Comparing with the effect of BI1003 (1  $\mu\text{M}$ ), compounds **5**–**7**, **11**, **15**–**17**, **19**, and **24** (10

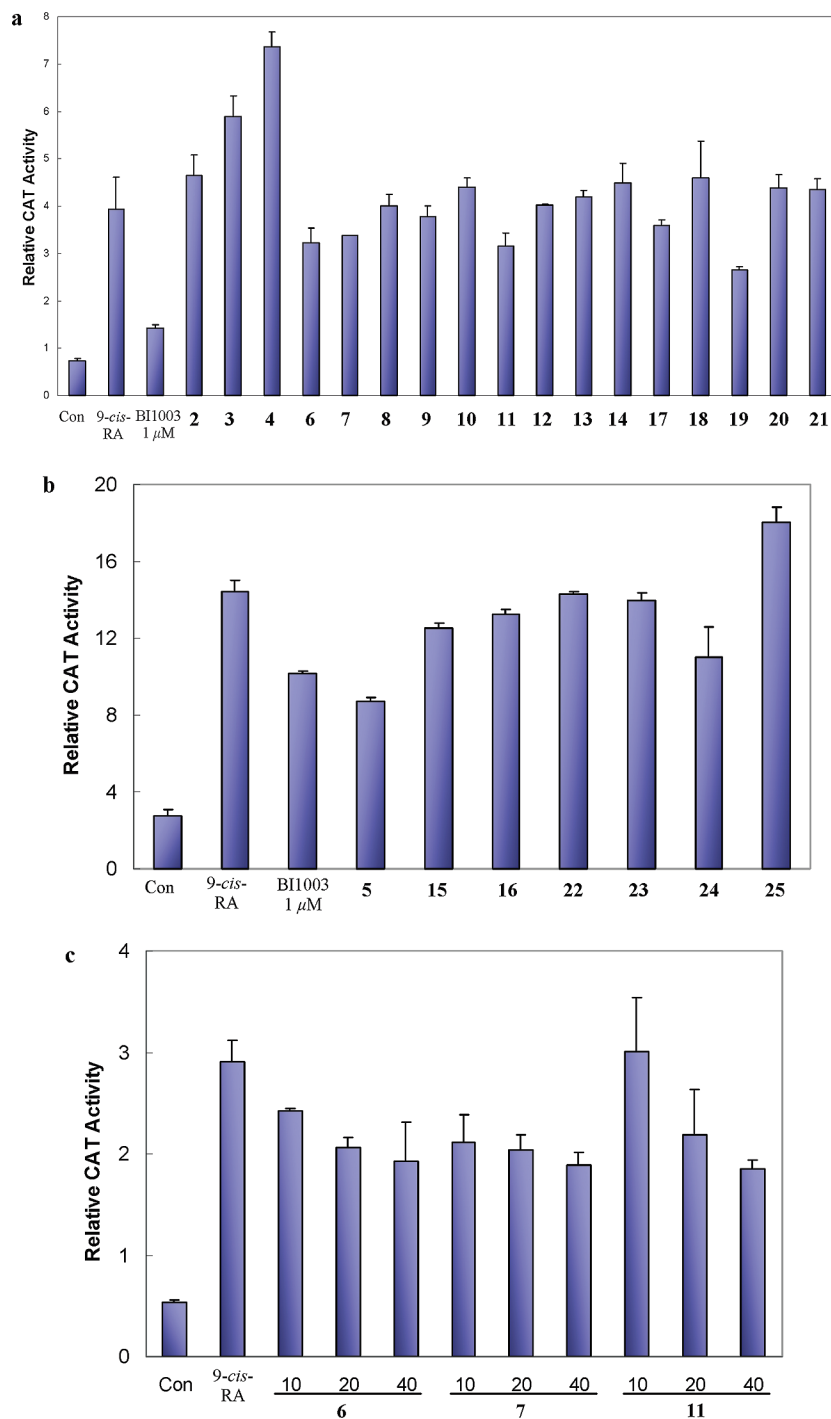
$\mu\text{M}$ ) showed transcriptional-inhibitory activities of RXR $\alpha$  to various degrees. Among them, compounds **6**, **7**, and **11** exhibited concentration-dependent activities. These compounds are currently being evaluated for their effects on RXR-mediated growth inhibition and apoptosis induction in cancer cells as well as RXR-dependent regulation of gene expression.

## Experimental Section

**General Experimental Procedures.** UV spectra were measured on a JASCO V-550 UV/vis spectrophotometer. IR spectra were recorded on a JASCO FTIR-400 spectrometer. 1D and 2D NMR spectra were recorded on a Bruker AV-400 spectrometer (400 MHz for  $^1\text{H}$ , 100 MHz for  $^{13}\text{C}$ ). ESIMS data were recorded on a Finnigan LCQ Advantage MAX mass spectrometer. HRESIMS data were determined by an Agilent 6210 LC/MSD TOF mass spectrometer. Open column chromatography (CC) was performed using silica gel (200–300 mesh, Qingdao Haiyang Chemical Goup Corp., Qingdao, People's Republic of China), ODS (50  $\mu\text{m}$ , YMC), and Sephadex LH-20 (Pharmacia). Thin-layer chromatography (TLC) was performed using precoated silica gel plates (silica gel GF<sub>254</sub>, 1 mm, Yantai).

**Plant Material.** The plant material was collected in Jinghong City, Yunnan Province, People's Republic of China in August 2008 and was identified as the stems of *Cratoxylum formosum* (Jack) Dyer ssp. *pruniflorum* (Kurz) Gogel by Jing-yun Cui, Xishuangbanna Tropical Botanic Garden of the Chinese Academy of Sciences. A voucher specimen (20080911) was deposited in the Institute of Traditional Chinese Medicine and Natural Products, Jinan University, Guangzhou, China.

**Extraction and Isolation.** The chopped, dried stems of *C. formosum* (5.0 kg) were refluxed with 40 L of 60% (v/v) EtOH–H<sub>2</sub>O twice, for two hours each time. After filtration, the filtrate was concentrated under reduced pressure to yield a brownish extract (675.0 g). The extract (600.0 g) was then separated over a Diaion HP-20 column, using EtOH–H<sub>2</sub>O as mobile phase, to give three fractions (A–C). Fraction C (90% EtOH–H<sub>2</sub>O eluent, 117.0 g) was chromatographed over silica gel eluted with cyclohexane–EtOAc in a gradient to yield eight fractions (C1–C8). Fraction C4 (9:1 cyclohexane–EtOAc as eluent, 4.0 g) was further separated over Sephadex LH-20 eluted with cyclohexane–CHCl<sub>3</sub> (1:1), CHCl<sub>3</sub>, and CHCl<sub>3</sub>–CH<sub>3</sub>OH (1:1), succes-



**Figure 3.** Effects of compounds 2–25 (10 μM) on the transcriptional activities of RXRα (a and b), effects of compounds 6, 7, and 11 (10, 20, and 40 μM) on the transcriptional activities of RXRα (c).

sively, to give eight subfractions (C4A–C4H). Subfraction C4B (CHCl<sub>3</sub> eluent) was submitted to silica gel CC, eluted with cyclohexane–acetone (9:1), to give **8** (12.8 mg) and **11** (13.3 mg). Subfraction C4C (CHCl<sub>3</sub> eluent) was purified over Sephadex LH-20 eluted with CHCl<sub>3</sub>–CH<sub>3</sub>OH (4:1), yielding **2** (14.2 mg) and **24** (15.5 mg). Subfraction C4D (CHCl<sub>3</sub> eluent) was subjected to silica gel CC, eluted with CHCl<sub>3</sub>–CH<sub>3</sub>OH (50:1), to afford **23** (42.5 mg). After purification with repeated silica gel CC, eluted with CHCl<sub>3</sub> and CHCl<sub>3</sub>–CH<sub>3</sub>OH (100:1), subfractions C4E and C4F (CHCl<sub>3</sub> eluents) yielded **4** (15.8 mg), **5** (27.5 mg), **7** (15.0 mg), **1** (13.6 mg), **6** (10.3 mg), **9** (20.9 mg), **10** (11.7 mg), and **14** (15.3 mg). Subfraction C4G [CHCl<sub>3</sub>–CH<sub>3</sub>OH (1:1) as eluent] was subjected to Sephadex LH-20 CC, eluted with CHCl<sub>3</sub>–CH<sub>3</sub>OH (3:2), to give **12** (9.5 mg) and **3** (11.6 mg). Compound **13** (11.6 mg) was recrystallized in CHCl<sub>3</sub>–CH<sub>3</sub>OH (4:1) from subfraction C4H [CHCl<sub>3</sub>–CH<sub>3</sub>OH (1:1) as eluent].

Fraction C6 (8:2 cyclohexane–EtOAc as eluent; 8.0 g) was further separated by passage over Sephadex LH-20, eluted with CHCl<sub>3</sub>–CH<sub>3</sub>OH (4:1), to give four subfractions (C6A–C6D). Subfraction C6D was applied to silica gel CC eluted with cyclohexane–EtOAc in gradient to afford eight subfractions (C6D1–C6D8). Subfraction C6D2 (9:1 cyclohexane–EtOAc) was subjected to silica gel CC, using CHCl<sub>3</sub>–CH<sub>3</sub>OH (50:1 and 20:1) as mobile phase, to give **22** (9.3 mg) and **20** (45.0 mg). Compound **16** (14.7 mg) was recrystallized in CHCl<sub>3</sub>–CH<sub>3</sub>OH (4:1) from subfraction C6D3 (9:1 cyclohexane–EtOAc). Subfraction C6D4 (8:2 cyclohexane–EtOAc) was subjected to Sephadex LH-20 CC, eluted with CHCl<sub>3</sub>–CH<sub>3</sub>OH (4:1), to give **17** (180.0 mg) and **21** (16.5 mg). Subfraction C6D6 (8:2 cyclohexane–EtOAc) was purified by silica gel CC, eluted with CHCl<sub>3</sub> and CHCl<sub>3</sub>–CH<sub>3</sub>OH (100:1), to give **18** (26.4 mg) and **19** (25.0 mg). Subfraction C6D7 (8:2 cyclohexane–EtOAc) was subjected to Sephadex LH-20 CC, eluted

with  $\text{CHCl}_3$ – $\text{CH}_3\text{OH}$  (3:2), to yield **15** (9.4 mg). Subfraction C6D8 (EtOAc eluent) was purified by ODS CC, eluted with  $\text{CH}_3\text{OH}$ – $\text{H}_2\text{O}$  (8:2), to afford **25** (37.2 mg).

**Pruniflorone M (1)**: yellow, amorphous powder; UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 205 (4.31), 228 (4.25), 255 (4.23), 281 (4.09), 379 (3.82) nm; IR (KBr)  $\nu_{\text{max}}$  3202, 1650, 1605, 1480, 1293, 797  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data (see Table 1); HRESIQTOFMS  $m/z$  287.0551 [ $\text{M} - \text{H}$ ] $^-$  (calcd for  $\text{C}_{15}\text{H}_{11}\text{O}_6$ , 287.0561).

**Pruniflorone N (2)**: brownish, amorphous powder; UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 205 (4.40), 226 (4.22), 299 (4.25), 312 (4.29) nm; IR (KBr)  $\nu_{\text{max}}$  3424, 1629, 1502, 1237, 1064, 818  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data (see Table 1); HRESIQTOFMS  $m/z$  243.0302 [ $\text{M} - \text{H}$ ] $^-$  (calcd for  $\text{C}_{13}\text{H}_7\text{O}_5$ , 243.0299).

**Pruniflorone O (3)**: yellow, amorphous powder; UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 205 (4.78), 238 (4.50), 269 (4.41), 281 (4.09), 309 (4.23), 372 (4.23) nm; IR (KBr)  $\nu_{\text{max}}$  3435, 1619, 1473, 1293, 1081, 782  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data (see Table 1); HRESIQTOFMS  $m/z$  303.0509 [ $\text{M} - \text{H}$ ] $^-$  (calcd for  $\text{C}_{15}\text{H}_{11}\text{O}_7$ , 303.0510).

**Pruniflorone P (4)**: reddish-brown gum; UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 205 (4.55), 241 (4.54), 265 (4.48), 316 (4.24), 379 (3.77) nm; IR (KBr)  $\nu_{\text{max}}$  3414, 2923, 1645, 1458, 1167, 820  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data (see Table 2); HRESIQTOFMS  $m/z$  447.2184 [ $\text{M} - \text{H}$ ] $^-$  (calcd for  $\text{C}_{28}\text{H}_{31}\text{O}_5$ , 447.2177).

**Pruniflorone Q (5)**: pale yellow, amorphous powder; UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 206 (4.73), 235 (4.57), 263 (4.59), 321 (4.34), 374 (4.15) nm; IR (KBr)  $\nu_{\text{max}}$  3349, 2914, 1618, 1481, 1292, 808  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data (see Table 2); HRESIQTOFMS  $m/z$  463.2132 [ $\text{M} - \text{H}$ ] $^-$  (calcd for  $\text{C}_{28}\text{H}_{31}\text{O}_6$ , 463.2126).

**Pruniflorone R (6)**: yellow, amorphous powder; UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 206 (4.56), 243 (4.51), 261 (4.51), 318 (4.30), 340 (4.02), 361 (4.02) nm; IR (KBr)  $\nu_{\text{max}}$  3374, 2969, 2926, 1648, 1579, 1285, 1113, 825  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data (see Table 2); HRESIQTOFMS  $m/z$  427.1748 [ $\text{M} - \text{H}$ ] $^-$  (calcd for  $\text{C}_{24}\text{H}_{27}\text{O}_7$ , 427.1762).

**Cell Culture and Reporter Gene Assay.** CV-1 green monkey kidney cells were grown in DME medium supplemented with 10% fetal bovine serum (FBS). The expression vectors for RXR $\alpha$  and reporter TREpal-tk-CAT have been described previously.<sup>28</sup> For reporter assays, CV-1 cells were seeded at  $5 \times 10^4$  cells/well in 24-well plates. Cells were transfected with 50 ng of TREpal-tk-CAT plasmid, 20 ng of  $\beta$ -galactosidase expression vector (pCH 110; Amersham), and 20 ng of RXR $\alpha$  expression vectors for receptors using Lipofectamine 2000 (Invitrogen). Cells were treated with compound for 20 h. CAT activity was normalized with  $\beta$ -galactosidase activity for transfection efficiency.

**Acknowledgment.** We thank J.-y. Cui (Xishuangbanna Tropical Botanic Garden of the Chinese Academy of Sciences) for collecting the plant material. We are grateful to H.-n. Zhao and Z.-q. Mu for measuring the HRESIMS and NMR data. This work was supported by grants from the National Natural Science Foundation of China (NSFC-30873146).

**Supporting Information Available:** NMR spectra of compounds **1–6**. Structures of compounds **7–25**. This information is available free of charge via the Internet at <http://pubs.acs.org>.

## References and Notes

- De Lera, A. R.; Bourguet, W.; Altucci, L.; Gronemeyer, H. *Nat. Rev. Drug Discovery* **2007**, *6*, 811–820.
- Moore, J. T.; Collins, J. L.; Pearce, K. H. *ChemMedChem* **2006**, *1*, 504–523.
- Giguere, V. *Endocr. Rev.* **1999**, *20*, 689–725.
- Desvergne, B. *Vitam. Horm.* **2007**, *75*, 1–32.
- Iinuma, M.; Tosa, H.; Ito, T.; Tanaka, T.; Madulid, D. A. *Phytochemistry* **1996**, *42*, 1195–1198.
- Na, Z. *J. Plant Resour. Environ.* **2007**, *16*, 75–77.
- Boonnak, N.; Karalai, C.; Chantrapromma, S.; Ponglimanont, C.; Fun, H. K.; Kanjana-opas, A.; Laphookhieo, S. *Tetrahedron* **2006**, *62*, 8850–8859.
- Boonnak, N.; Karalai, C.; Chantrapromma, S.; Ponglimanont, C.; Kanjana-opas, A.; Chantrapromma, K.; Kato, S. *Chem. Pharm. Bull.* **2010**, *58*, 386–389.
- Laphookhieo, S.; Syers, J. K.; Kiattansakul, R.; Chantrapromma, K. *Chem. Pharm. Bull.* **2006**, *54*, 745–747.
- Boonnak, N.; Karalai, C.; Chantrapromma, S.; Ponglimanont, C.; Fun, H. K.; Kanjana-Opas, A.; Chantrapromma, K.; Kato, S. *Tetrahedron* **2009**, *65*, 3003–3013.
- Boonsri, S.; Karalai, C.; Ponglimanont, C.; Kanjana-Opas, A.; Chantrapromma, K. *Phytochemistry* **2006**, *67*, 723–727.
- Mahabusarakam, M.; Nuangnaowarat, W.; Taylor, W. C. *Phytochemistry* **2006**, *67*, 470–474.
- Silva, A. M. S.; Pinto, D. C. G. A. *Curr. Med. Chem.* **2005**, *12*, 2481–2497.
- Seo, E. K.; Kim, N. C.; Wani, M. C.; Wall, M. E.; Navarro, H. A.; Burgess, J. P.; Kawanishi, K.; Kardon, L. B. S.; Riswan, S.; Rose, W. C.; Fairchild, C. R.; Farnsworth, N. R.; Kinghorn, A. D. *J. Nat. Prod.* **2002**, *65*, 299–305.
- Zou, Y. S.; Hou, A. J.; Zhu, G. F.; Chen, Y. F.; Sun, H. D.; Zhao, Q. S. *Bioorg. Med. Chem.* **2004**, *12*, 1947–1953.
- Deachathai, S.; Mahabusarakam, W.; Phongpaichit, S.; Taylor, W. C. *Phytochemistry* **2005**, *66*, 2368–2375.
- Kijjoa, A.; Jose, M.; Gonzalez, T. G.; Pinto, M. M. M.; Damas, A. M.; Mondranon, I. O.; Silva, A. M. S.; Herz, W. *Phytochemistry* **1998**, *49*, 2159–2162.
- Marston, A.; Hamburger, M.; Sordat-Diserens, I.; Msonthi, J. D.; Hostettmann, K. *Phytochemistry* **1993**, *33*, 809–812.
- Kato, L.; De Oliveira, C. M. A.; Vencato, I.; Lauriucci, C. *J. Chem. Crystallogr.* **2005**, *35*, 23–26.
- Gonzalez, M. J.; Nascimento, M. S. J.; Cidade, H. M.; Pinto, M. M. M.; Kijjoa, A.; Anantachoke, C.; Silva, A. M. S.; Herz, W. *Planta Med.* **1999**, *65*, 368–371.
- Zhang, Z.; Elsohly, H. N.; Jacob, M. R.; Pasco, D. S.; Walker, L. A.; Clark, A. M. *Planta Med.* **2002**, *68*, 49–54.
- Patnaik, M.; Sharma, V. K.; Parmar, V. S.; Boll, P. M. *Acta Chem. Scand. Ser.* **1987**, *B41*, 210–212.
- Wang, H.; Ye, G.; Ma, C. H. *J. Pharm. Biomed. Anal.* **2007**, *45*, 793–798.
- Iinuma, M.; Tosa, H.; Tanaka, T.; Riswan, S. *Chem. Pharm. Bull.* **1996**, *44*, 232–234.
- Sordat-Diserens, I.; Rogers, C.; Sordat, B.; Hostettmann, K. *Phytochemistry* **1992**, *31*, 313–316.
- Chen, L. G.; Yang, L. L.; Wang, C. C. *Food Chem. Toxicol.* **2008**, *46*, 688–693.
- Harrison, L. J.; Leong, L. S.; Sia, G. L.; Sim, K. Y.; Tan, H. T. W. *Phytochemistry* **1993**, *33*, 727–728.
- Zhang, X. K.; Lehmann, J.; Hoffmann, B.; Dawson, M. I.; Cameron, J.; Graupner, G.; Hermann, T.; Tran, P.; Pfahl, M. *Nature* **1992**, *358*, 587–591.
- Lu, J. Y.; Dawson, M. I.; Hu, Q. Y.; Xia, Z. B.; Dambacher, J. D.; Ye, M.; Zhang, X. K.; Li, E. *Magn. Reson. Chem.* **2009**, *47*, 1071–1080.

NP1001797