

## Macrophyllionium and Macrophyllines A and B, Oxindole Alkaloids from *Uncaria macrophylla*

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An unusual oxindole alkaloid inner salt, macrophyllionium (**1**), and a pair of new tetracyclic oxindole alkaloids, macrophyllines A (**2**) and B (**3**), together with six known alkaloids, were isolated from the aerial parts of *Uncaria macrophylla*. Corynantheidine (**8**) exhibited moderate cytotoxicity against HL-60 and SW480 cells with IC<sub>50</sub> values of 13.96 and 23.28  $\mu$ M, respectively. Dihydrocorynantheine (**9**) exhibited significant vasodilating activity against phenylephrine-induced contraction in rat thoracic aorta rings (IC<sub>50</sub> = 6.73  $\mu$ g/mL). In addition, compounds **2**, **6**, and **9** showed weak inhibitory action on KCl-induced contraction.

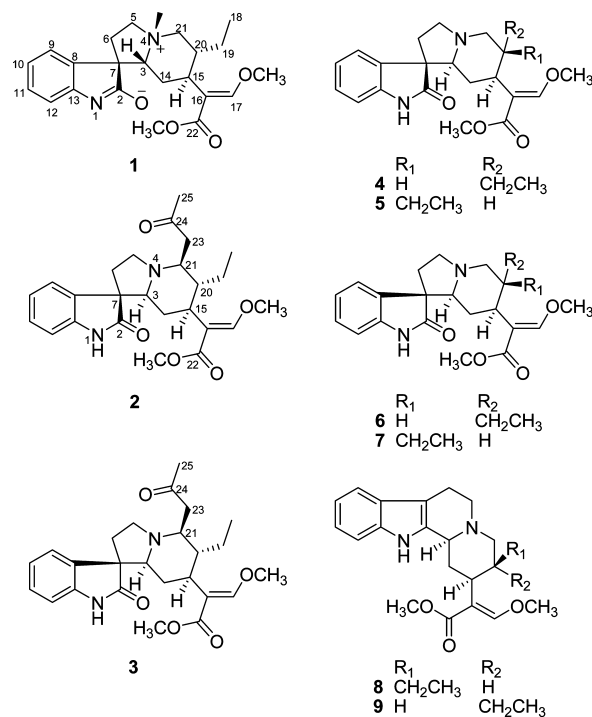
The genus *Uncaria* (Rubiaceae) comprises 34 species worldwide with 11 of them endemic to China.<sup>1</sup> The dried hooks of some *Uncaria* species have been used as the well-known traditional Chinese medicine “Gou-teng” to relieve hypertension, epilepsy, preeclampsia, and associated symptoms such as headaches and dizziness.<sup>2</sup> These activities have been linked to the presence of oxindole alkaloids.<sup>3</sup>

*U. macrophylla* Wall., a dominant plant widely distributed in the Yunnan Province, is identified as one origin of “Gou-teng” in the Chinese Pharmacopoeia.<sup>2</sup> However, only eight oxindole alkaloids were reported from this plant.<sup>4</sup> HPLC analysis of *U. macrophylla* collected in Yunnan showed the existence of some minor alkaloids, indicating the need for further investigation. The present study led to the discovery of an unusual oxindole alkaloid inner salt, macrophyllionium (**1**), and a pair of new tetracyclic oxindole alkaloids, macrophyllines A (**2**) and B (**3**), together with the known rhynchophylline (**4**),<sup>4a</sup> corynoxine B (**5**),<sup>4a</sup> isorhynchophylline (**6**),<sup>4a</sup> corynoxine (**7**),<sup>4a</sup> corynantheidine (**8**),<sup>5</sup> and dihydrocorynantheine (**9**).<sup>5</sup> This paper describes the isolation and structural elucidation of these alkaloids, as well as their cytotoxicity and vasodilating activities.

### Results and Discussion

The MeOH extract of the aerial parts of *U. macrophylla* was separated into a H<sub>2</sub>O-soluble fraction, a CHCl<sub>3</sub>-soluble fraction, and emulsion layers. Macrophyllionium (**1**) was obtained from the emulsion layer, while macrophyllines A (**2**) and B (**3**) were isolated from the CHCl<sub>3</sub>-soluble fraction.

Compound **1**, obtained as a light yellow oil, had a molecular formula of C<sub>23</sub>H<sub>30</sub>N<sub>2</sub>O<sub>4</sub> based on the HRESIMS ([M + H]<sup>+</sup> at *m/z* 399.2290, calcd 399.2283). The UV absorptions at 209, 242, and 289 nm indicated an oxindole chromophore.<sup>6</sup> The <sup>1</sup>H, <sup>13</sup>C, and DEPT NMR spectra displayed a substituted oxindole ring [ $\delta_C$  59.1 (s, C-7), 114.0 (d, C-12), 121.6 (d, C-10), 126.9 (d, C-9), 130.2 (d, C-11), 132.9 (d, C-8), 153.2 (s, C-13), and 187.4 (s, C-2);  $\delta_H$  6.94 (d, *J* = 7.6 Hz, H-12), 6.96 (t, *J* = 7.6 Hz, H-10), 7.22 (t, *J* = 7.6 Hz, H-11), 7.46 (d, *J* = 7.6 Hz, H-9)] and 15 additional carbon signals including three methines ( $\delta_C$  32.1, 33.6, and 78.7), five



methylenes ( $\delta_C$  24.9, 25.1, 33.4, 61.3, and 67.0), one methyl ( $\delta_C$  11.0), one carbonyl ( $\delta_C$  168.9), two *O*-methyls ( $\delta_C$  51.6 and 62.1), one *N*-methyl ( $\delta_C$  50.4), and a set of olefinic carbons ( $\delta_C$  110.1 and 163.1). These data were similar to those of rhynchophylline (**4**), but an additional *N*<sub>4</sub>-methyl group in **1** was deduced from the HMBC correlations of *N*<sub>4</sub>-CH<sub>3</sub> ( $\delta_H$  3.44, s, 3H) to C-3 ( $\delta_C$  78.7, d), C-5 ( $\delta_C$  67.0, t), and C-21 ( $\delta_C$  61.3, t). A noticeable difference was also observed in the chemical shift of C-13 with a downfield shift by almost 10 ppm in **1** ( $\delta_C$  153.2) [in comparison to **4** ( $\delta_C$  142.8)], suggesting the presence of an iminium double bond between N-1 and C-2.<sup>7</sup> Furthermore, according to the MS analysis and the chemical shift of C-2 ( $\delta_C$  187.4), an oxygen anion rather than a hydroxy group should be connected to C-2. The presence of an oxygen anion and a quaternary nitrogen atom (N-4) was essential for stability and led to the inference that compound **1** was an inner salt.

According to their biosynthetic origin, the corynantheine-type alkaloids are distinguished by the orientation of H-15 being  $\alpha$ .<sup>8</sup> The ROESY correlations of H-15/H<sub>2</sub>-19, H-15/H-21b, and H-21b/

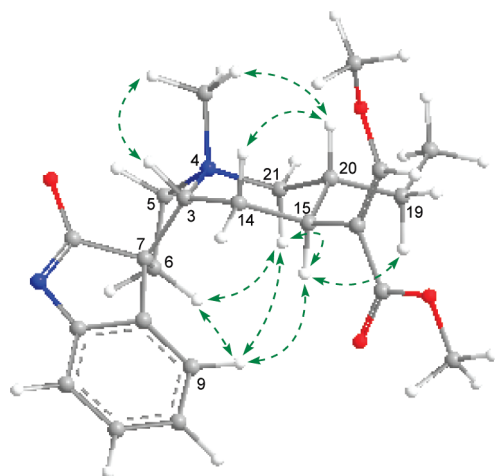
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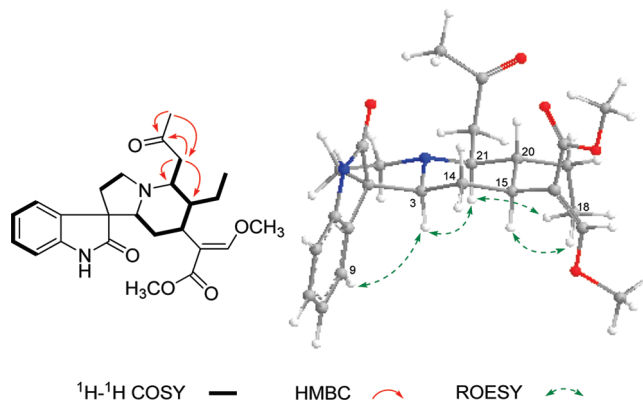
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**Figure 1.** Key ROESY correlations of **1**.

H-6a indicated the  $\alpha$ -orientation of H-6a, H-21b, and the 20-ethyl group, while the correlations of H-3/ $N_4$ -CH<sub>3</sub>, H-20/ $N_4$ -CH<sub>3</sub>, and H-14a/H-20 suggested the  $\beta$ -orientation of H-3, H-14a, H-20, and  $N_4$ -CH<sub>3</sub>. In addition, the C-7 configuration was determined by the ROESY correlations of H-9 with H-6a, H-15, and H-21b (Figure 1). Thus, the structure of macrophyllionium was established as **1**. It represents the first example of a zwitterionic oxindole alkaloid and extends the structural variety of the known betaines.<sup>9</sup>

Compound **2** was obtained as colorless crystals with a molecular formula of C<sub>25</sub>H<sub>32</sub>N<sub>2</sub>O<sub>5</sub> as deduced from HRESIMS ([M + H]<sup>+</sup> at *m/z* 441.2393). The IR absorption bands at 3441, 1708, and 1631 cm<sup>-1</sup> revealed the presence of amino, carbonyl, and olefinic



**Figure 2.** Key 2D NMR correlations of **2**.

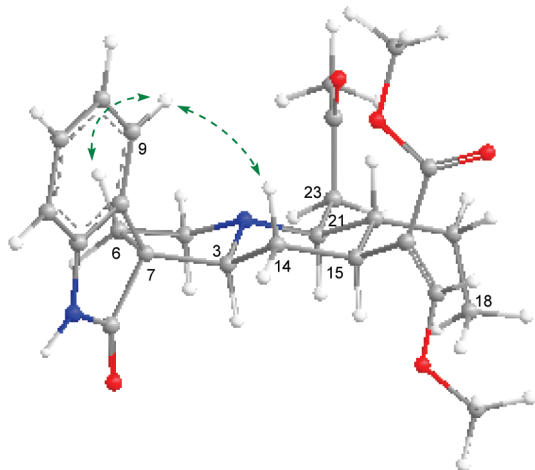
functionalities. The <sup>1</sup>H and <sup>13</sup>C NMR data of **2** (Table 1) were closely related to those of **4**, except for the appearance of three additional carbon signals at  $\delta_C$  30.2 (q, C-25), 47.2 (t, C-23), and 207.7 (s, C-24) and two sets of proton signals at  $\delta_H$  2.24 (3H, s, H-25), 2.71 (1H, dd,  $J = 2.0, 17.9$  Hz, H-23b), and 2.84 (1H, dd,  $J = 6.9, 17.9$  Hz, H-23a). On the basis of the strong HMBC correlations of H-23/C-24, H-25/C-23, and H-25/C-24, the additional signals were due to a CH<sub>3</sub>-CO-CH<sub>2</sub>- moiety attached to C-21 ( $\delta_C$  60.7) based on the <sup>1</sup>H-<sup>1</sup>H COSY cross-peak between H-21 and H-23.

The relative configuration of **2** was established on the basis of a ROESY experiment (Figure 2). H-3, H-15, H-21, and the 20-ethyl group were assigned as  $\alpha$ -oriented by the strong ROESY correlations of H-3/H-21, CH<sub>3</sub>-18/H-21, and H-15/CH<sub>3</sub>-18. The *R* configuration of C-7 was deduced from the ROESY correlations

**Table 1.** <sup>1</sup>H and <sup>13</sup>C NMR Data for Compounds **1**–**3**

position	<b>1</b> <sup>a</sup>		<b>2</b> <sup>b</sup>		<b>3</b> <sup>c</sup>	
	$\delta_H$ ( $J$ in Hz)	$\delta_C$	$\delta_H$ ( $J$ in Hz)	$\delta_C$	$\delta_H$ ( $J$ in Hz)	$\delta_C$
NH			7.91		8.44	
2		187.4, qC		180.9, qC		181.5, qC
3	4.08, d (6.8)	78.7, CH	2.50, t (11.5)	74.2, CH	2.66 <sup>d</sup>	71.3, CH
5a	3.98, ddd (11.6, 8.5, 2.0)	67.0, CH <sub>2</sub>	3.21, td (8.3, 2.4)	52.1, CH <sub>2</sub>	3.31, br t (8.4)	52.0, CH <sub>2</sub>
5b	3.87, td (11.6, 8.2)		2.46, br t (8.3)		2.47, dd (17.4, 8.6)	
6a	2.61, m	33.4, CH <sub>2</sub>	2.41, m	34.4, CH <sub>2</sub>	2.37, br t (11.0)	34.7, CH <sub>2</sub>
6b	2.52, dd (14.0, 8.0)		1.96, ddd (12.0, 6.3, 2.0)		1.96, dd (11.0, 8.4)	
7		59.1, qC		56.2, qC		57.0, qC
8		132.9, qC		134.5, qC		134.1, qC
9	7.46, d (7.6)	126.9, CH	7.22, br. d (7.7)	123.4, CH	7.40, d (7.5)	125.1, CH
10	6.96, t (7.6)	121.6, CH	7.01, td (7.7, 1.0)	122.5, CH	7.00, t (7.5)	122.2, CH
11	7.22, t (7.6)	130.2, CH	7.15, td (7.7, 1.0)	127.7, CH	7.15, t (7.5)	127.4, CH
12	6.94, d (7.6)	114.0, CH	6.81, br d (7.7)	109.1, CH	6.85, d (7.5)	109.3, CH
13		153.2, qC		140.8, qC		140.4, qC
14a	2.30, ddd (16.0, 11.9, 7.1)	24.9, CH <sub>2</sub>	2.00 <sup>d</sup>	29.2, CH <sub>2</sub>	1.47, td (11.1, 8.0)	29.8, CH <sub>2</sub>
14b	1.24, dd (16.0, 5.6)		1.14, br d (11.5)		1.03, br d (11.1)	
15	2.91, td (11.9, 5.6)	32.1, CH	2.59, td (11.9, 4.1)	37.3, CH	2.68, td (12.0, 3.0)	36.0, CH
16		110.1, qC		112.8, qC		113.0, qC
17	7.34, s	163.1, CH	7.21, s	159.6, CH	7.17, s	159.4, CH
18	0.94, t (7.5)	11.0, CH <sub>3</sub>	0.73, t (7.6)	9.5, CH <sub>3</sub>	0.75, t (7.5)	9.2, CH <sub>3</sub>
19	1.50, m	25.1, CH <sub>2</sub>	1.27, m	21.8, CH <sub>2</sub>	1.36, m	21.7, CH <sub>2</sub>
	1.26, dd (15.1, 7.5)				1.28, m	
20	2.57, m	33.6, CH	2.05, tt (10.7, 3.6)	40.3, CH	1.99, m	40.7, CH
21a	3.57, dd (13.2, 3.1)	61.3, CH <sub>2</sub>	2.71 <sup>d</sup>	60.7, CH	2.64 <sup>d</sup>	61.5, CH
21b	3.48, t (13.2)					
22		168.9, qC		168.6, qC		168.1, qC
23a			2.84, dd (17.9, 6.9)	47.2, CH <sub>2</sub>	2.76, (br d, 2.7)	47.4, CH <sub>2</sub>
23b			2.71, dd (17.9, 2.0)			
24				207.7, qC		207.3, qC
25			2.24, s	30.2, CH <sub>3</sub>	2.30, s	30.5, CH <sub>3</sub>
OCH <sub>3</sub>	3.68, s	62.1, CH <sub>3</sub>	3.71, s	61.4, CH <sub>3</sub>	3.68, s	61.1, CH <sub>3</sub>
COOCH <sub>3</sub>	3.59, s	51.6, CH <sub>3</sub>	3.61, s	51.0, CH <sub>3</sub>	3.57, s	50.7, CH <sub>3</sub>
$N_4$ -CH <sub>3</sub>	3.44, s	50.4, CH <sub>3</sub>				

<sup>a</sup> Recorded in methanol-*d*<sub>4</sub>, 500 MHz for  $\delta_H$ , 125 MHz for  $\delta_C$ . <sup>b</sup> Recorded in CDCl<sub>3</sub>, 500 MHz for  $\delta_H$ , 100 MHz for  $\delta_C$ . <sup>c</sup> Recorded in CDCl<sub>3</sub>, 400 MHz for  $\delta_H$ , 100 MHz for  $\delta_C$ . <sup>d</sup> Overlapped, without designating multiplicity.



**Figure 3.** Significant ROESY correlations of **3**.

**Table 2.** Vasodilating Effects of Compounds **2**, **6**, and **9** on the Contracted Thoracic Aorta Smooth Muscle of Rat Induced by KCl (60 mmol/L) or Phenylephrine (1  $\mu$ mol/L) in Vitro

	IC <sub>50</sub> ( $\mu$ g/mL)			
	<b>2</b>	<b>6</b>	<b>9</b>	verapamil <sup>a</sup>
KCl	15.88	18.41	21.81	0.32
phenylephrine	>30	>30	6.73	1.41

<sup>a</sup> Positive control.

between H-9 and H-3, as well as from the <sup>13</sup>C NMR shift of C-3 ( $\delta_C$  74.2),<sup>5,10</sup> because the chemical shift of C-3 ( $\delta$  74–75) in 7R alkaloids may be readily distinguished from that of 7S alkaloids ( $\delta$  70–72).<sup>6,11</sup> Therefore, the structure of macrophylline A was established as **2**.

Compound **3** was also isolated as colorless crystals. The molecular formula C<sub>25</sub>H<sub>32</sub>N<sub>2</sub>O<sub>5</sub> was determined by the positive HRESIMS ([M + H]<sup>+</sup> at *m/z* 441.2400), suggesting that **3** is an isomer of **2**. The UV, IR, and NMR data were closely related to those of **2**, except for the chemical shifts of C-3, C-9, H-14a (Table 1) and some key ROESY correlations. The ROESY correlations of H-9 with H-6b and H-14a as well as the chemical shift of C-3 ( $\delta_C$  71.3) indicated the *S* configuration of C-7 (Figure 3), which was in accord with the upfield shift of H-14a by 0.53 ppm ( $\delta_H$  1.47) [in comparison to **2** ( $\delta_H$  2.00)] due to H-14a in **3** being located within the shielding zone of the aromatic ring. Thus, the structure of **3** was determined as shown.

Macrophyllines A (**2**) and B (**3**) are a pair of C-7 diastereomers with an additional 2-oxopropyl group compared with other analogues, and they were proved to be natural products by LC-MS analysis of the MeOH extract (Supporting Information). This is also the first report of the presence of compounds **8** and **9** in this plant.

All compounds were evaluated in vitro for their cytotoxicity against five human cancer cell lines using the MTT method as reported previously.<sup>12</sup> Cisplatin (Sigma, USA) was used as the positive control. Compound **8** exhibited moderate cytotoxic activity toward HL-60 and SW480 cell lines with IC<sub>50</sub> values of 13.96 and 23.28  $\mu$ M, respectively, while the other compounds were inactive (IC<sub>50</sub> > 40  $\mu$ M).

Vasodilating activity is relevant to the antihypertensive effect of oxindole alkaloids from *Uncaria*.<sup>3b</sup> With the exception of compound **8**, all other alkaloids were investigated for their relaxation effects on KCl- or phenylephrine-induced contraction in rat thoracic aorta rings (Table 2). Dihydrocorynantheine (**9**) exhibited significant vasodilating activity against phenylephrine-induced contraction in rat thoracic aorta rings (IC<sub>50</sub> = 6.73  $\mu$ g/mL). In addition, macrophylline A (**2**), isorhynchophylline (**6**), and **9** showed weak

inhibitory action on KCl-induced contraction. The other compounds were considered inactive, with IC<sub>50</sub> > 30  $\mu$ g/mL.

## Experimental Section

**General Experimental Procedures.** Melting points were obtained on an X-4 micro melting point apparatus. Optical rotations were measured on a Horiba SEPA-300 polarimeter. UV spectra were recorded on a Shimadzu UV-2401PC spectrophotometer. IR spectra were obtained with a Tensor 27 FT-IR spectrometer with KBr pellets. NMR spectra were acquired with Bruker AV-400 and DRX-500 spectrometers with TMS as an internal standard at room temperature ( $\delta$  in ppm, *J* in Hz). ESIMS (including HRESIMS) and FABMS were carried out on API QSTAR Pulsar i and VG Autospec-3000 mass spectrometers, respectively. Silica gel (100–200 and 200–300 mesh), silica gel H (Qingdao Marine Chemical Ltd., China), and Sephadex LH-20 (Amersham Pharmacia Biotech, Sweden) were used for column chromatography (CC). Preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with a Waters XTerra Prep RP-18 column (5  $\mu$ m, 19  $\times$  300 mm). MPLC was performed on a Lisui EZ Purify III System including pump manager P03, detector modules P02, and fraction collector P01 (Shanghai Li Sui Chemical Engineering Co., Ltd., China) and columns packed with LiChroprep RP-18 silica gel (40–63  $\mu$ m, Merck, Darmstadt, Germany). Fractions were monitored by TLC, and spots were visualized using Dragendorff's reagent. Solvents were distilled prior to use.

**Plant Material.** The aerial parts of *U. macrophylla* were collected from Xishuangbanna, Yunnan Province, People's Republic of China, in April 2009, and were identified by Prof. Xiao Cheng of the Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. 200904U01) has been deposited in 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 plant material (28 kg) was extracted with MeOH at room temperature. After evaporation of the solvent in vacuo, the residue was dissolved in 5% HCl, and then partitioned with EtOAc. The acidic solution was basified using 10% ammonia/water to pH 9–10, followed by exhaustive extraction with CHCl<sub>3</sub>, to afford a H<sub>2</sub>O-soluble fraction (A), a CHCl<sub>3</sub>-soluble fraction (B, 95 g), and emulsion layers (C, 500 g). Fraction B was subjected to silica gel CC (100–200 mesh) and eluted with gradient petroleum ether (PE)–acetone (1:0 to 0:1) to give four further fractions, B<sub>1</sub>–B<sub>4</sub>. Fraction B<sub>2</sub> (60 g) was further subjected to MPLC to obtain two subfractions, B<sub>21</sub> (MeOH–H<sub>2</sub>O, 55%) and B<sub>22</sub> (MeOH). Subfraction B<sub>22</sub> was purified by repeated silica gel CC (PE–EtOAc, 7:3, for **4**–**7**; PE–acetone–Et<sub>2</sub>NH, 40:10:1, for **8**) to afford **4** (10 g), **5** (7.5 g), **6** (4 g), **7** (15 g), **8** (6 mg), and a mixture (1.1 g). The mixture was further purified by preparative HPLC to yield **2** (95 mg) and **3** (220 mg) [MeOH–H<sub>2</sub>O (containing 5 mmol/L (NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub>), 45:55 and 40:60, respectively]. Fraction C (500 g) was fractionalized into three subfractions, C<sub>1</sub>, C<sub>2</sub>, and C<sub>3</sub>, by silica gel CC eluted with a solvent system of CHCl<sub>3</sub>–MeOH, and subfractions C<sub>1</sub> and C<sub>2</sub> were alkaloid positive by Dragendorff's reagent. Subfraction C<sub>1</sub> (80 g) was further isolated and purified by repeated silica gel CC and Sephadex LH-20 CC (MeOH–H<sub>2</sub>O, 80%) to afford **9** (150 mg). Subfraction C<sub>2</sub> (30 g) was separated on an RP-18 column to give a fraction (MeOH–H<sub>2</sub>O, 20:80), which was further isolated and purified by repeated silica gel CC (CHCl<sub>3</sub>–MeOH, 7:3) to yield **1** (820 mg).

**Macrophyllionium (1):** light yellow, viscous oil; [ $\alpha$ ]<sub>D</sub><sup>26</sup> +53.5 (*c* 0.30, MeOH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 289 (3.43), 242 (4.12), 209 (4.41) nm; IR (KBr)  $\nu_{\max}$  2964, 1709, 1699, 1632, 1565, 1455, 1246, 1116, 759 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 1; ESIMS (pos.) *m/z* 399 [M + H]<sup>+</sup>, 797 [2M + H]<sup>+</sup>; HRESIMS (pos.) *m/z* 399.2290 (calcd for C<sub>23</sub>H<sub>31</sub>N<sub>2</sub>O<sub>4</sub>, 399.2283).

**Macrophylline A (2):** colorless crystals (MeOH); mp 181–183 °C; [ $\alpha$ ]<sub>D</sub><sup>26</sup> –11.7 (*c* 0.16, MeOH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 285 (3.57), 244 (4.31), 208 (4.55) nm; IR (KBr)  $\nu_{\max}$  3441, 2924, 1708, 1631, 1470, 1240, 1119, 750 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 1; HRESIMS (pos.) *m/z* 441.2393 (calcd for C<sub>25</sub>H<sub>33</sub>N<sub>2</sub>O<sub>5</sub>, 441.2389).

**Macrophylline B (3):** colorless crystals (MeOH); mp 114–115 °C; [ $\alpha$ ]<sub>D</sub><sup>26</sup> –15.0 (*c* 0.13, MeOH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 286 (3.56), 243 (4.36), 208 (4.57) nm; IR (KBr)  $\nu_{\max}$  3439, 2924, 1706, 1630, 1470, 1242, 1119, 756 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 1; HRESIMS (pos.) *m/z* 441.2400 (calcd for C<sub>25</sub>H<sub>33</sub>N<sub>2</sub>O<sub>5</sub>, 441.2389).

**Cytotoxicity Assay.** Five human cancer cell lines, human myeloid leukemia HL-60, lung cancer A549, hepatocellular carcinoma SMMC-7721, breast cancer MCF-7, and colon cancer SW480 cells, were used in the cytotoxic assay. Cells were cultured in RPMI-1640 or in DMEM medium (Hyclone, USA), supplemented with 10% fetal bovine serum (Hyclone, USA) in 5% CO<sub>2</sub> at 37 °C. The cytotoxicity assay was performed according to the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) method in 96-well microplates.<sup>12</sup> Briefly, 100 μL of adherent cells was seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before addition of test compounds, while suspended cells were seeded just before drug addition with initial density of 1 × 10<sup>5</sup> cells/mL. Each tumor cell line was exposed to the test compound at concentrations of 0.0625, 0.32, 1.6, 8, and 40 μM in triplicates for 48 h, with cisplatin (Sigma, USA) as a positive control. After compound treatment, cell viability was detected and a cell growth curve was graphed. IC<sub>50</sub> values were calculated by Reed and Muench's method.<sup>13</sup>

**Vasodilating Activity Assays.** Male Sprague–Dawley rats weighing 280–350 g were used in the test. Rats were sacrificed by stunning and exsanguination. Then, the thoracic aorta was isolated and cut into 3 mm wide ring segments. Each aortic ring was mounted vertically in an organ bath, which contained 15 mL of Krebs-Henseleit (K-H) solution kept at 37 °C, and gassed continuously with 95% O<sub>2</sub> and 5% CO<sub>2</sub>. The rings were attached to isometric transducers connected to a PowerLab data acquisition system (AD Instruments, Australia). A resting tension of 1.0 g was applied to each ring. The rings were allowed to equilibrate for 60 min and washed every 20 min. After an equilibration, contractions were induced by repeated administrations of 60 mmol/L KCl or 1 μmol/L phenylephrine for testing the contractions of the aorta until a stable response was achieved. Then, the sample or control drug was added cumulatively into the organ bath. Consecutive concentrations were given when the response of the previous dose had reached a plateau. The contractile force was determined before and after sample or control drug administration. The results were expressed as a percent inhibition of KCl or phenylephrine-induced contraction by sample or control drug, which was calculated as compared to the maximal contraction with KCl or phenylephrine alone (= 100%). IC<sub>50</sub> is defined as the dose of sample or control drug leading to a 50% relaxation of KCl or phenylephrine-induced contraction.

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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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