

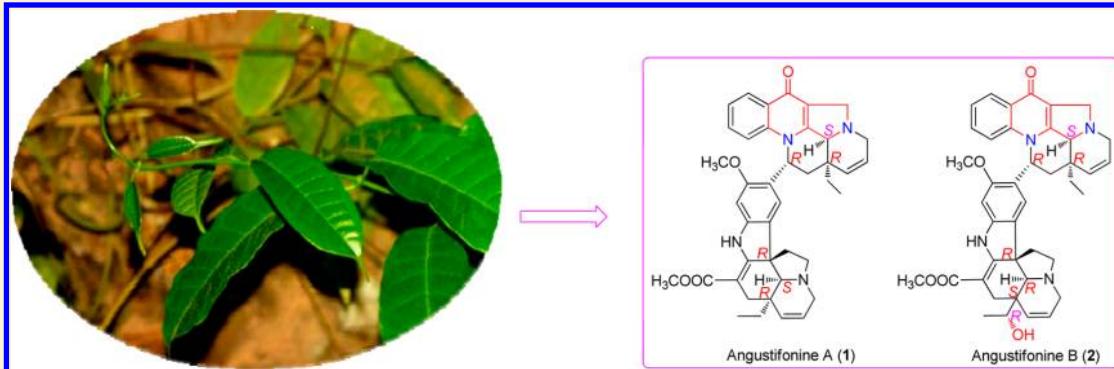
## Angustifonines A and B, Cytotoxic Bisindole Alkaloids from *Bousigonia angustifolia*

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Supporting Information



**ABSTRACT:** Two new bisindole alkaloids, angustifonines A (1) and B (2), comprising the union of a rearranged monoterpenoid quinoline and an aspidospermine alkaloid, as well as 27 known indole alkaloids were isolated from the twigs and leaves of *Bousigonia angustifolia*. Their structures and absolute configurations were elucidated by a combination of MS, NMR, and computational methods. Angustifonines A and B exhibited cytotoxicity against various human cancer cell lines with IC<sub>50</sub> values of 2.71–16.22 μM. A possible biosynthesis pathway toward the new bisindole alkaloids 1 and 2 is presented.

Plants of the Apocynaceae family are notable for producing structurally and biologically interesting indole alkaloids, such as reserpine, vinblastine, and catharanthine.<sup>1</sup> The genus *Bousigonia*, comprising two species, belongs to the Apocynaceae family and is distributed mainly in China, Laos, and Vietnam.<sup>2</sup> Previous chemical investigations of *B. mekongensis* resulted in the isolation of two new eburnamine-aspidospermine-type bisindole alkaloids, mekongenines A and B.<sup>3</sup> In the course of our ongoing work,<sup>4</sup> we have investigated the constituents of *B. angustifolia*. As a result, 29 indole alkaloids including the new angustifonines A (1) and B (2) were isolated from the twigs and leaves. The structures of the new bisindoles comprise the union of a rearranged monoterpenoid quinoline and an aspidospermine alkaloid. This paper describes the isolation and structural elucidation of the new isolates, as well as examines their cytotoxicities against five human tumor cell lines.

### RESULTS AND DISCUSSION

Angustifonine A (1) had a molecular formula of C<sub>41</sub>H<sub>44</sub>N<sub>4</sub>O<sub>4</sub>, as deduced from <sup>13</sup>C NMR data and the HRESIMS ion at *m/z* 657.3433 ([M + H]<sup>+</sup>, calcd 657.3440), with 22 indices of hydrogen deficiency. IR absorption bands at 3379 and 1678 cm<sup>-1</sup> indicated the presence of amino or hydroxy groups and ester carbonyl functionalities, respectively. The UV absorption

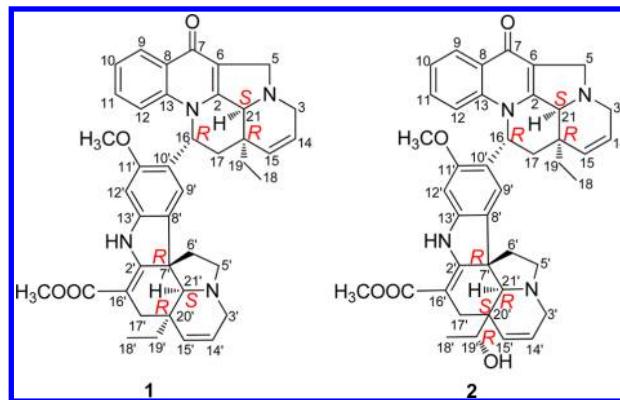


Figure 1. Structures of angustifonines A (1) and B (2).

maxima at 242 and 334 nm suggested the presence of a β-aminoacrylate chromophore and are characteristic of the aspidospermine indole alkaloids.<sup>5</sup> The <sup>13</sup>C NMR and DEPT data (Table 1) revealed 41 carbon resonances, which were classified as four methyl, nine methylene, 13 methine, and 15 quaternary carbons. The connectivities of the A and B moieties

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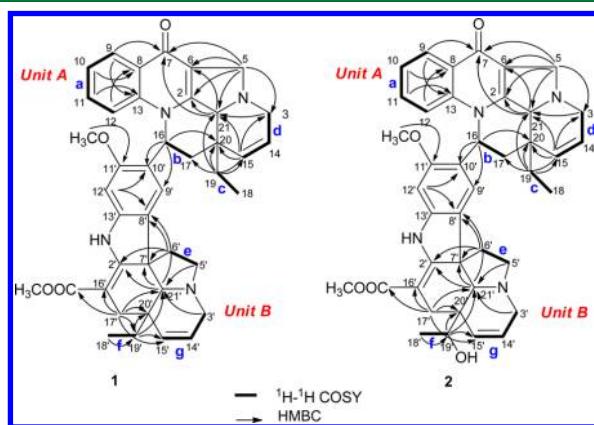
**Table 1.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR Data of Angustifonine A (1) in Methanol- $d_4$

no.	unit A		unit B	
	$\delta_{\text{H}}^a$	$\delta_{\text{C}}^b$	$\delta_{\text{H}}^a$	$\delta_{\text{C}}^b$
2		158.0 s	2'	167.6 s
3a	2.69 d (17.1)	48.8 t	3'a	2.82 d (15.8)
3b	3.33 dd (17.1, 2.9)		3'b	3.21 dd (15.8, 3.9)
5a	3.88 d (13.7)	58.2 t	5'a	2.43 m
5b	4.14 d (13.7)		5'b	2.87 dd (14.8, 7.0)
6		117.9 s	6'a	1.57 dd (11.6, 4.2)
			6'b	1.88 m
7		176.3 s	7'	55.9 s
8		128.7 s	8'	131.6 s
9	8.30 d (8.1)	126.8 d	9'	6.50 s
10	7.29 t (8.1)	125.4 d	10'	
11	7.34 t (8.1)	132.3 d	11'	
12	7.22 d (8.1)	118.6 d	12'	6.76 s
13		141.2 s	13'	
14	5.73 m	127.3 d	14'	5.60 dd (9.7, 3.9)
15	5.76 d (11.6)	130.4 d	15'	5.44 d (9.7)
16	5.78 br d (12.0)	56.2 d	16'	
17a	2.26 m	42.1 t	17'a	2.28 d (15.3)
17b	2.26 m		17'b	2.34 d (15.3)
18	0.86 t (7.4)	8.8 q	18'	0.22 t (7.3)
19a	1.37 q (7.4)	35.5 t	19'a	0.55 q (7.3)
19b	1.53 q (7.4)		19'b	0.71 q (7.3)
20		36.9 s	20'	
21	4.25 s	68.1 d	21'	2.12 s
				70.1 d
				169.9 s
				51.6 q
				56.6 s

<sup>a</sup>Measured at 400 MHz. <sup>b</sup>Measured at 100 MHz.

were established from analyses of the 2D NMR spectra (HSQC,  $^1\text{H}$ - $^1\text{H}$  COSY, and HMBC) as shown in Figure 2.

In unit A, four deshielded protons ( $\delta_{\text{H}}$  7.22 (d,  $J$  = 8.1 Hz, H-12), 7.29 (t,  $J$  = 8.1 Hz, H-10), 7.34 (t,  $J$  = 8.1 Hz, H-11), 8.30 (d,  $J$  = 8.1 Hz, H-9)) suggested the presence of a nonsubstituted quinolone moiety,<sup>6</sup> which was supported by the correlations of H-5b and H-21 with C-2 (158.0, C-6

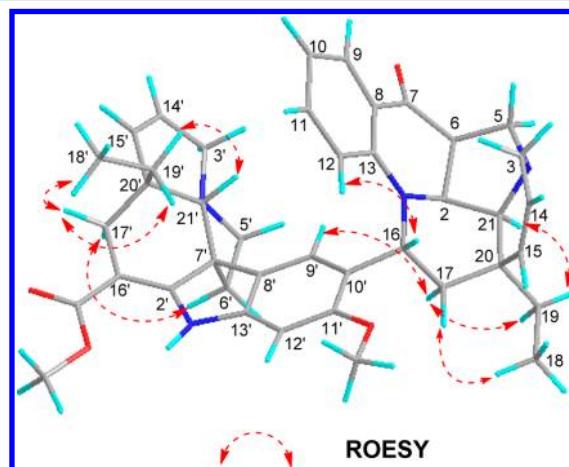


**Figure 2.** Selected 2D NMR correlations for angustifonines A (1) and B (2).

( $\delta_{\text{C}}$  117.9), and C-7 ( $\delta_{\text{C}}$  176.3) and H-9 with C-7. The HMBC cross-peaks of H-5b with C-3 ( $\delta_{\text{C}}$  48.8) and C-21 ( $\delta_{\text{C}}$  68.1) suggested a connection among C-3, C-5 ( $\delta_{\text{C}}$  58.2), and C-21 through a nitrogen atom. The correlations of H-19a with C-15 ( $\delta_{\text{C}}$  130.4), C-20 ( $\delta_{\text{C}}$  36.9), and C-21 and H-15 with C-3, C-17 ( $\delta_{\text{C}}$  42.1), C-20, and C-21 established linkages among C-15, C-17, C-19, and C-21 through C-20, suggesting the connectivity of partial structures b-d. These data indicated that unit A possessed a quinolone moiety, as found in meloyunine C,<sup>7</sup> featuring a 6/6/5/6/6 pentacyclic rearranged ring system.

The remaining chemical shift patterns from the  $^1\text{H}$  and  $^{13}\text{C}$  NMR data in unit B were characteristic of an aspidospermine skeleton,<sup>8</sup> which was confirmed by HSQC,  $^1\text{H}$ - $^1\text{H}$  COSY, and HMBC data. HMBC correlations of H-6'a with C-7' ( $\delta_{\text{C}}$  55.9), C-8' ( $\delta_{\text{C}}$  131.6), and C-21' ( $\delta_{\text{C}}$  70.1), H-6'b with C-2' ( $\delta_{\text{C}}$  167.6), and H-21' with C-8' indicated the connectivity of C-6' and C-21' through C-7' of the indole moiety. The linkage of C-3', C-5', and C-21' to a nitrogen atom was confirmed by HMBC correlations of H-5'b and H-3'b with C-21' ( $\delta_{\text{C}}$  70.1), which revealed the connections of the partial structures e and g. Partial fragments g and f and C-17' were mutually linked through C-20' as determined from the HMBC correlations of H-19'a with C-15' ( $\delta_{\text{C}}$  134.0), C-20' ( $\delta_{\text{C}}$  42.2), and C-21' and H-17'a with C-15', C-19' ( $\delta_{\text{C}}$  27.7), C-20', and C-21'. The ester carbonyl carbon at  $\delta_{\text{C}}$  169.9 was linked to C-16' as deduced from the HMBC correlation of H-2-17' with C-16' ( $\delta_{\text{C}}$  93.8) and the ester carbonyl carbon. Finally, the HMBC correlations of H-16 with C-10' ( $\delta_{\text{C}}$  120.6) and C-9' ( $\delta_{\text{C}}$  119.7) established a linkage between C-16 in unit A and C-10' in unit B. The gross structure of angustifonine A (1) was thus elucidated as indicated in Figure 2.

The relative configuration of 1 was deduced from analysis of ROESY correlations and  $^1\text{H}$ - $^1\text{H}$  coupling constants in combination with molecular modeling studies (Figure 3). The



**Figure 3.** Stereostructure of angustifonine A (1) with selected ROESY correlations.

$\beta$ -orientation of H-16 was assigned on the basis of its coupling constant (br d, 12.0 Hz).<sup>9</sup> The  $\alpha$ -orientation of H-21 and the C-20 ethyl group was established by ROESY correlations of H-17b/H<sub>3</sub>-18 and -19a and H-21/H<sub>2</sub>-19. Similarly, H-21' and the C-20' ethyl also had  $\alpha$ -orientations as determined from the correlations of H-17'b/H<sub>3</sub>-18' and -19'b and H-21'/H<sub>2</sub>-19'. The observed ROESY correlations of H<sub>3</sub>-18/H-21 and H<sub>3</sub>-18'/H-21' indicated that several conformational interconversions occur in 1 as reflected by a variation in the dihedral angle

between C-21-C-20-C-19-C-18 and C-21'-C-20'-C-19'-C-18' (see Supporting Information for a detailed conformational analysis).

Angustifonine B (**2**) was obtained as a yellowish, amorphous solid. The IR and UV absorption data generally matched those of **1**, indicating that **2** also possesses the  $\beta$ -anilinoacrylate chromophore found in the aspidospermine indole alkaloids.<sup>5</sup> The molecular formula of **2** was established as  $C_{41}H_{44}N_4O_5$  by the  $^{13}\text{C}$  NMR data and the HRESIMS ion at  $m/z$  673.3392 ( $[M + H]^+$ ; calcd 673.3389), which is 16 mass units greater than that of **1**. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR data (Table 2) revealed that **2**

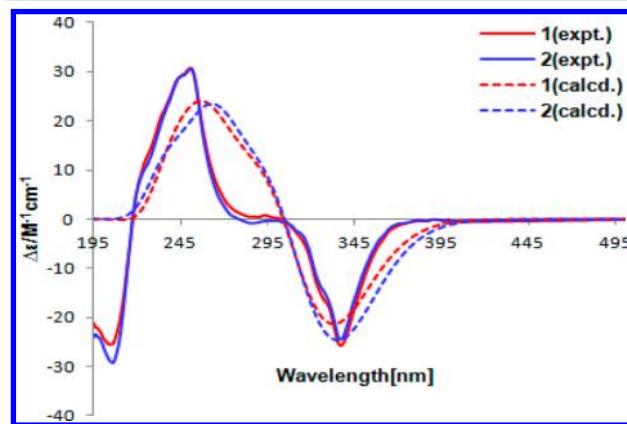
**Table 2.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR Data of Angustifonine B (**2**) in Methanol- $d_4$

no.	unit A		unit B	
	$\delta_{\text{H}}^a$	$\delta_{\text{C}}^b$	$\delta_{\text{H}}^a$	$\delta_{\text{C}}^b$
2		158.2 s	2'	167.4 s
3a	2.71 d (17.3)	48.9 t	3'a	2.83 d (15.7)
3b	3.38 dd (17.3, 2.9)		3'b	3.27 dd (15.7, 4.2)
5a	3.90 d (14.0)	58.3 t	5'a	2.42 m
5b	4.17 d (14.0)		5'b	2.90 overlap
6		118.0 s	6'a	1.62 overlap
			6'b	1.96 dd (11.4, 6.6)
7		176.3 s	7'	55.6 s
8		128.7 s	8'	131.0 s
9	8.33 dd (8.1, 1.4)	126.7 d	9'	6.48 s
10	7.32 t (8.1, 1.4)	125.4 d	10'	120.8 s
11	7.40 t (8.1, 1.4)	132.3 d	11'	157.7 s
12	7.21 d (8.1)	118.6 d	12'	6.81 s
13		141.2 s	13'	146.4 s
14	5.76 dd (3.6, 2.8)	127.6 d	14'	5.73 dd (10.0, 3.6)
15	5.80 overlap	130.0 d	15'	5.44 d (10.0)
16	5.79 dd (12.0, 5.4)	55.8 d	16'	130.5 d
17a	2.30 dd (14.7, 5.4)	42.2 t	17'a	2.26 d (15.6)
17b	2.35 overlap		17'b	2.87 d (15.6)
18	0.91 t (7.4)	8.7 q	18'	0.20 d (7.5)
19a	1.44 q (7.4)	35.5 t	19'	3.00 q (7.5)
19b	1.62 q (7.4)			66.2 d
20		36.8 s	20'	47.7 s
21	4.30 s	68.2 d	21'	2.15 s
			COOCH <sub>3</sub> '	67.8 d
			COOCH <sub>3</sub> '	170.0 s
			OCH <sub>3</sub> '	51.5 q
				3.95 s
				56.6 q

<sup>a</sup>Measured at 400 MHz. <sup>b</sup>Measured at 100 MHz.

has 41 carbon atoms, which were analogous to those of **1** except for the chemical shifts attributed to the C-20 ethyl group in unit B. Analysis of the spectroscopic data, particularly the HMBC correlations of H<sub>3</sub>-18' with C-19' ( $\delta_{\text{C}}$  66.2), suggested a hydroxy group located at C-19' in **2**. The ROESY correlations of **2**, similar to those of **1**, indicated that these compounds had the same relative configurations, except for C-19'. However, the absolute configuration at C-19' of **2** was confirmed to be 19'R according to NMR data that paralleled those of (19R)-hydroxytabersonine.<sup>4c,10</sup>

The absolute configurations at C-16, C-20, C-21, C-7', C-20', and C-21' of **1** and **2** were resolved by the calculated results from electronic circular dichroism (ECD) spectra using time-dependent density functional theory (TDDFT) performed with the Gaussian09 program.<sup>11</sup> The overall calculated ECD spectra of the optimized geometries of **1** and **2** and their experimentally recorded ECD spectra match closely, as shown in Figure 4 (Supporting Information). Therefore, the absolute configurations of **1** and **2** may be defined unequivocally as shown in Figure 1.



**Figure 4.** Comparison of the experimental ECD (solid line) and calculated ECD (dotted line) spectra of angustifonines A (**1**) and B (**2**).

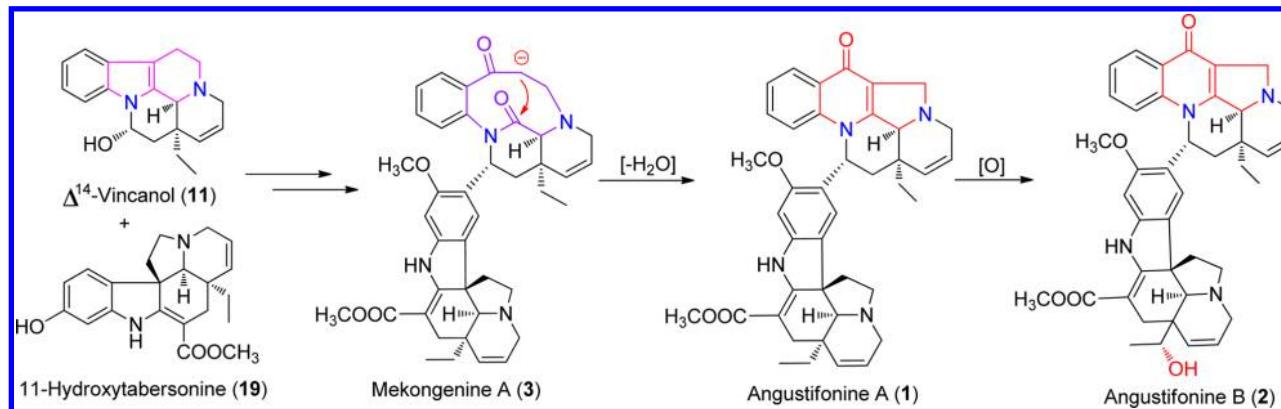
Angustifonines A (**1**) and B (**2**) are new bisindole alkaloids constituting rearranged monoterpenoid quinoline alkaloid and aspidospermine alkaloid moieties.<sup>12</sup> A plausible biogenetic pathway for angustifonines A (**1**) and B (**2**) is shown in Scheme 1. We postulate that **1** and **2** might originate from  $\Delta^{14}$ -vincanol (**11**)<sup>13</sup> and 11-hydroxytabersonine (**19**)<sup>14</sup> through the key intermediate mekongenine A (**3**).<sup>3</sup> Subsequent, intramolecular aldol condensation and oxidation of **3** could generate the two unprecedented alkaloids **1** and **2**.

Angustifonines A (**1**) and B (**2**) were evaluated for their cytotoxicity against five human cancer cell lines, HL-60, SMMC-7721, A-549, MCF-7, and SW480, by the MTT method. Angustifonine A (**1**) showed significant cell growth inhibitory activity against all cell lines with IC<sub>50</sub> values of  $3.03 \pm 0.04$ ,  $10.55 \pm 0.32$ ,  $7.23 \pm 0.23$ ,  $3.61 \pm 0.08$ , and  $2.71 \pm 0.06 \mu\text{M}$ , respectively. Angustifonine B (**2**) showed a moderate cell growth inhibitory activity against all cancer cell lines with IC<sub>50</sub> values of  $9.60 \pm 0.10$ ,  $15.28 \pm 0.65$ ,  $16.22 \pm 0.28$ ,  $12.08 \pm 0.36$ , and  $13.88 \pm 0.28 \mu\text{M}$ , respectively, while the cisplatin control showed cytotoxicity with IC<sub>50</sub> values of  $1.56 \pm 0.02$ ,  $10.82 \pm 0.22$ ,  $14.26 \pm 0.18$ ,  $19.28 \pm 0.26$ , and  $25.69 \pm 0.88 \mu\text{M}$ , respectively. The paclitaxel control exhibited a powerful inhibitory activity against all examined tumor cells (IC<sub>50</sub> <  $0.008 \mu\text{M}$ ).

## EXPERIMENTAL SECTION

**General Experimental Procedures.** Optical rotations were measured on a Perkin-Elmer 241 polarimeter. ECD spectra were recorded with an Applied Photophysics Chirascan spectrometer. UV spectra were recorded on a UV 210A spectrophotometer. IR spectra were recorded on a Bio-Rad FTS-135 spectrophotometer (KBr). HRESIMS data were measured on a VG Auto Spec-3000 spectrometer. ESIMS data were obtained on a Finnigan MAT 90

Scheme 1. Plausible Biosynthesis Pathway for Angustifonines A (1) and B (2)



spectrometer. NMR spectra were recorded on a Bruker AM-400 NMR spectrometer with TMS as internal standard.

**Plant Material.** The twigs and leaves of *B. angustifolia* were collected from Mengla County, Yunnan Province, P. R. China, and identified by Mr. Jing-Yun Cui, Xishuangbanna Tropical Plant Garden. A voucher specimen (No. CUI20100418) was deposited at Kunming Institute of Botany, Chinese Academy of Sciences.

**Extraction and Isolation.** The dried twigs and leaves of *B. angustifolia* (8 kg) were extracted with MeOH, and the pH of the crude extract was adjusted with saturated tartaric acid to 2. The acidic mixture was defatted with petroleum ether (PE) and subsequently extracted with CHCl<sub>3</sub>. The aqueous phase was adjusted to pH ~10 with saturated Na<sub>2</sub>CO<sub>3</sub> and extracted with CHCl<sub>3</sub> to obtain the crude alkaloids. The crude alkaloids (50 g) were separated on a silica gel column (300 g, 200–300 mesh; CHCl<sub>3</sub>/MeOH, 1:0 → 0:1), yielding five major fractions (Fr 1–5). Fraction 1 (8.8 g) was chromatographed over a series of silica gel columns (200 g, 200–300 mesh; CHCl<sub>3</sub>/acetone, 1:0 → 6:4; 100 g, 300–400 mesh; CHCl<sub>3</sub>/MeOH, 1:0 → 9:1; 50 g, 300–400 mesh; CHCl<sub>3</sub>/acetone, 1:0 → 5:5) to afford melodinine J (5, 10 mg),<sup>15</sup> eburnamenine (6, 12 mg),<sup>16</sup> and O-methylvincanol (8, 9 mg).<sup>17</sup> Fraction 2 (10.2 g) was further chromatographed over a reversed-phase C<sub>18</sub> silica gel medium-pressure column (400 g, 40–63  $\mu$ m; MeOH/H<sub>2</sub>O, 1:1 → 1:0) to give four fractions (Fr 2A–2D). Fraction 2A (2.2 g) was chromatographed over a series of silica gel columns (120 g, 200–300 mesh; CHCl<sub>3</sub>/acetone, 1:0 → 5:5; 80 g, 200–300 mesh; CHCl<sub>3</sub>/MeOH, 9:1) to afford 16-vincamine (9, 20 mg),<sup>17</sup>  $\Delta^{14}$ -vincanol (11, 28 mg),<sup>13</sup> and  $\Delta^{14}$ -vincamone (12, 14 mg).<sup>13</sup> Fraction 2B (1.8 g) was subjected to a Sephadex LH-20 column (i.d. 2.8 × 180 cm, 40–70  $\mu$ m) eluted with MeOH, and the fractions were further separated by semipreparative HPLC (Waters XBridge C<sub>18</sub>, i.d. 250 × 10 mm, 5  $\mu$ m, 85% MeOH with 0.1% v/v diethylamine, 3.0 mL/min, t<sub>R</sub> 17.3 and 22.2 min, UV 254 nm) to give angustifonines A (1, 48 mg) and B (2, 36 mg). Fraction 2C (2.4 g) was separated on a silica gel column (150 g, 300–400 mesh; PE/acetone, 3:1), yielding three fractions (Fr 2C1–2C3). Fraction 2C1 (400 mg) was purified using a Sephadex LH-20 column (i.d. 1.5 × 150 cm, 40–70  $\mu$ m) eluted with MeOH, followed by semipreparative HPLC (Waters XBridge C<sub>18</sub>, i.d. 250 × 10 mm, 5  $\mu$ m, 85% MeOH with 0.1% v/v diethylamine, 3.0 mL/min, t<sub>R</sub> 9.9, 16.9, and 26.8 min, UV 254 nm) to afford mekongenine A (3, 12 mg),<sup>3</sup> mekongenine B (4, 38 mg),<sup>3</sup> and vincanol (7, 46 mg).<sup>18</sup>  $\Delta^{14}$ -Vincamenine (10, 8 mg),<sup>13</sup> 14,15-dehydroepivincine (14, 16 mg),<sup>19</sup> and vincadiformine (18, 66 mg)<sup>20</sup> were separated from fraction 2C2 (428 mg) by semipreparative HPLC (Waters XBridge C<sub>18</sub>, i.d. 250 × 10 mm, 5  $\mu$ m, 70% MeCN/H<sub>2</sub>O with 0.1% v/v diethylamine, 3.5 mL/min, t<sub>R</sub> 13.1, 19.5, and 28.3 min, UV 254 nm). O-Methyl- $\Delta^{14}$ -vincanol (13, 15 mg),<sup>21</sup> 11-methoxytabersonine (20, 26 mg),<sup>22</sup> and meloscine (29, 28 mg)<sup>23</sup> were obtained from fraction 2C3 (258 mg) by semipreparative HPLC (Waters XBridge C<sub>18</sub>, i.d. 250 × 10 mm, 5  $\mu$ m, 70% MeOH/H<sub>2</sub>O with 0.1% v/v diethylamine, 3.5 mL/min, t<sub>R</sub> 8.0, 16.2, and 31.5 min, UV 254 nm). Fraction 3 (8.6 g) was further chromatographed over a reversed-phase C<sub>18</sub> silica gel medium-pressure

column (400 g, 40–63  $\mu$ m; MeOH/H<sub>2</sub>O, 1:1 → 1:0) to give four fractions (Fr 3A–3D). Fraction 3A (360 mg) was separated by semipreparative HPLC (Waters XBridge C<sub>18</sub>, i.d. 250 × 10 mm, 5  $\mu$ m, 45% MeOH/H<sub>2</sub>O, 3.0 mL/min, t<sub>R</sub> 9.2 and 19.6 min, UV 254 nm) to give 19-hydrovincanol (16, 12 mg)<sup>24</sup> and tabersonine (17, 10 mg).<sup>25</sup> 11-Hydroxytabersonine (19, 11 mg)<sup>14</sup> and (19R)-acetoxy-11-hydroxytabersonine (22, 16 mg)<sup>26</sup> were obtained from fraction 3B (540 mg) by semipreparative HPLC (Waters XBridge C<sub>18</sub>, i.d. 250 × 10 mm, 5  $\mu$ m, 50% MeOH/H<sub>2</sub>O, 3.0 mL/min, t<sub>R</sub> 17.3 and 19.3 min, UV 254 nm). Fraction 3C (780 mg) was purified using a Sephadex LH-20 column (i.d. 2.8 × 180 cm, 40–70  $\mu$ m) eluted with MeOH, followed by semipreparative HPLC (Waters XBridge C<sub>18</sub>, i.d. 250 × 10 mm, 5  $\mu$ m, 65% MeOH/H<sub>2</sub>O, 4.0 mL/min, t<sub>R</sub> 16.0, 20.8, and 27.7 min, UV 254 nm) to afford (19R)-hydroxy-11-methoxytabersonine (21, 22 mg),<sup>27</sup> vindolinine (25, 48 mg),<sup>28</sup> and scandine (26, 38 mg),<sup>29</sup> respectively. Fraction 4 (7.8 g) was further chromatographed over a reversed-phase C<sub>18</sub> silica gel medium-pressure column (400 g, 40–63  $\mu$ m; MeOH/H<sub>2</sub>O, 1:1 → 1:0) to give four fractions (Fr 4A–4D). Fraction 4A (420 mg) was separated by semipreparative HPLC (Waters XBridge C<sub>18</sub>, i.d. 250 × 10 mm, 5  $\mu$ m, 30% MeOH/H<sub>2</sub>O, 3.5 mL/min, t<sub>R</sub> 14.3 and 17.8 min, UV 254 nm) to give 14,15 $\alpha$ -epoxy-11-hydroxytabersonine (23, 23 mg)<sup>30</sup> and 14,15 $\alpha$ -epoxy-11-methoxytabersonine (24, 18 mg).<sup>31</sup> 10-Hydroxscandine (27, 280 mg)<sup>32</sup> was crystallized in acetone from fraction 4B (1.2 g). Fraction 4C (550 mg) was purified using a Sephadex LH-20 column (i.d. 2.0 × 160 cm, 40–70  $\mu$ m) eluted with MeOH, followed by semipreparative HPLC (Waters XBridge C<sub>18</sub>, i.d. 250 × 10 mm, 5  $\mu$ m, 35% MeOH/H<sub>2</sub>O, 3.0 mL/min, t<sub>R</sub> 11.0 and 18.8 min, UV 254 nm) to afford 14,15-dehydrovincine (15, 18 mg)<sup>33</sup> and scandine-N<sup>4</sup>-oxide (28, 26 mg).<sup>34</sup>

**Angustifoline A (1):** yellowish, amorphous solid;  $[\alpha]^{19}_{\text{D}} -132$  (*c* 0.2, CH<sub>3</sub>OH); IR (KBr)  $\nu_{\text{max}}$  3379, 2924, 1678, 1618, 1460  $\text{cm}^{-1}$ ; UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 207 (4.54), 242 (4.52), 334 (4.46) nm; ECD (0.00019 M, MeOH)  $\lambda_{\text{max}}$  ( $\Delta\epsilon$ ) 251 (+30.9), 337 (-25.6) nm; <sup>1</sup>H and <sup>13</sup>C NMR data (Table 1); ESIMS *m/z* 657 [M + H]<sup>+</sup>; HRESIMS *m/z* 657.3433 (M + H; calcd for C<sub>41</sub>H<sub>45</sub>N<sub>4</sub>O<sub>4</sub>, 657.3440).

**Angustifoline B (2):** yellowish, amorphous solid;  $[\alpha]^{19}_{\text{D}} -118$  (*c* 0.3, CH<sub>3</sub>OH); IR (KBr)  $\nu_{\text{max}}$  3424, 2925, 1671, 1619, 1461  $\text{cm}^{-1}$ ; UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 211 (4.58), 243 (4.55), 333 (4.45) nm; ECD (0.00016 M, MeOH)  $\lambda_{\text{max}}$  ( $\Delta\epsilon$ ) 251 (+30.5), 337 (-24.4) nm; <sup>1</sup>H and <sup>13</sup>C NMR data (Table 1); ESIMS *m/z* 673 [M + H]<sup>+</sup>; HRESIMS *m/z* 673.3392 (M + H; calcd for C<sub>41</sub>H<sub>45</sub>N<sub>4</sub>O<sub>5</sub>, 673.3389).

**Cytotoxic Bioassays.** The following human tumor cell lines were used: HL-60 human myeloid leukemia, SMMC-7721 human hepatocarcinoma, A-549 lung cancer, MCF-7 breast cancer, and SW480 human pancreatic carcinoma (all cancer cell lines were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China)). All cells were cultured in RPMI-1640 or DMEM medium (Hyclone, Logan, UT, USA), supplemented with 10% fetal bovine serum (Hyclone, Logan, UT, USA), in 5% CO<sub>2</sub> at 37 °C. The cytotoxicity assay was performed using the MTT method in 96-well microplates.<sup>35</sup> Briefly, 100  $\mu$ L of adherent cells was seeded into each well of a 96-well cell culture plate and allowed to adhere for 12 h

before introduction of the drug, while suspended cells were seeded just before introduction of the drug, each with an initial density of  $1 \times 10^5$  cells/mL in 100  $\mu\text{L}$  of medium. Each tumor cell line was exposed to the tested compound at concentrations of 0.0625, 0.32, 1.6, 8, and 40  $\mu\text{M}$  in triplicate for 48 h, with cisplatin and paclitaxel (Sigma, St. Louis, MO, USA) as positive controls. After incubation, MTT (100  $\mu\text{g}$ ) was added to each well, and the incubation was allowed to continue for 4 h at 37 °C. The cells were next lysed with 100  $\mu\text{L}$  of 20% SDS/50% DMF after removal of 100  $\mu\text{L}$  of medium. The optical density of the lysate was measured at 595 nm in a 96-well microtiter plate reader (Bio-Rad 680). The IC<sub>50</sub> value of each compound was calculated by Reed and Muench's method.<sup>36</sup>

## ■ ASSOCIATED CONTENT

### Supporting Information

This material (detailed computational methods, 1D and 2D NMR, ESIMS, HRESIMS, UV, IR, and ECD spectra of angustifonines A (1) and B (2)) is available free of charge via the Internet at <http://pubs.acs.org>.

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### Notes

The authors declare no competing financial interest.

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