

## Novel *seco*-phenanthroquinolizidine alkaloids from Indonesian *Boehmeria virgata*

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### ABSTRACT

Two novel *seco*-phenanthroquinolizidine alkaloids, (–)-(14aR,15R)-hydroxyjulandine (**1**) and (+)-(S)-3-O-demethyljulandine (**2**), together with eleven known compounds (**3**–**13**) were isolated from the leaves of Indonesian *Boehmeria virgata*. The structures of these compounds were assigned based on the interpretation of 1D/2D NMR and HRMS data. The absolute configurations of *seco*-phenanthroquinolizidines **1** and **2** were determined from their ECD spectra by comparison with the known phenanthroquinolizidine **3**. The isolated *seco*-phenanthroquinolizidines **1**–**3** were evaluated for antiproliferative activity against five tumor cell lines. Compound **3** exhibited potent cytotoxic activity, while **1** and **2** were not active against all tested cell lines.

### 1. Introduction

Phenanthroquinolizidine compounds, a subtype of quinolizidine alkaloids, are a small group of herbal metabolites found in several plant families, such as Urticaceae (Hart et al., 1968); (Cai et al., 2006); (Hoffmann et al., 1978); (Krpmotic et al., 1972); (Al-Shamma et al., 1982); (Doan Thi Thuy et al., 2019); (Luo et al., 2003), Vitaceae (Saifah et al., 1983), Lauraceae (Hoffmann et al., 1978), and Acanthaceae (Abdel-Şattar et al., 2020). An overview of the literature (Jia et al., 2020) and the latest report by Abdel-Şattar et al. (2020) revealed no more than ten naturally occurring phenanthroquinolizidine alkaloids with promising biological properties, including cytotoxic (Hoffmann et al., 1978); (Doan Thi Thuy et al., 2019); (Luo et al., 2003), antiviral (Krpmotic et al., 1972), antimicrobial (Al-Shamma et al., 1982), and antimalarial (Abdel-Şattar et al., 2020) have been reported.

*Boehmeria virgata* (G. Forst.) Guill. (Urticaceae), locally known as “Parangromang”, is a medicinal plant traditionally used by the Makassar ethnic group in South Sulawesi, Indonesia to treat cancer diseases (Manggau et al., 2018); (Wardihan et al., 2013). Previous studies reported that an ethanolic extract of *B. virgata* leaves exhibited cytotoxic activity against HeLa, WiDr, and T4T7D cell lines (Wardihan et al., 2013) and alkaloid compounds were predicted as the pharmacologically

active substances (Manggau et al., 2018). Although no details on isolated active constituents of this plant were subsequently reported, alkaloid components especially phenanthroquinolizidine analogues have been isolated from related species including *B. pinnosa* Nakai & Satake ex Oka (Cai et al., 2006), *B. cylindrica* (L.) Sw. (Al-Shamma et al., 1982), *B. siamensis* Craib (Luo et al., 2003), *B. caudata* Sw. (Hoffmann et al., 1978), and *B. rugolosa* Wedd. (Semwal et al., 2009). Furthermore, triterpenoid compounds, such as boehmerone and boehmerol (Oyarzún et al., 1986), as well as a lignan, boehmeran (Takemoto et al., 1975), were obtained from *B. excelsa* (Bertero ex Steud.) Wedd. and *B. tricuspis* (Hance) Makino, respectively. The isolations of flavonoid glycosides (Semwal et al., 2009) and other compounds (Doan Thi Thuy et al., 2018) have also been reported. In our continuing phytochemical studies of Indonesian plants collected in South Sulawesi (Yamashita et al., 2020); (Rahim et al., 2020); (Rahim et al., 2018), we herein report the isolation and structure elucidation of two novel *seco*-phenanthroquinolizidine alkaloids **1** and **2**, along with eleven known compounds (**3**–**13**) from the Indonesian plant *B. virgata*, as well as the antiproliferative activity of the isolated *seco*-phenanthroquinolizidines **1**–**3**.

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## 2. Results and discussion

Leaves of *B. virgata* were extracted with methanol, and the extract was further partitioned and fractionated using various chromatographic techniques to give two new *seco*-phenanthroquinolizidine alkaloids, (–)-(14a*R*,15*R*)-hydroxyjulandine (**1**) and (+)-(S)-3-*O*-desmethyljulandine (**2**) (Fig. 1). Eleven known compounds (Figure S1, Supporting Information), including (–)-(*R*)-julandine (**3**) (Suzuki et al., 1995), (–)-cryptopleurine (**4**) (Kim et al., 2004), ixerol B (**5**) (Hu and Wang, 2013), 4-oxo- $\beta$ -ionol (**6**) (Hartman et al., 1988), (3*R*,6*R*,7*E*)-3-hydroxy-4,7-megastigmadien-9-one (**7**), (–)-3-hydroxy- $\beta$ -ionone (**8**) (Machida and Kikuchi, 1996), (–)-dehydrovomifoliol (**9**) (Mori, 1974), (–)-3-oxo- $\alpha$ -ionone (**10**) (Ito et al., 1997), methyl trans-ferulate (**11**) (Song et al., 2008), *p*-coumaric acid methyl ester (**12**) (Seifert and Unger, 1994), and (S)-5-methoxyprolridin-2-one (**13**) (Song et al., 2008), were also isolated and elucidated by comparison of their spectroscopic data with those in the literature.

Compound **1** was isolated as a pale-yellow oil and showed a positive response with Dragendorff reagent. The molecular formula, C<sub>24</sub>H<sub>30</sub>NO<sub>4</sub>, was determined based on a protonated molecular ion peak at *m/z* 396.2176 [M+H]<sup>+</sup>, (calcd 396.2175) by HRMS. The <sup>1</sup>H NMR data of **1** (Table 1) indicated the presence of three methoxy groups at  $\delta_{\text{H}}$  3.58 (3H, s, OCH<sub>3</sub>-2), 3.71 (3H, s, OCH<sub>3</sub>-6), and 3.81 (3H, s, OCH<sub>3</sub>-3). A 1,2,4-trisubstituted phenyl ring (ring-A) was postulated from proton signals at  $\delta_{\text{H}}$  6.53 (1H, d, *J* = 1.7 Hz, H-1), 6.70 (1H, dd, *J* = 1.7, 8.3 Hz, H-4a), and 6.72 (1H, d, *J* = 8.3 Hz, H-4), while a 1,4-disubstituted benzene ring (ring-B) was proposed from proton signals at  $\delta_{\text{H}}$  6.67 (2H, dd, *J* = 2.1, 8.6 Hz, H-5 and H-7) and 6.92 (2H, dd, *J* = 2.1, 8.6 Hz, H-4a and H-8). A signal at  $\delta_{\text{H}}$  4.40 (1H, dd, *J* = 1.7, 7.6 Hz, H-15) was characterized as a hydroxylated methine. The <sup>13</sup>C NMR data (Table 1) showed 24 carbon signals (two overlapped carbon signals at  $\delta_{\text{C}}$  129.3 and 113.3), which corresponded to three methoxy carbons ( $\delta_{\text{C}}$  55.1, 55.6, and 55.7), five methylene carbons ( $\delta_{\text{C}}$  59.7, 55.4, 30.6, 25.3, and 23.9), nine methine carbons including seven aromatic ( $\delta_{\text{C}}$  129.3, 129.3, 121.4, 113.3, 113.3, 113.5, and 110.8) and two carbons bearing oxygen and nitrogen ( $\delta_{\text{C}}$  73.8 and 64.4, respectively), as well as seven non-hydrogenated olefinic and aromatic carbons ( $\delta_{\text{C}}$  158.1, 148.4, 147.7, 134.5, 133.8, 132.5, and 130.1) based on the HMQC and DEPT spectra. In the HMBC spectrum of **1** (Fig. 2), the correlations of H-1 with C-3/C-4a/C-15a and H-4 with C-2/C-15b characterized a 1,2,4-trisubstituted phenyl ring-A linked to a quinolizidine moiety at C-15a, while the correlations of H-4b/H-8 with C-6/C-8b and H5/H7 indicated a C-8a 1,4-disubstituted benzene ring-B connected at C-8b. Based on these data and the presence of COSY correlations encompassing H-11 to H-15, compound **1** is likely a *seco*-phenanthroquinolizidine alkaloid. When the NMR spectroscopic data were compared with those of the known phenanthroquinolizidine, (–)-14a*R*-julandine (**3**) (Suzuki et al., 1995), differences were observed at only the C-14, C-14a, and C-15 positions and an additional methine signal at  $\delta_{\text{H}}$  4.40 (1H, dd, *J* = 1.7, 7.6 Hz, H-15) was present. These findings together

**Table 1**  
<sup>1</sup>H and <sup>13</sup>C NMR Spectroscopic Data of **1** and **2**.

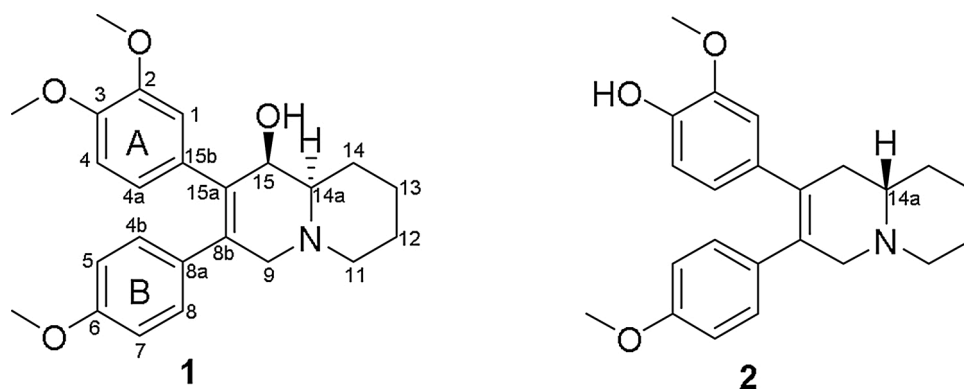
Position	1		2	
	$\delta_{\text{H}}$ (J in Hz) <sup>a</sup>	$\delta_{\text{C}}$ <sup>a</sup>	$\delta_{\text{H}}$ (J in Hz) <sup>a</sup>	$\delta_{\text{C}}$ <sup>b</sup>
1	6.53 d (1.7)	113.5	6.41 d (1.7)	112.4
2		148.4		145.6
3		147.7		143.8
4	6.72 d (8.3)	110.8	6.72 d (8.2)	113.7
4a	6.70 dd (1.7, 8.3)	121.4	6.61 dd (1.7, 8.2)	121.3
4b	6.92 dd (2.1, 8.6)	129.3	6.97 dd (1.8, 8.7)	130.2
5	6.67 dd (2.1, 8.6)	113.3	6.69 dd (1.8, 8.7)	113.4
6		158.1		157.9
7	6.67 dd (2.1, 8.6)	113.3	6.69 dd (1.8, 8.7)	113.4
8	6.92 dd (2.1, 8.6)	129.3	6.97 dd (1.8, 8.7)	130.2
8a		132.5		133.3
8b		133.8		131.4
9	a. 3.29 d (16.5) b. 3.37 d (16.5)	59.7	a. 3.02 d (16.5) b. 3.56 d (16.5)	60.4
11	a. 2.17 m b. 3.01 m	55.4	a. 2.09 m b. 3.06 m	55.6
12	1.69 m	25.3	1.71 m	25.9
13	a. 1.37 overlap b. 1.86 m	23.9	a. 1.32 m b. 1.79 m	24.4
14	a. 1.36 overlap b. 2.31 m	30.6	a. 1.31 m b. 1.83 m	33.3
14a	2.23 m	64.4	2.22 m	57.9
15	4.40 dd (1.7, 7.6)	73.8	a. 2.31 m b. 2.51 m	39.6
15a		134.5		131.3
15b		130.1		133.9
OCH <sub>3</sub> -2	3.58 s	55.6	3.53 s	55.6
OCH <sub>3</sub> -3	3.81 s	55.7		
OCH <sub>3</sub> -6	3.71 s	55.1	3.72	55.2

<sup>a</sup><sup>1</sup>H NMR: 600 MHz in CDCl<sub>3</sub>, <sup>13</sup>C NMR: 100 MHz in CDCl<sub>3</sub>.

<sup>b</sup><sup>13</sup>C NMR: 150 MHz in CDCl<sub>3</sub>.

with the fact that the molecular weight of **1** is 16 amu more than that of **3** suggested that **1** is a hydroxylated julandine analogue. A NOESY correlation between H-14a and H-9 $\alpha$  indicated an  $\alpha$  orientation for H-14a (Fig. 3). Also, correlations of H-15 with H-14 $\alpha$ , H-14a, and H-1 indicated OH-15 and H-14a *trans* to each other and in axial positions. These NOESY correlations also supported the connection of ring-A to C-15a. Compound **1** exhibited a negative optical rotation, [ $\alpha$ ]<sub>D</sub><sup>23</sup> –137.6 (CHCl<sub>3</sub>, *c* 0.14), and a negative Cotton effect at 284 nm in the CD spectrum, which were the same as those of (–)-*R*-julandine (**3**, [ $\alpha$ ]<sub>D</sub><sup>23</sup> –57.9 (CHCl<sub>3</sub>, *c* 0.16)) isolated in this study (Fig. 4). The data indicated that **1** and **3** have the same *R*-orientation at C-14a. Therefore, compound **1** was determined to be (–)-(14a*R*,15*R*)-hydroxyjulandine.

Compound **2** was obtained as a pale-yellow oil. In the HRMS of **2**, a protonated molecular ion peak at *m/z* 366.2073 [M+H]<sup>+</sup> (calcd 366.2069) indicated a molecular formula of C<sub>22</sub>H<sub>28</sub>NO<sub>3</sub>, which is 30 amu less than that of **1** indicating loss of OCH<sub>2</sub>. The <sup>1</sup>H NMR data of **2** were like those of **1**, except for the absence of the hydroxylated methine



**Fig. 1.** Structures of compounds **1** and **2** isolated from *B. virgata* leaves.

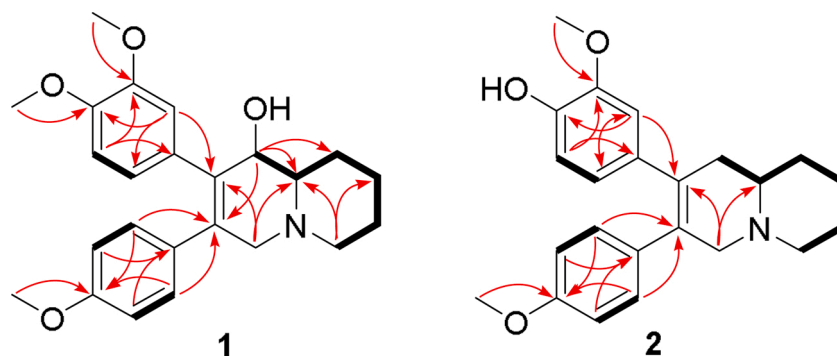


Fig. 2.  $^1\text{H}$ - $^1\text{H}$  COSY (bold lines) and selected key HMBC (arrows) correlations of compounds 1 and 2.

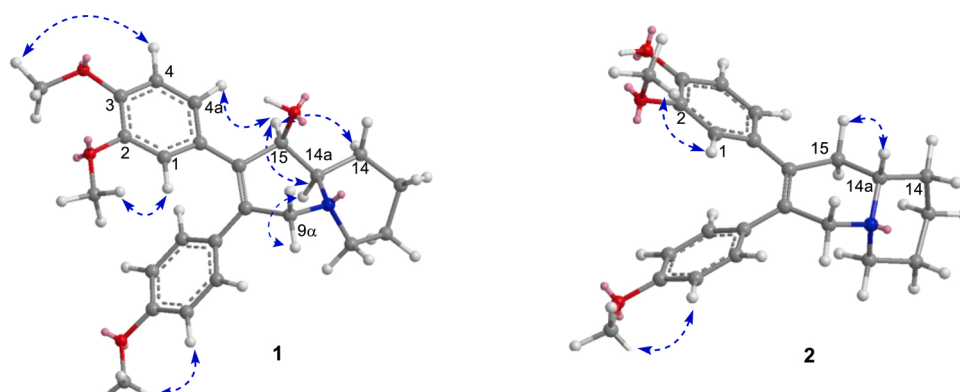


Fig. 3. Key NOESY correlations (dotted arrows) of compounds 1 and 2.

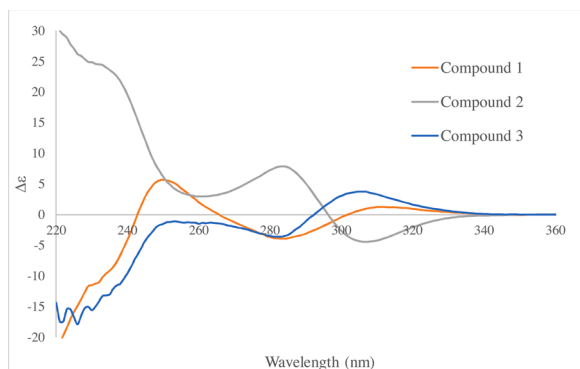


Fig. 4. Experimental ECD spectra of compounds 1–3 in MeOH.

signal ( $\delta_{\text{H}}$  4.40) found in 1 and the presence of two, rather than three, methoxy signals in 2. The differences in the  $^{13}\text{C}$  NMR data (Table 1) of 1 and 2 at C-2, C-3, and C-4 indicated that a methoxy group on the 1,2,4-trisubstituted phenyl ring-A of 1 was changed to a hydroxy group in 2. The HMBC correlations of the methoxy signals at  $\delta_{\text{H}}$  3.52 (3H, s) with  $\delta_{\text{C}}$  145.6 (C-2),  $\delta_{\text{H}}$  6.41 (d,  $J = 1.7$  Hz, H-1) with  $\delta_{\text{C}}$  143.8 (C-3)/ $\delta_{\text{C}}$  121.3 (C-4a),  $\delta_{\text{H}}$  6.72 (d,  $J = 8.2$  Hz, H-4) with  $\delta_{\text{C}}$  145.6 (C-2)/  $\delta_{\text{C}}$  133.9 (C-15b), and  $\delta_{\text{H}}$  3.72 (3H, s) with  $\delta_{\text{C}}$  157.9 (C-6) suggested that 2 is a 3-*O*-desmethyl analogue of julandine. Compound 2 showed a positive optical rotation with  $[\alpha]_{\text{D}}^{23} +44.1$  ( $\text{CHCl}_3$ ,  $c$  0.03) and positive Cotton effect at 284 nm in CD spectrum (Fig. 4). Based on these results, compound 2 has an *S*-configuration at C-14a and was determined to be (+)-(*S*)-3-*O*-desmethyljulandine.

The *seco*-phenanthroquinolizidines 1, 2, and 3 were evaluated for their antiproliferative activity against five human tumor cell lines (HTCLs), A549 (lung cancer), MCF-7 (breast cancer: estrogen receptor

(ER) positive and HER2 negative), MDA-MB-231 (breast cancer: ER, progesterone receptor (PR), and HER2 negative), KB (epidermoid carcinoma: HeLa derivative) and KB-VIN (vincristine resistant KB subline: P-gp-over-expressing). The known *seco*-phenanthroquinolizidine, (*R*)-julandine (3), showed potent cytotoxicity against all cell lines including MDR (KB-VIN) (Table 2), while the newly identified *seco*-phenanthroquinolizidines 1 and 2 displayed no activity. The greater potency of 3 compared with 1 was consistent with prior reports that hydroxylation at C-15 of the phenanthroquinolizidine skeleton decreases cytotoxicity (Cai et al., 2006). Although compound 2 was inactive, the related pileamartine D with a bond between C-4a and C-4b showed selective cytotoxic activity in a recent study (Doan Thi Thuy et al., 2019) suggesting that a rigid phenanthrene structure is beneficial for cytotoxicity. In addition, although cryptopleurine (4) was not tested in this study due to insufficient quantity, it exhibits nanomolar cytotoxic activity against many HTCLs as reported in other studies (Doan Thi Thuy et al., 2019); (Banwell et al., 2006). Similarly, reduced cytotoxicity was previously reported for some *seco*-phenanthroquinolizidine (Banwell et al., 2006) and phenanthroindolizidine derivatives (Lykkeberg et al., 2002); (Stark et al., 2000)

### 3. Experimental

#### 3.1. General experimental

Optical rotations were measured on a JASCO P-2200 digital polarimeter in  $\text{CHCl}_3$ . NMR spectra were recorded on JEOL JNM-ECS400 and JNM-ECA600 NMR spectrometers with tetramethylsilane as an internal standard. Chemical shifts ( $\delta$ ) are stated in part per million (ppm). HRMS data were obtained from JMS-700 (FAB) mass spectrometer. Analytical and preparative TLC were performed on precoated silica gel 60 F<sub>254</sub> plates (0.25 or 1 mm thickness; Merck) and  $\text{NH}_2$  silica gel F<sub>254</sub> (0.5 mm; Wako). Column chromatography was performed with silica gel 60 N

**Table 2**  
Antiproliferative Data of Compounds 1–3.

compound	cell lines (IC <sub>50</sub> μM) <sup>a</sup>				
	A549	MDA-MB-231	MCF-7	KB	KB-VIN
<b>1</b>	24.4 ± 0.8	28.8 ± 1.2	36.2 ± 1.8	31.8 ± 0.3	44.5 ± 3.0
<b>2</b>	>40	>40	>40	>40	>40
<b>3</b>	0.8 ± 0.1	0.9 ± 0.01	4.6 ± 0.3	3.6 ± 0.4	4.5 ± 0.4
PXL (nM) <sup>b</sup>	6.4 ± 0.1	7.7 ± 0.5	7.6 ± 0.5	5.2 ± 0.1	1538.4 ± 116.5

<sup>a</sup> Antiproliferative activity is stated as IC<sub>50</sub> values for each cell line, the concentration of compound that caused 50 % reduction relative to untreated cells evaluated by the SRB assay, mean ± SD (n = 6).

<sup>b</sup> The IC<sub>50</sub> of paclitaxel (PXL) is stated in nM.

(spherical, 63–210 μm, neutral, Kanto Chemical). Analytical and reversed-phase preparative TLC (PTLC) was conducted on Silica gel 60 RP-18 F254S (0.25 mm, Merck). All cell lines were obtained from the Lineberger Comprehensive Cancer Center (UNC–CH) or from ATCC, except KB-VIN, which was obtained from Professor Y.-C. Cheng (Yale University).

### 3.2. Plant material

Leaves of *B. virgata* were collected at Lengkesa Village, Parigi Sub-district, Gowa, South Sulawesi, Indonesia in November 2019 and authenticated by Djoko Santoso, Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Indonesia. A voucher specimen was deposited in the Pharmacognosy-Phytochemistry Laboratory, Hasanuddin University (2019\_AR\_FFUH\_01).

### 3.3. Extraction and isolation compounds

Air-dried leaves of *B. virgata* (1.45 kg) were powdered and extracted three times (8.5 L for each time) with MeOH at room temperature for 48 h. The volatile solvent was evaporated to afford a crude MeOH extract (90.8 g). The MeOH extract was further partitioned between *n*-hexane and MeOH–H<sub>2</sub>O (9:1) to give *n*-hexane and MeOH–H<sub>2</sub>O extracts, 69.8 g and 20.4 g, respectively. The MeOH–H<sub>2</sub>O extract in EtOAc (250 mL) was subjected to ultrasound sonication for 15 min to separate EtOAc soluble and insoluble parts. The same process was repeated five times on the insoluble part to give an EtOAc soluble part (1.86 g) and insoluble material (18.45 g). The EtOAc soluble part (1.8 g) was subjected to column chromatography (CC) on a silica gel eluting with *n*-hexane–acetone (10:1, 5:1, 1:1, 1:2, 1:5, v/v), acetone 100 %, and MeOH 100 % to give 12 fractions (FA–FL). Fraction J (291 mg) was re-chromatographed by silica gel CC eluted successively with CHCl<sub>3</sub>–MeOH (40:1, 30:1, 25:1, 20:1, 10:1, v/v) and MeOH 100 % to afford nine subfractions (J1–J9). The subfraction J4 (18.5 mg) was separated by NH<sub>2</sub> silica gel PTLC developed with *n*-hexane–acetone (3:1, v/v) to give compounds **3** (3.1 mg), **4** (0.5 mg), and **1** (6.8 mg). Compounds **2** (0.7 mg) and **6** (2.4 mg) were isolated from subfractions J6 and J7, respectively, by NH<sub>2</sub> silica gel PTLC developed with CHCl<sub>3</sub>–MeOH (20:1, v/v). Repeated purifications of subfraction J5 by NH<sub>2</sub> silica gel PTLC developed with CHCl<sub>3</sub>–MeOH (25:1, v/v) gave compound **5** (2.3 mg). Fraction I (500 mg) was further fractionated with reversed-phased medium pressure liquid chromatography (MPLC) eluted with MeOH–H<sub>2</sub>O (4:1, v/v), MeOH 100 %, and acetone to give 9 subfractions, I1–I9. Repeated chromatography of subfraction I2 (188.6 mg) by silica gel CC eluting with *n*-hexane–EtOAc (5:1, 4:1, 3:1, 1:1, 1:5, v/v), EtOAc 100 %, and MeOH 100 % yielded 15 further subfractions (I2A–I2O). Compounds **7** and **8** were obtained from subfraction I2E as a mixture (3.0 mg). Compound **9** (1.6 mg) was isolated from subfraction I2G (4.7 mg) by PTLC on silica gel eluted with *n*-hexane–EtOAc (2:1, v/v). A mixture (3.1 mg) of **10** and **11** was also obtained from subfraction I2H (11.3 mg) by reversed-phase PTLC eluted with MeOH–H<sub>2</sub>O (3:1, v/v). Subfraction I2K was separated by the repeated reversed-phase PTLC eluted with MeOH–H<sub>2</sub>O (2:1, v/v) and normal phase PTLC eluted with *n*-

hexane–EtOAc (2:1, v/v) to give compounds **12** (1.7 mg) and **13** (0.9 mg).

#### 3.3.1. (–)-(14aR,15R)-hydroxyjulandine (1)

Pale-yellow oil; [α]<sub>D</sub><sup>23</sup> –137.6 (CHCl<sub>3</sub>, c 0.14); ECD (c 21 μg/mL, CH<sub>3</sub>OH) Δε<sub>284</sub> –3.91, Δε<sub>310</sub> +1.24; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 600 MHz) and <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz), see Tables 1 and 2; HR-FAB-MS *m/z* 396.2176 [M+H]<sup>+</sup>, (calcd for C<sub>24</sub>H<sub>30</sub>NO<sub>4</sub>, 396.2175).

#### 3.3.2. (+)-(14aS)-3-O-desmethyljulandine (2)

Pale-yellow oil; [α]<sub>D</sub><sup>23</sup> +44.1 (CHCl<sub>3</sub>, c 0.03); ECD (c 40 μg/mL, CH<sub>3</sub>OH) Δε<sub>284</sub> +7.88, Δε<sub>307</sub> –4.43; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 600 MHz) and <sup>13</sup>C NMR (CDCl<sub>3</sub>, 150 MHz), see Tables 1 and 2; HR-FAB-MS *m/z* 366.2073 [M+H]<sup>+</sup>, (calcd for C<sub>23</sub>H<sub>28</sub>NO<sub>3</sub>, 366.2069).

### 3.4. Antiproliferative activity assay

The antiproliferative activity assay using five human tumor cell lines, including A549 (lung carcinoma), MDA-MB-231 (estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, HER2-negative breast cancer), MCF-7 (ER-positive, HER2-negative breast cancer), KB (epidermoid carcinoma, HeLa derivative), and KBVIN (vincristine resistant KB subline: P-gp-over-expressing) as previously presented (Saito et al., 2021). Briefly, 4000 – 12 000 freshly trypsinized cells were seeded in 96-well microtiter plates with each test compound prepared in DMSO, which were cultured for 72 h. The treated cells were fixed in 10 % trichloroacetic acid followed by staining with 0.04 % sulforhodamin B. The highest concentration of vehicle (DMSO) was 0.1 % v/v, which displayed no effect on cell growth. Paclitaxel (Sigma-Aldrich, purity >95 %) was used as a reference compound. The mean IC<sub>50</sub> is the concentration of compound that inhibited cell growth by 50 % compared with a vehicle control under the experimental conditions used and is the average from at least three independent experiments with duplicate samples (n = 6).

#### Declaration of Competing Interest

The authors declare no competing financial interest.

#### Declaration of Competing Interest

The authors report no declarations of interest.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.phytol.2021.08.003>.

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