Cytotoxic Bisbenzylisoquinoline Alkaloids from *Stephania epigaea*

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ABSTRACT: Six new bisbenzylisoquinoline alkaloids (1–6) and seven known compounds (8–14) were isolated from the tubers of *Stephania epigaea*, in addition to the major alkaloid, cepharanthine (7). The structures of 1–6 were elucidated by combined spectroscopic data analysis and chemical methods, with their configurations determined from their optical rotation values and confirmed using circular dichroism. Compounds 1–6 belong to the cepharanthine type of bisbenzylisoquinoline alkaloids and have a rare methylenedioxy substituent. Compound 1, a dimer composed of benzylisoquinoline and seco-aristolactam units, represents a new type of bisbenzylisoquinoline alkaloid, while compounds 3–6 are bisbenzylisoquinoline N-oxides. These compounds were evaluated for their in vitro cytotoxicities against six human cancer cell lines (A-549, ECA109, HL-60, MCF-7, SMMC-7721, and SW480). Cepharanthine (7), the major component of *S. epigaea*, exhibited cytotoxicity against all of these cancer cell lines except ECA109, while its known analogue, 10, displayed cytotoxicity against all six cancer cell lines.

The genus *Stephania* (Menispermaceae), containing 60 species, is distributed mainly in the warmer parts of Asia and Africa, with about two-thirds of the number of this genus growing in mainland China.¹ These species have been utilized in folk medicine for the treatment of asthma, cancer, dysentery, fever, hyperglycemia, intestinal complaints, inflammation, sleep disturbances, and tuberculosis.² Several chemical studies on *Stephania* spp. have been carried out over the past five decades, which have led to the identification of more than 200 hasubanan,³ aporphine,⁴ protoberberine,⁵ and bisbenzylisoquinoline⁶ alkaloids as the major constituents. Among these, cepharanthine (7) was reported as a main bisbenzylisoquinoline alkaloid having various biological activities, such as antitumor activity,⁷ suppression of cytokine production,⁸ and induction of apoptosis.⁹

*Stephania epigaea* H. S. Lo (Menispermaceae) is a herbaceous liana mainly growing in the southwest and southeast of Yunnan Province, People’s Republic of China. Its tubers have been used by local people to treat fever and for sedation. Previous studies showed that it produces cepharanthine (7) and the other alkaloids cephethylene, delaivaline, isochondodendrine, (−)-norcepharaline, and runanine.¹⁰ In order to explore a new source and further investigate the bioactivities of cepharanthine (7) and its analogues, a detailed chemical investigation on the tubers of *S. epigaea* was carried out. This led to the identification of 13 minor bisbenzylisoquinoline alkaloids (1–6, 8–14), in addition to the main component, cepharanthine (7). Compounds 1–6 are new cepharanthine analogues, and their structures were elucidated on the basis of detailed spectroscopic analysis and chemical methods. The isolated compounds 3–14 were evaluated for their cytotoxicity against six human cancer cell lines (A-549 human lung carcinoma, ECA109 human esophagus cancer, HL-60 human myeloid leukemia, MCF-7 human breast adenocarcinoma, SMMC-7721 hepatocellular carcinoma, and SW480 colon cancer), and the results obtained are discussed herein.

RESULTS AND DISCUSSION

The alkaloid portion from the tubers of *S. epigaea* was subjected to repeated column chromatography over silica gel, followed by preparative thin-layer chromatography on silica gel (GF254) and recrystallization, to afford the major component cepharanthine (7), together with 13 bisbenzylisoquinoline alkaloids (1–6, 8–14). All showed a positive reaction to Dragendorff’s reagent. The known compounds (7–14) (see Supporting Information) were identified as cepharanthine (7),⁶ secocepharanthine (8),¹¹ cepharaline (9),¹² (+)-2-norcepharanthine (10),¹⁰ cepharaline-2/β-N-oxide (11),⁹ 3′,4′-dihydrosopharaline (12),¹³ homaromoline (13),¹⁴ and fangchinoline (14),¹⁵ respectively, using authentic samples and by comparison of their spectroscopic and physical data with literature values.

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Compound 1 was obtained as a white, amorphous powder. Its molecular formula was established as $C_{36}H_{32}N_2O_7$ on the basis of the positive HREIMS ($m/z$ 606.2379 [M]+, calc'd for $C_{36}H_{32}N_2O_7$ 606.2366), corresponding to 21 degrees of unsaturation. In the $^{13}$C NMR and DEPT spectra of 1 (Table 1), 36 carbon signals were observed, assigned as four methyls, of which two are methoxy groups ($\delta_C$ 55.5, 56.2), five methylenes, including one bearing a heteroatom ($\delta_C$ 51.0) and one bearing two oxygen atoms ($\delta_C$ 102.3), two aliphatic methines bearing heteroatoms ($\delta_C$ 64.4, 64.5), a carbonyl ($\delta_C$ 168.8), and 24 aromatic carbons arising from four benzene rings. The $^1$H NMR spectrum of 1 (Table 2) showed the presence of one para-disubstituted benzene ring ($\delta_H$ 7.41, 6.99 (each 1H, dd, $J = 2.5, 8.3$ Hz), 7.10, 6.39 (each 1H, brd, $J = 8.4$ Hz)], a 1,3,4-trisubstituted benzene ring ($\delta_H$ 6.76 (d, $J = 8.2$ Hz), 6.80 (dd, $J = 1.8, 8.2$ Hz), 5.64 (brs)], three aromatic singlet protons ($\delta_H$ 6.41, 6.67, 6.92 (each 1H, s)] due to two benzene rings, four heteroatom-bearing singlet methyls ($\delta_H$ 2.53, 3.24, 3.84, 3.69), and one methylenedioxy group ($\delta_H$ 5.69 and 5.74, five 1H each, $J = 12$ Hz). These NMR spectroscopic features of 1 were closely related to those of cephanepharine (7). However, instead of six aliphatic methylenes ($\delta_C$ 51.2, 45.9, 41.2, 38.6, 28.6, 25.9) in 7, only four methylene signals at $\delta_C$ 31.0, 38.6, 37.7, and 28.2, together with an additional carbonyl carbon at $\delta_C$ 168.8, were observed for 1. All these characteristics suggested that compound 1 is a norcephanepharine analogue.

In the HMBC spectrum of 1 (Figure 1), three aromatic singlet protons were assigned as H-5 ($\delta_H$ 6.41), H-8 ($\delta_H$ 6.67), and H-5′ ($\delta_H$ 6.92) of the A and A′ aromatic rings, respectively, based on the correlations of $\delta_H$ 6.41 (s) with C-4/C-8a/C-6/C-7, $\delta_H$ 6.67 (s) with C-6/C-4a/C-7, and $\delta_H$ 6.92 (s) with C-6′/C-7′/C-8′a, and from the correlations of H-8 with $\delta_C$ 64.5 (CH, C-1), H-5 with $\delta_C$ 28.2 (CH$_2$, C-4), and the N-CH$_3$ ($\delta_C$ 2.53) group with $\delta_C$ 31.0 (CH$_2$, C-3). These observations allowed the B-ring of 1 to be constructed. In addition, HMBC correlations of the ABX-coupled aromatic proton at $\delta_H$ 6.80 (dd, $J = 1.8, 8.2$ Hz, H-14) with C-12 ($\delta_C$ 147.1)/C-α ($\delta_C$ 37.7, CH$_2$), H-1 ($\delta_H$ 3.63) with C-9, and H-α with C-1 revealed the connectivity of the 1,3,4-trisubstituted benzene C-ring to C-1 of the B-ring through a methylene group (C-α). Cross-peaks of the aromatic proton at $\delta_H$ 6.99 (H-11′) with C-9′ ($\delta_C$ 134.9), both H-10′ ($\delta_H$ 7.41) and H-14′ ($\delta_H$ 7.10) with C-12′ ($\delta_C$ 153.8), both H-α′ ($\delta_H$ 2.55, 3.56) and H-1′ ($\delta_H$ 4.75) with C-9′, and H-1′ with C-8′a ($\delta_C$ 130.9) confirmed the connection of the para-disubstituted benzene C-ring via another methylene group, C-α′, to the heteroatom-bearing methine ($\delta_C$ 64.4, C-1′), which was connected to the C-8′a position of the A′-ring. The above key HMBC correlations were used to construct the cephanepharine skeleton in 1. Also, HMBC correlations of the downfield shifted methylenedioxy protons ($\delta_H$ 5.69 and 5.74) with C-6′ and C-7′ and of the two methoxy groups ($\delta_C$ 3.69 and 3.84) with C-6 ($\delta_C$ 149.1) and C-12 (147.1), respectively, could be observed. These, together with the ROESY correlations (Figure 1) of the methoxy group signals at $\delta_H$ 3.69 with H-S and of the other methoxy group signal at $\delta_H$ 3.84 with H-13, revealed the locations of the methylenedioxy group and two methoxy groups in 1, which were the same as those in 7. Furthermore, HMBC correlations of both H-S′ and 2′-N-CH$_3$ ($\delta_H$ 3.24) with the carbonyl carbon ($\delta_C$ 168.8, C-3′), and 2′-N-CH$_3$ also with C-1′, were used to determine the B′-ring in 1. This was supported by the IR band at 1687 cm$^{-1}$, produced by the characteristic absorption of a secondary amide.

In the EIMS of 1, a fragment ion peak at $m/z$ 380 corresponded to the upper half of the molecule, as a result of the cleavage of two benzylic bonds, C-1/C-α and C-1′/C-α′. These data confirmed that compound 1 is a head-to-head bisbenzyl-isooquinoline alkaloid. It was concluded that the diphenyl ether bridge occurs between C-11/C-12 and C-7′/C-8′. The positive optical rotation value of 1 ($[\alpha]_D^{25}$ +172.8 ($c$ 1.1, MeOH)) and a circular dichroism (CD) curve (Supporting Information) similar to that of 7 indicated the 1R, 1′S configurations in 1, the same as those of cephanepharine (7).

On the basis of the above evidence, the structure of compound 1 was elucidated as 3′-nor-4′-oxocephanepharine, which is a dimer consisting of benzylisoquinoline and seco-aristolactam units.

Compound 2, a white, amorphous powder, gave a molecular formula of $C_{35}H_{30}N_2O_9$ as deduced by the positive HREIMS ($m/z$ 589.2338 [M + H]$^+$, calc'd for $C_{35}H_{30}N_2O_9$ 589.2338), implying 22 degrees of unsaturation. The NMR data were closely related to those of cephanepharine (7), except for the signals arising from the B- or B′-ring. Instead of two aliphatic methylenes ($\delta_C$ 28.9, 51.2), one N-bearing methine ($\delta_C$ 64.2), and two N-CH$_3$ ($\delta_C$ 42.0, 43.9) groups in 7, only one N-CH$_3$ ($\delta_C$ 41.9) group were observed in 2, indicating that the B- or B′-ring in 2 is an aromatic ring. On comparison with 7, the $^1$H NMR spectrum of 2 displayed two additional mutually coupled aromatic protons at $\delta_H$ 8.18 and 7.53 (each 1H, $d, J = 5.9$ Hz). In the HMBC spectrum, correlations of the signal at $\delta_H$ 7.53 with C-5 (C-106.5) and C-8a (C-123.2), of $\delta_H$ 8.18 with C-4a (137.7), and of both aromatic protons at $\delta_H$ 8.18 and 7.67 (H-8) with the downfield shifted aromatic quaternary carbon at $\delta_C$ 160.5 confirmed that the B- or B′-ring in 2 is dehydrogenated to form a pyridine ring. Thus, the aromatic proton signals at $\delta_H$ 8.18 and 7.53 were assigned at H-3 and H-4, respectively. Furthermore, the signal at $\delta_C$ 160.5 was assigned to C-1 based
on the HMBC correlations of H-α (δH 4.52 and 4.11) with δC 160.5, C-9 (δC 132.8), and C-10 (δC 122.5). Other HMBC, 1H–1H COSY, and ROESY correlations (Figure 1) were used to confirm the planar structure of 2. In the EIMS of 2, an ion peak at m/z 482 [M – 106]+, together with a corresponding base peak at m/z 481 [M – 107]– due to the loss of a C-ring, indicated the characteristics of a bisbenzylisoquinoline with C-7/C-8 diphenyl ether bridge linkages, the negative optical rotation value of 2 ([α]D –13.5 (c 1.1, MeOH)) was used to confirm the 1R configuration. Therefore, compound 2 was elucidated as (−)-1,3,4-dehydrocochranine.

Compound 3 was obtained as a white, amorphous powder. Its molecular formula was established as C29H32N2O2 according to the positive HREIMS (604.2198 [M]+, calcd for C29H32N2O2 604.2210), 16 Da more than that of 2. The 1H NMR and 13C NMR spectroscopic data were very similar to those of 2, except for the significantly downfield chemical shifts of C-1’, 2’-N-CH3, C-3’, and C-4’ with Δδ of 14.4, 14.9, 14.6, and 2.8 ppm, respectively, suggesting that N-2’ in 3 is oxygenated. This was confirmed by the EIMS, in which a weak molecular ion peak at m/z 604 [M]+ (25%) and a major fragment ion peak at m/z 588 [M – 16]+ (100%) were observed, accompanied by the base peak at m/z 587, due to the loss of oxygen. A somewhat weak ion peak at m/z 379 corresponded to the upper half of 3. On comparing with compound 7, the proton signals of H-1’ (δH 4.96) and 2’-N-CH3 (δH 3.31) were shifted downfield by 0.35 and 0.70 ppm, respectively, suggesting a trans relationship between the N-oxygen and H-1’ in 3.16 The ROESY correlation of 2’-N-CH3 with H-1’ (Figure 1) also supported the opposite orientation of H-1’ with the N-oxygen in 3. The 1S configuration of 3 was determined by its same positive [α]D value (+40.7) to that of (+)-cochranine ([α]D +130 (c 0.5, CHCl3))19 and confirmed by the different CD spectrum of 3 with that of (−)-1,3,4-dehydrocochranine (2) (Supporting Information).
fore, compound 3 was determined to be (+)-1,3,4-dehydrocepharanthine-2′/β-N-oxide.

The molecular formula of compound 4 was assigned as C_{16}H_{17}N_{2}O_{3}, according to the HREIMS (m/z 606.2366 [M]+, calc for C_{16}H_{17}N_{2}O_{3} 606.2366), with 21 degrees of unsaturation. The 1^\text{H} NMR spectrum of 4 was very close to that of 1,3,4-dehydrocepharanthine-2′/β-N-oxide (3), except that the aromatic methines of C-3 and C-4 in 3 were replaced by two aliphatic methylenes at δ_{C} 47.0 and 26.5 in 4. Their corresponding mutually coupled proton signals were at δ_{H} 3.61 and 2.67, respectively. In addition, the chemical shift of C-1 in 4 was shifted downfield to δ_{C} 169.3 (δ_{C} 160.6 for 3). The EIMS of 4 exhibited a fragment ion peak at m/z 588 [M − 16]^+ (100%), suggesting the presence of a bisbenzylisoquinoline N-oxide functionality. The two aliphatic methylenes at δ_{C} 47.0 and 26.5 were assigned at C-3 and C-4, respectively, due to the HMBC correlation of H-5 (δ_{H} 6.76, s) with δ_{C} 26.5 (C-4), while the downfield shifted carbon signal at δ_{C} 169.3 was assigned at C-1, based on its HMBC correlations with H-8 (δ_{H} 7.21) and H-3 (δ_{H} 3.61). Other 1^\text{H}−1^\text{H} COSY and HMBC correlations (Figure 1) were used to confirm the structure of 4. The trans relationship between the 2′-N-oxide and H-1′ was revealed by the ROESY correlation of 2′-N-CH_{3} with H-1′. The similar positive [α]_{D}^{25} (+40.7) and CD Cotton effects (Supporting Information) to those of 3 supported the 1′S configuration in 4. Consequently, compound 4 was deduced as (+)-1-dehydrocepharanthine-2′/β-N-oxide.

Compound 5 was obtained as a white, amorphous powder and gave a molecular formula of C_{17}H_{18}N_{2}O_{4}, as deduced from the positive HREIMS (m/z 623.2757 [M + 1]^+), calc for C_{17}H_{18}N_{2}O_{4} 623.2757), 16 Da more than that of cepharanthine (7). The 1^\text{H} and 13^\text{C} NMR spectra of 5 displayed two sets of signals with an integral ratio of 6.5:3.5, implying the occurrence of a pair of compounds, 5a (major) and 5b (minor). The protons and their corresponding carbons of 5a and 5b were fully separated and assigned on the basis of detailed analysis of the 1D- and 2D-NMR spectra. The 1^\text{H} and 13^\text{C} NMR features of 5a and 5b were closely related to those of cepharanthine (7), except for the chemical shifts arising from the B-ring. On comparing to those of 7 (δ_{H} 64.5, 44.1, and 51.0), 1, 2-N-CH_{3}y, and 3 of 5a and 5b were downfield shifted to δ_{C} 78.2/81.4, 58.7/60.2, and 60.3/60.8, respectively, suggesting that both 5a and 5b are N-oxides of 7. This was supported by the reduction of 5 with zinc powder and HCl at room temperature, which yielded only cepharanthine (7) as the product. The N-oxide positions for 5a and 5b were both determined to be at the 2-N position from the HMBC correlations of H-8 (δ_{H} 6.79/6.93) and 2-N-CH_{3} (δ_{H} 3.45/2.98) with δ_{C} 78.2/81.4 (C-1), H-5 (δ_{H} 6.64/6.72) with δ_{C} 25.2/28.1 (C-4), H-1 (δ_{H} 4.54/4.25) with C-α (δ_{C} 39.0/35.2), and H-α (δ_{H} 3.23/2.70, 3.46/4.14) with C-9 (δ_{C} 128.3/133.9) and C-10 (δ_{C} 117.1/125.4). Other 1^\text{H}−1^\text{H} COSY and HMBC correlations (Figure 1) helped confirm the same planar structures of 5a and 5b. The only difference between 5a and 5b was the oxygen orientation at the 2′-N position. In the 1^\text{H} NMR spectrum, the chemical shifts of 2′-N-CH_{3} for 5a and 5b were downfield shifted by 0.89 and 0.42 ppm, respectively, compared with that of 7, suggesting that 5a is cepharanthine-2′e-N-oxide and 5b is cepharanthine-2′/β-N-oxide.\textsuperscript{35} This was confirmed by the weak ROESY correlation of H-1 with 2-N-CH_{3} in 5b, but no correlation between H-1 and 2-N-CH_{3} was observed in 5a (Figure 1). The large positive optical rotation

Table 2. 1^\text{H} NMR Spectroscopic Data for Compounds 1–6 in CD_{3}OD (δ in ppm)

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*Data were recorded at 600 MHz. \textsuperscript{3}Data were recorded at 500 MHz. *Data were detected in CD_{3}OD + CDCl_{3} (1:1). \textsuperscript{4}Overlapped with singlet 2-N-CH_{3} signal.
value of $\alpha_{25}^{25}D +204.2$ (c 1.0, MeOH) together with the chemical reduction of 5 with zinc power yielding only 7 implied the same 1R, 1’S configurations in both compounds 5a and 5b as those in 7. Therefore, 5 was determined to be a mixture of cepharanthine-2α-N-oxide (5a) and -2β-N-oxide (5b).

Compound 6 was obtained as a white, amorphous powder. Its molecular formula was determined as C_{37}H_{38}N_{2}O_{7} due to the positive HREIMS ($m/z$ 622.2387 [M] +, calcd for C_{36}H_{32}N_{2}O_{7}, 622.2679), which was also 16 Da more than that of cepharanthine (7). Comparison the 1H and 13C NMR spectroscopic data (Table 2) with those of cepharanthine-2β-N-oxide revealed that compound 6 has a similar structure. However, the downfield shifted H-1′ [$\delta_H 4.84$ (6), $\delta_H 4.63$ (11)] and 2′-N-CH$_3$ [$\delta_H 3.69$ (6), $\delta_H 3.31$ (11)] signals suggested that compound 6 is a 2′α-N-oxide of cepharanthine (7). The proton signals at $\delta_H 4.84$ and 3.69 were assigned to H-1′ and 2′-N-CH$_3$, respectively, on the basis of their HMBC correlations with C-4′a ($\delta_C$ 125.1)/C-8′ ($\delta_C$ 139.2)/C-9′ ($\delta_C$ 136.6) and C-3′ ($\delta_C$ 58.8)/C-1′ ($\delta_C$ 77.2). Since no NOE effects between H-1′ and 2′-N-CH$_3$ were observed, this proved indirectly that H-1′ is oriented on the same side of the molecule as the oxygen of N-oxide. The large positive optical rotation value of 6 ($\alpha_{25}^{25}D +229.0$ (c 1.0, MeOH)) and its similar CD spectrum to that of cepharanthine (7) implied that compound 6 has the same 1R, 1’S configurations as 7. The reduction of 6 with zinc power and HCl at room temperature yielded cepharanthine (7). Consequently, compound 6 was determined to be cepharanthine-2α-N-oxide.

The isolated compounds 3–14 were evaluated for their cytotoxicity against human lung carcinoma (A-549), human esophagus cancer (ECA109), human myeloid leukemia (HL-60), human breast adenocarcinoma (MCF-7), hepatocellular carcinoma (SMMC-7721), and colon cancer (SW480) cell lines. Tanespimycin (17-AAG) and cisplatin were used as positive control substances. Among these, compounds 4, 7, 10, 13, and 14 showed cytotoxic potency against the above six human cancer cell lines (Table 3), and the other compounds tested were inactive (IC$_{50} > 10 \mu$M). It is noted that cepharanthine (7) as the major component of S. epigaea exhibited inhibitory activity against all cancer cell lines except ECA109. The known analogue (+)-2-norcepharanthine (10) also showed cytotoxicity against all six cancer cell lines, with

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*Not determined.
IC$_{50}$ values ranging from 2.3 to 4.8 μM. In turn, the new compound (+)-1-dehydrocepharanthine-2'-β-N-oxide (4) displayed selective cytotoxicity for the ECA109 cell line (Table 3). Both 4 and 10 are cepharanthine analogues bearing only one N-CH$_3$ in their respective structures.

### EXPERIMENTAL SECTION

**General Experimental Procedures.** Optical rotations were performed on a P-1020 polarimeter (JASCO, Tokyo, Japan). IR spectra was measured on a Bruker Tensor 27 spectrometer with KBr pellets. 1D- and 2D-NMR spectra were run on Bruker AM-400, DRX-500, and AVANCE III-600 NMR spectrometers operating at 400, 500, and 600 MHz for $^1$H and 13C, respectively. Coupling constants are expressed in Hz, and chemical shifts are given on a ppm scale with tetramethylsilane as internal standard. The MS data were recorded on a VG Auto Spec-3000 spectrometer (VG, Manchester, U.K.) with 400, 500, and 600 MHz for $^1$H and 100, 125, and 150 MHz for 13C, respectively.

**Plant Material.** The tubers of *S. epigaea* (350 kg) were extracted with 1% hydrochloric acid solution (700 L) at room temperature. The extract was adjusted to pH 10 with 5% NaOH to give a precipitate (72.8 kg). The precipitate was re-extracted with diethylamine (2:1:0.02) and petroleum ether (30:1:0.3), and then zinc powder (100 mg) was added. After being stirred at room temperature for 2 h, the reaction mixture was extracted with CHCl$_3$, three times. The organic layer was subjected to preparative TLC (CHCl$_3$) and then subjected to preparative TLC (CHCl$_3$–CH$_3$OH–NH$_3$–H$_2$O, 10:1:0.1), while MTT, [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was added instead of MTS for the ECA109 cell line. The incubation was continued for another 4 h to give a formazan product. In each well, 100 μL of 20% SDS was added after 100 μL of medium was removed and then incubated overnight for the formazan product to dissolve completely. The absorbance of the solution was measured at 570 nm using a Bio-Rad 680 instrument. Compound concentrations inhibiting 50% of cell growth (IC$_{50}$ values) were performed on a P-1020 polarimeter (JASCO, Tokyo, Japan). The MS data were recorded on a VG Auto Spec-3000 spectrometer (VG, Manchester, U.K.) with 400, 500, and 600 MHz for $^1$H and 100, 125, and 150 MHz for 13C, respectively.

**Cytotoxicity Assay.** The six cancer cell lines (A-549 lung cancer, ECA109 human esophagus cancer, HL-60 human myeloid leukemia, MCF-7 breast cancer, SW480 colon cancer) were cultured in RPMI 1640 medium containing 10% fetal bovine serum and 100 U/mL penicillin/streptomycin in a humidified incubator in a 5% CO$_2$ atmosphere at 37°C. Cells (5 $\times$ 10$^3$/well) were plated in 96-well plates in 100 μL of medium, in which the test samples were added at various concentrations. After 48 h incubation, MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] solution (5 mg/mL in phosphate-buffered saline) was added (20 μL/well), while MTT [(3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide] was added instead of MTS for the ECA109 cell line. The incubation was continued for another 4 h to give a formazan product. In each well, 100 μL of 20% SDS was added after 100 μL of medium was removed and then incubated overnight for the formazan product to dissolve completely. The absorbance of the solution was measured at 570 nm using a Bio-Rad 680 instrument. Compound concentrations inhibiting 50% of cell growth (IC$_{50}$ values) were calculated by the Reed and Muench method. The 1H and 13C NMR, HSQC, HMBC, $^1$H NMR, and $^1$H NMR, and $^{13}$C NMR (CHCl$_3$, 150 MHz) for the formazan product. In each well, 100 μL of 20% SDS was added after 100 μL of medium was removed and then incubated overnight for the formazan product to dissolve completely. The absorbance of the solution was measured at 570 nm using a Bio-Rad 680 instrument. Compound concentrations inhibiting 50% of cell growth (IC$_{50}$ values) were calculated by the Reed and Muench method. The 1H and 13C NMR, HSQC, HMBC, $^1$H NMR, and $^{13}$C NMR (CHCl$_3$, 150 MHz) for the formazan product. In each well, 100 μL of 20% SDS was added after 100 μL of medium was removed and then incubated overnight for the formazan product to dissolve completely. The absorbance of the solution was measured at 570 nm using a Bio-Rad 680 instrument. Compound concentrations inhibiting 50% of cell growth (IC$_{50}$ values) were calculated by the Reed and Muench method. The 1H and 13C NMR, HSQC, HMBC, $^1$H NMR, and $^{13}$C NMR (CHCl$_3$, 150 MHz) data, see Tables 1 and 2; EIMS m/z 606 [M$^+$] + (calcd for C$_{36}$H$_{32}$N$_2$O$_7$, 606.2366). The IC$_{50}$ values ranging from 2.3 to 4.8 μM. In turn, the new compound (+)-1-dehydrocepharanthine-2'-β-N-oxide (4) displayed selective cytotoxicity for the ECA109 cell line (Table 3). Both 4 and 10 are cepharanthine analogues bearing only one N-CH$_3$ in their respective structures.

## ASSOCIATED CONTENT

**Supporting Information**

Structures of the known compounds 8–14 from *S. epigaea* and the 1H and 13C NMR, HSQC, HMBC, $^1$H NMR, and $^{13}$C NMR (CHCl$_3$, 150 MHz) data, see Tables 1 and 2; EIMS m/z 606 [M$^+$] + (calcd for C$_{36}$H$_{32}$N$_2$O$_7$, 606.2366).

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**Notes**

The authors declare no competing financial interest.
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