Alkaloids from the stems of *Clausena lansium* and their neuroprotective activity†

Jie Liu,a Yi-Qian Du,a Chuang-Jun Lia, Li Lia, Fang-You Chen,a Jing-Zhi Yang,a Nai-Hong Chenb and Dong-Ming Zhangd,a

Eight new alkaloids, including three pairs of enantiomers (+)-(2'S,3'R)-clauselansine A (1a) and (−)-(2'R,3'S)-clauselansine A (1b); (+)-(2'S,3'R)-clauselansine B (2a) and (−)-(2'R,3'S)-clauselansine B (2b); (+)-(35,4R,5S,6S)-clauselansine C (3a) and (−)-(35,4S,5R,6R)-clauselansine C (3b), (+)-(1'R,2'R,6'R)-clauselansine B (4a), and (+)-(1'R,2'R)-clauselansine D (5a), together with twelve known alkaloids (4b, 5b, 6a, 6b, 7a, 7b and 8–13) were isolated from the stems of *Clausena lansium*. Their structural elucidation and stereochemistry determination were achieved by spectroscopic and chemical methods including 1D and 2D NMR experiments, especially the employment of electronic circular dichroism calculation spectra, Mosher’s method, and Snatzke’s method expressed by the induced circular dichroism spectrum. Compounds 1b, 2a, 3b, 5a, and 5b inhibited PC12 cell damage induced by Okadaic Acid, and increased cell viability from 70.5 ± 5.4% to 83.4 ± 4.1%, 91.2 ± 10.1%, 83.5 ± 7.8%, 89.7 ± 4.8%, 83.3 ± 5.9% at 10 μM, respectively.

Results and discussion

Compound 1 (1a/1b) was obtained as colourless oil. Its molecular formula was assigned as C_{18}H_{25}NO_{4} based on the 13C NMR spectroscopic data and HRESIMS (m/z 320.1854 [M + H]+, cale for C_{18}H_{26}NO_{4}, 320.1856), implying seven indices of hydrogen deficiency. The IR spectrum displayed absorptions characteristic of amino (3313 cm⁻¹), amide (1640 cm⁻¹) and aromatic ring (1671, 1612, and 1439 cm⁻¹) groups, and the UV spectrum showed absorptions at λ max 202, 223, 282 nm. The 1H NMR (Table 1) spectrum showed a set of signals for 1,2,3-
trisubstituted benzene ring at 7.43 (1H, d, J = 7.6 Hz, H-4), 6.90 (1H, t, J = 7.4 Hz, H-5), 6.85 (1H, d, J = 6.8 Hz, H-6), together with a doublet at δH 7.18 (1H, d, J = 2.4 Hz, H-2) and a broad signal at δH 10.80 (1H, NH), which indicated a 3,7-disubstituted indole moiety. In addition, a double bond group at δH 7.35 (JH 7.6 Hz, H-4), a methylene at 3.55 (2H, d, J = 7.3 Hz, H-1″) and two methyls at δH 1.70 (3H, s, H-5″), 1.00 (3H, s, H-5′′) were also exhibited in the 1H NMR spectrum. The 13C NMR and DEPT spectra exhibited a double bond, and four methylenes, two methines, two methyls and the remaining eight in indole moiety. The above information indicated 1 was a diprenylated indole. A comparison of the 1H and 13C NMR of 1 with those of hexalobines suggested that their structures are closely related, except for 1,3,4-trihydroxy-3-methylbut-2-yl and 4-hydroxy-3-methyl-2-butenyl at C-3 and C-7 in 1. In the HMBC spectrum, the cross-peaks between H-1′/C-3 (δC 114.2), C-3′ (δC 75.0), H-2′/C-2 (δC 123.0), C-3a (δC 128.3), C-5′ (δC 22.0), H-4′/C-3′ (δC 75.0), C-5′′ (δC 22.0) demonstrated 1,3,4-trihydroxy-3-methylbut-2-yl group attached to C-3 of the indole moiety (Fig. 2); the cross-peaks between H-1″/C-6 (δC 119.7), C-7a (δC 134.4), C-3″ (δC 136.3), H-2″/C-7 (δC 123.7), C-4″ (δC 66.3), C-5″ (δC 13.7) demonstrated 4-hydroxy-3-methyl-2-butenyl group attached to C-7 of the indole moiety (Fig. 2). The NOE difference experiment displayed that a strong enhancement of H-2″ was observed when H-4″ was irradiated while H-2′′ was no enhanced on irradiation of H-5″, indicating an E configuration of the double bond. Thus, the structure of 1 was elucidated. The specific rotation of 1 approached zero, and no Cotton effect was found in the electronic circular dichroism (ECD) spectrum of 1, indicating a racemic mixture. Subsequent chiral resolution of 1 afforded the anticipated enantiomers 1a and 1b, which showed mirror image-like ECD curves and specific rotations {1a: [α]20D +18.7 (c 0.1 MeOH); 1b: [α]20D −17.0 (c 0.1 MeOH)}. In order to confirm the absolute configuration of the 1′,3′,4′-triol of 1b, 1b was treated with 2,2-dimethoxypropane and pyridinium-2-toluene sulfonate and converted into its acetonide 1c (Fig. 3). According to 1D and 2D NMR, 1c was determined similar to pyrido[3,4-b]pyrano[3,4-b]indoles. In order to confirm the absolute configuration of 1c, the ECD calculations were also performed for the four configurations 2′R,3′S, 2′R,3′R, 2′S,3′R, and 2′S,3′S-1c using the time-dependent density functional theory (TDDFT) method at the B3LYP/6-31G (d) level. The calculated ECD spectrum for 2′R,3′S-1c and 2′R,3′R-1c enantiomer agreed with the experimental ECD data (Fig. 4) of 1c. Thus, the absolute configuration at C-2′ of 1c was 2′R. In addition, the absolute configuration of the 3′,4′-diol moiety of 1c was determined using induced CD spectra by Snatzke’s method. A positive Cotton effect at 323 nm (Fig. 5) in the induced CD spectrum indicated the 3′S configuration for 1c by means of the empirical helicity rule. According to the above information, the

Fig. 1 Alkaloid derivatives (1–13) obtained from the stems of C. lansium.
Table 1 1H and 13C NMR spectroscopic data of compounds 1, 1c, and 2 (δ in ppm, J in Hz)

<table>
<thead>
<tr>
<th>Position</th>
<th>δH a</th>
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a In DMSO-d6 (600 MHz). b In DMSO-d6 (150 MHz). Coupling constants (J) in Hz are given in parentheses. The assignments were based on HSQC and HMBC experiments.

The isolation of 1 and 2 led to the elucidation of their structures. The NOE difference experiment displayed that a strong enhancement of H-2' was observed when H-4'' was irradiated, while H-2'' was no enhanced on irradiation of H-5'', indicating an E configuration of the double bond. Thus, the structure of 2 was elucidated.

The specific rotation of 2 approached zero, and no Cotton effect was found in the electronic circular dichroism (ECD) spectrum of 2, indicating a racemic mixture. Subsequent chiral resolution of 2 afforded the anticipated enantiomers 2a and 2b, which showed mirror image-like ECD curves and specific rotations [2a: [α]20 D +28.0 (c 0.1 MeOH); 2b: [α]20 D −32.6 (c 0.1 MeOH)]. In order to confirm the absolute configuration of the enantiomers 2a and 2b, a systematic conformational analysis and optimization were performed for 2a and 2b using the same method applied to 1a and 1b. A comparison of the theoretically

![Fig. 2](image-url)  
Fig. 2 Key 1H, 1H-COSY and HMBC correlations of compound 1 and 2.

![Fig. 3](image-url)  
Fig. 3 The isolation of 1 to 1a and 1b and the action of compound 1b to its acetonide derivative 1c.
calculated and experimental ECD curves (Fig. 6) demonstrated that the configuration of 2a was 2'S,3'R and the configuration of 2b was 2'R,3'S. According to the structures of 1a and 2a, we speculate that 2a was possibly generated by dehydration of 1a, which means the configuration of 2a was the same as 1a. And 2b was also possibly generated by dehydration of 1b, which means the configuration of 2b was the same as 1b. Thus, 2a has a 2'S,3'R-configuration and 2b has a 2'R,3'S-configuration. Therefore, compounds 2a and 2b were given the trivial names (+)(2'S,3'R)-clauselansine B and (+)(2'R,3'S)-clauselansine B, respectively.

Compound 3 (3a/3b) was obtained as a white solid. Its molecular formula was assigned as C_{18}H_{17}NO_{2} based on the $^{13}$C NMR spectroscopic data and HRESIMS (m/z 280.1333 [M + Na]$^+$, calcd for C_{18}H_{17}NO_{2}Na 280.1332), implying eleven indices of hydrogen deficiency. The IR spectrum displayed absorptions characteristic of hydroxyl (3320 cm$^{-1}$), carbonyl (1678 cm$^{-1}$) and aromatic groups (1601, 1483, and 1454 cm$^{-1}$). The $^1$H NMR spectrum showed nine aromatic protons [ring A: $\delta_H$ 6.92 (1H, d, $J = 7.6$ Hz, H-3'), 7.23 (1H, overlapped, H-4'), 7.31 (1H, m, H-5') and 6.23 (1H, d, $J = 7.6$ Hz, H-6'); ring B: $\delta_H$ 6.98 (2H, d, $J = 7.1$ Hz, H-2', 6')], 7.27 (2H, m, H-3', 5'), 7.23 (1H, overlapped, H-4')] together with four methine groups at $\delta_H$ 4.11 (1H, d, $J = 8.3$ Hz, H-3), 3.77 (1H, dd, $J = 8.3, 5.3$ Hz, H-4); 4.66 (1H, t, $J = 8.3$ Hz, H-5), 4.77 (1H, d, $J = 8.3$ Hz, H-6), and a hydroxyl group at $\delta_H$ 5.97 (1H, s). The $^{13}$C NMR spectrum exhibited twelve aromatic carbons, one carbonyl $\delta_C$ 174.1, one oxymethine $\delta_C$ 75.0, three methines ($\delta_C$ 52.3, 53.3, 65.2), and one methyl ($\delta_C$ 29.6). The $^1$H, $^1$H-COSY correlations (Fig. 7) suggested one OCH-CH-CH fragment. The $^1$H and $^{13}$C NMR data (Table 2) also indicated 1,2-disubstituted and monosubstituted aromatic units (ring A and B) and one methyl group. The HMBC correlations (Fig. 6) of H-4 with C-2' ($\delta_C$ 144.7) and C-6' ($\delta_C$ 124.3); H-5 with C-2' ($\delta_C$ 144.7) and C-1'' ($\delta_C$ 140.7); H-6 with C-3' ($\delta_C$ 125.8) and C-2''(C-6') ($\delta_C$ 129.6) showed that ring A was connected at C-4 and C-6, and ring B was connected at C-6. The above information coupled with biogenetic considerations and literature references indicated 3 was similar to the dehydro-derivative of neoclausenamide. Its relative configuration was established on the basis of NOESY correlations (Fig. 7). The NOESY correlations of H-4 with H-5, H-5 with H-4, H-6, N-CH$_3$ and H-6 with H-5, H-6'', H-3 with H-2'' showed that H-4, H-5 and H-6 were $\beta$ orientation, while H-3 was $\alpha$ orientation. Thus, compound 3 (clauselansine C) was fully identified.
The specific rotation of 3 approached zero, and no Cotton effect was found in the electronic circular dichroism (ECD) spectrum of 3, indicating a racemic mixture. Subsequent chiral resolution of 3 afforded the anticipated enantiomers 3a and 3b, which showed mirror image-like ECD curves and specific rotations (3a: $[\alpha]_{D}^{20} +17.9$ (c 0.1 MeOH); 3b: $[\alpha]_{D}^{20} -22.0$ (c 0.1 MeOH)). Compound 3a was treated with (R)- and (S)-$\alpha$-methoxy-$\alpha$-(trifluoromethyl)phenylacetyl chloride (MTPA-Cl) in anhydrous CH2Cl2 to afford the 3a-(S)-MTPA ester (3aa) and 3a-(R)-MTPA ester (3ab), respectively. The $\Delta\Omega_{M}$ values were calculated as shown in Fig. 8. Application of Mosher’s rule revealed that 3a had the $3R,4R,5S,6S$ configuration. Meanwhile, the absolute configuration of 3b was assigned with the $3R,4S,5R,6R$-configuration. In order to confirm the absolute configuration of the enantiomers 3a and 3b, the ECD calculations were also performed for the two configurations $3S,4R,5S,6S$ and $3R,4S,5R,6R$-3 using the time-dependent density functional theory (TDDFT) method at the B3LYP/6-31G (d) level. The calculated ECD spectrum for $3S,4R,5S,6S$-3 agreed with the experimental ECD data (Fig. 9) of 3a. The calculated ECD spectrum for $3R,4S,5R,6R$-3 was in good accordance with the experimental spectrum of 3b (Fig. 9). Thus, 3a has a $3S,4R,5S,6S$ configuration and 3b has a $3R,4S,5R,6R$ configuration. Therefore, compounds 3a and 3b were given the trivial names (+)-(3S,4R,5S,6S)-clauselansine C and (−)-(3R,4S,5R,6R)-clauselansine C, respectively.

Compound 4 (4a/4b) was obtained as a white powder. Its molecular formula was determined as $C_{19}H_{19}NO_{5}$ on the basis of its $^{13}$C NMR and HRESIMS ($m/z$ 326.1398 [M + H]+, calcd for $C_{19}H_{19}NO_{5}$ 326.1387), corresponding with eleven indices of hydrogen deficiency. Its NMR data (Table 3) was almost identical to those of claulansine B. Chiral separation of 4 afforded a pair of enantiomer 4a ($[\alpha]_{D}^{20} +142.7$ (c 0.1 MeOH)) and 4b ($[\alpha]_{D}^{20} -99.5$ (c 0.1 MeOH)), which had opposite ECD curves. By comparison of the calculated ECD spectra of the $1R,2R,6'R$ and $1'S,2'S,6'S$ configurations of 4 with the experimental data of 4a and 4b (Fig. 10), the absolute configurations of 4a and 4b were assigned as $1R,2R,6'R$ and $1'S,2'S,6'S$. Thus, 4a was defined as (+)-(1R,2R,6'R)-clauselansine B, and 4b was identified as the known compound (−)-(1S,2S,6'S)-clauselansine B.

Compound 5 (5a/5b) was obtained as a white powder. The HRESIMS displayed $m/z$ 364.1163 [M + Na]$^+$ (calcd for $C_{19}H_{19}NO_{5}$Na 364.1155), which was consistent with a molecular formula of $C_{19}H_{19}NO_{5}$ with eleven indices of hydrogen deficiency. Its NMR data (Table 3) was almost identical to those of claulansine D. Chiral isolation of 5 afforded the enantiomer 5a ($[\alpha]_{D}^{20} +62.0$ (c 0.11 MeOH)) and 5b ($[\alpha]_{D}^{20} -99.2$ (c 0.1 MeOH)).
which had opposite ECD curves (Fig. 11). The absolute configurations of 5a and 5b were determined using the same methods as described in 4a and 4b. Thus, 5a was defined as (+)-(1R,2R)-clauselansine D, and 5b was identified as the known compound (−)-(1S,2S)-clauselansine D.

The structures of nine known compounds were also identified by comparing their spectroscopic data to those found in the literature. The known compounds isolated were claulansine B (4b), claulansine D (5b), (−)-(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (6a), (+)-(1R,3S)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (6b), (R)-isoplatydesmine (7a), (S)-isoplatydesmine (7b), clauselansine A (8), ribaline (9), γ-fagarine (10), dictamnine (11), N-(2-hydroxy-2-phenylethyl)-cinnamamide (12), N-phenylethylbenzamide (13).

Compounds 1–13 were evaluated for their neuroprotective effect on neuron-like PC12 cells induced by Aβ25-35, and Okadaic Acid (OKA) in vitro using the MTT method. The neuron growth factor (NGF) was used as a positive control. At 10 μM, 1b, 2a, 3b, 5a, 5b increased the cell survival rate of the Okadaic acid-treated group, other compounds were inactive, while all the compounds failed to protect cells from Aβ25-35.

Clausenamide is one of the novel compounds isolated from Clausena lansium (Lour) skel. Clausenamide is unusual in that it contains 4 chiral centers yielding 8 pairs of enantiomers. In pharmacological studies numerous models and indicators showed that (−)-clausenamide is the active enantiomer, while (+)-clausenamide is inactive and elicits greater toxicity than (−)-clausenamide. Compounds 3 and clausenamide are very similar in structure and therefore have the same biological activity. Similarly, 3b is the active enantiomer, while 3a is inactive. The carbazole and quinolone alkaloids having neuroprotective effects were exhibited in previous researches, and carbazole alkaloids may be derived from indole alkaloids. As we all know, thalidomide as a chiral racemic compound, its R-configuration has inhibitory activity of pregnancy, while S-configuration has teratogenic. One of the isomers of the enantiomer is highly active and the other isomer is inactive, or both isomers are active, or both isomers are inactive. Thus, it’s reasonable that compound 1b, 2a, 5a, and 5b are active while 1a, 2b are inactive. All in all, alkaloids isolated from C. lansium are worthy of study to find more potential effects in the further (Table 4).

**Conclusions**

In summary, we have reported the isolation, identification and biological study of twenty compounds (1–13) including three new enantiomers (1a, 1b, 2a, 2b, 3a, 3b), two new ones (4a, 5a), and several of them inhibited PC12 cell damage induced by Okadaic Acid. Furthermore, compounds 6–7 were isolated from the genus Clausena for the first time. The occurrence of alkaloids derivatives from C. lansium is in agreement with the previous findings, 1–7 indicating that the isolation of these enantiomer compounds might be a useful chemotaxonomic for screening activity. The results of preliminary neuroprotective effect assays suggested that several isolated alkaloids derivatives showed moderate activity. Moreover, previous findings exhibited that the carbazole, amide and quinolone alkaloids metabolites have neuroprotective effects and other biological activities such as anti-inflammatory, hepatoprotective, cytotoxic which indicating that the alkaloid compounds and their biological activities of C. lansium are worth studying in order to find compounds with potential activity.

**Experimental**

Optical rotations were measured on a JASCO P2000 automatic digital polarimeter. UV spectra were recorded on a JASCO V-650 spectrophotometer, CD spectra were measured on a JASCO J-815 spectropolarimeter. IR spectra were recorded on a Nicolet 5700 spectrometer using an FT-IR microscope transmission method. NMR spectra were acquired with Bruker AVIIIHD 600, VNS-600, and Mercury-400 spectrometers in DMSO-d6, and MeOD-d4. HREIMS spectra were collected on an Agilent 1100 series LC/MSD ion trap mass spectrometer. MPLC system was composed of two C-605 pumps (Büchi), a C-635 UV detector (Büchi), a C-660 fraction collector (Büchi), and an ODS column (450 mm × 60 mm, 50 μm, 400 g; YMC). Semi-preparative HPLC was conducted using a Shimadzu LC-6AD instrument with an SPD-20A detector and a Daicel Chiralpak AD-H column (250 × 10 mm, 5 μm). Preparative HPLC was also performed on

**Table 4 Neuroprotective effects of compounds 1–13 against Okadaic acid-induced injury in PC12 Cells (10 μM, means ± SD, n = 6)**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Control (10 μM)</th>
<th>1a (10 μM)</th>
<th>1b (10 μM)</th>
<th>2a (10 μM)</th>
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<th>4b (10 μM)</th>
<th>5a (10 μM)</th>
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<td>100.0 ± 2.64</td>
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<td>76.6 ± 7.5</td>
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*p < 0.001 vs. control, **p < 0.001 vs. model, *p < 0.01 vs. model.*
a Shimadzu LC-6AD instrument with a YMC-Pack ODS-A column (250 × 20 mm, 5 μm). Column chromatography (CC) was performed with silica gel (200–300 mesh, Qingdao Marine Chemical Inc., Qingdao, People’s Republic of China), SF-PRP 512A (100–200 mesh, Beijing Sunflower and Technology Development Co., Beijing, People’s Republic of China), ODS (50 μm, YMC, Japan), and Sephadex LH-20 (GE, Sweden). TLC was carried out on glass precoated silica gel GF254 plates. Spots were visualized under UV light or by spraying with 10% sulfuric acid in EtOH followed by heating. PC12 cells (adrenal gland; pheochromocytoma) were purchased from the American Type Culture Collection. Dimethyl sulfoxide (DMSO), Aβ$_{35-35}$, Okadaic acid, 3-(3,4-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) were obtained from Sigma (St Louis, MO, USA). Dulbecco’s Modified Eagle’s Medium (DMEM), fetal bovine serum (FBS) and equine serum were purchased from Gibco BRL (New York, NY, USA). All other chemicals were of analytical grade and were commercially available.

**Plant material.** The stems of *C. lansium* were collected in Liuzhou, Guangxi, China, in December 2008 and identified by Enginer Guangri Long, Forestry of Liuzhou. A voucher specimen has been deposited at the Herbarium of Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College (ID-S-2320).

**Extraction and isolation.** Air-dried, powdered stems of *C. lansium* (200 g) were macerated for 3 h with 20 L of 95% ETOH (aq) and refluxed for 4 h (20 L × 2). The filtrate was evaporated under reduced pressure to yield a dark brown residue (640 g). The residue was suspended in water (2000 mL) and then partitioned with CHCl$_3$ (3 × 2000 mL), EtOAC (3 × 2000 mL), and n-BuOH (3 × 2000 mL), successively. After removing the solvent, the n-BuOH-soluble portion (850 g) was fractionated via macroporous adsorbent resin (HPD-100) column with H$_2$O, 30% ETOH, 60% ETOH, and 95% ETOH to yield four corresponding fractions A-D.

Fraction B (180.6 g) was fractionated via silica gel column chromatography, eluting with CHCl$_3$–MeOH–H$_2$O (10 : 1 : 0.05, 9 : 1 : 0.1, 8 : 2 : 0.2, 7 : 3 : 0.3, 6 : 4 : 0.4) to afford twelve fractions B$_1$-B$_{12}$ on the basis of TLC analysis. Fraction B$_4$ (5.9 g) was further separated by reversed-phase silica MPLC with MeOH–H$_2$O (20–50%, 50 mL min$^{-1}$, 6 h) to afford B$_{4a}$-B$_{4g}$ fractions. Fractions B$_{4a}$-B$_{4b}$ were successively separated using preparative HPLC (detection at 210 nm, 18% CH$_3$CN, 8 mL min$^{-1}$) to yield 1 (20.3 mg, t$_R$ 37.50 min). Compound 1 was further separated by semipreparative chiral HPLC (n-hexane-2-propanol, 8 : 1, 3 mL min$^{-1}$) to give 2a (2.8 mg, t$_R$ 48.14 min) and 2b (2.1 mg, t$_R$ 49.16 min). Compound 2 was further separated by semipreparative chiral HPLC (n-hexane-2-propanol, 7 : 1, 3 mL min$^{-1}$) to give 7a (4.8 mg, t$_R$ 19.85 min) and 7b (5.3 mg, t$_R$ 25.86 min). Fraction C$_{1-5}$ was successively separated by Sephadex LH-20 and then using preparative HPLC (detection at 210 nm, 29% CH$_3$CN, 8 mL min$^{-1}$) to yield 2 (5.2 mg, t$_R$ 48.14 min). Compound 2 was further separated by semipreparative chiral HPLC (n-hexane-2-propanol, 8 : 1, 3 mL min$^{-1}$) to give 2a (2.8 mg, t$_R$ 25.81 min) and 2b (2.1 mg, t$_R$ 34.17 min). Fraction C$_{1-5}$ was further separated by reversed-phase silica MPLC with MeOH–H$_2$O (35–55%, 50 mL min$^{-1}$, 6 h) to afford C$_{1-5}$-1–C$_{1-5}$-8 fractions. Fraction C$_{1-5}$ was successively separated via silica gel column chromatography and then using preparative HPLC (detection at 210 nm, 32% CH$_3$CN, 8 mL min$^{-1}$) to yield 3 (10.7 mg, t$_R$ 53.75 min), 4 (9.2 mg, t$_R$ 39.05 min), 10 (3.1 mg, t$_R$ 52.10 min), 12 (2.4 mg, t$_R$ 49.16 min) and 13 (3.2 mg, t$_R$ 65.64 min). Compound 3 was further separated by semipreparative chiral HPLC (n-hexane-2-propanol, 9 : 1, 3 mL min$^{-1}$) to give 3a (4.1 mg, t$_R$ 19.87 min) and 3b (3.9 mg, t$_R$ 25.12 min). Compound 4 was further separated by semipreparative chiral HPLC (n-hexane-2-propanol, 7 : 1, 3 mL min$^{-1}$) to give 4a (1.5 mg, t$_R$ 21.98 min) and 4b (4.3 mg, t$_R$ 40.07 min). Fraction C$_6$ was further separated by reversed-phase silica MPLC with MeOH–H$_2$O (35–55%, 50 mL min$^{-1}$, 6 h) to afford C$_6$-1–C$_6$-60 fractions. Fraction C$_{1-5}$ was successively separated via silica gel column chromatography and then using preparative HPLC (detection at 210 nm, 30% CH$_3$CN, 8 mL min$^{-1}$) to yield 8 (5.2 mg, t$_R$ 33.31 min) and 11 (7.1 mg, t$_R$ 23.17 min).

**(+)-(2'S,3'R)-Clauselansine A (1a).** Colourless oil; [α]$_D^{20}$ +18.7 (c 0.1 MeOH); UV (MeOH) λ$_{max}$ (log ε) 202.8 (4.29), 220.6 (4.38), 281.8 (3.48) nm; ECD (MeOH) λ$_{max}$ (Δε) 308 (+0.75), 226 (+4.01) nm; IR (microscope) ν$_{max}$ 3358, 2917, 2851, 1615, 1377, 1379, 1109, 1040, 749 cm$^{-1}$; $^1$H NMR (DMSO-d$_6$, 400 MHz) and $^{13}$C NMR (DMSO-d$_6$, 100 MHz), see Table 1; HRESIMS m/z 320.1854 [M + H]$^+$ (calculated for C$_{18}$H$_{28}$NO$_4$, 320.1856).

**(+)-(2'R,3'S)-Clauselansine A (1b).** Colourless oil; [α]$_D^{20}$ +17.0 (c 0.1 MeOH); ECD (MeOH) λ$_{max}$ (Δε) 268 (−0.23), 226 (−0.79) nm; UV, IR, NMR, and HRESIMS were the same as those of 1a.

**(−)-(2'S,3'R)-Clauselansine B (2a).** White powder; [α]$_D^{20}$ +28.0 (c 0.1 MeOH); UV (MeOH) λ$_{max}$ (log ε) 203.0 (4.37), 220.0 (4.37), 281.6 (3.53) nm; ECD (MeOH) λ$_{max}$ (Δε) 276 (−0.18), 233 (+1.03) nm; IR (microscope) ν$_{max}$ 3358, 2917, 2851, 1615, 1377, 1039, 1002, 739 cm$^{-1}$; $^1$H NMR (DMSO-d$_6$, 600 MHz) and $^{13}$C NMR (DMSO-d$_6$, 150 MHz), see Table 1; HRESIMS m/z 302.1758 [M + H]$^+$ (calculated for C$_{18}$H$_{28}$NO$_4$, 302.1751).
(−)-(2'R,3'S)-Clauselansine B (2b). White powder; [α]D20 −32.6 (c 0.1 MeOH); ECD (MeOH) λ max (Δε) 280 (+0.35), 232 (−1.56) nm; UV, IR, NMR, and HRESIMS were the same as those of 2a.

(+)-(3S,4R,5S,6S)-Clauselansine C (3a). White powder; [α]D20 +17.9 (c 0.1 MeOH); UV (MeOH) λ max (log ε) 203.8 (4.28) nm; ECD (MeOH) λ max (Δε) 272 (−0.53), 234 (+1.27), 215 (−2.27) nm; IR (microscope) r max 3230, 2923, 1678, 1483, 1454, 1403, 1202, 1132, 1078, 753, 703 cm−1; 1H NMR (DMSO-d6, 400 MHz) and 13C NMR (DMSO-d6, 100 MHz), see Table 2; HRESIMS m/z 280.1333 [M + Na]+ (calcd for C18H17NaNO2, 280.1332).

(+)-(3R,4S,5R,6R)-Clauselansine C (3b). White powder; [α]D20 −22.0 (c 0.1 MeOH); ECD (MeOH) λ max (Δε) 272 (+0.27), 234 (−1.31), 215 (+1.84) nm; UV, IR, NMR, and HRESIMS were the same as those of 3a.

Preparation of acetonide derivative (1c).
To determine the relative configuration of 1b, compound 1b (3 mg) was treated with 2,2-dimethoxypropane (1 mL) and pyridinium p-toluene sulfonate (1 mg), then stirred at 30 °C for 5 h under N2 circumstance. The reaction solution was evaporated in vacuo and purified by reversed-phase preparative HPLC using CH3OH/H2O (60 : 40 v/v) to yield the acetonide (1c) as a white amorphous powder. [α]D20 +62.0 (c 0.11 MeOH); UV (MeOH) λ max (log ε) 195.8 (3.71), 231.0 (3.87), 278.2 (4.11) nm; ECD (MeOH) λ max (Δε) 335 (+0.28), 261 (−1.94), 243 (+1.89), 225 (−1.50) nm; IR (microscope) r max 3319, 2971, 1613, 1573, 1503, 1456, 1383, 1248, 1081, 1024, 745 cm−1; 1H NMR (DMSO-d6, 600 MHz) and 13C NMR (DMSO-d6, 150 MHz), see Table 3; HRESIMS m/z 326.1398 [M + H]+ (calcd for C19H19NNaO5, 326.1394).

Preparation of (R)- and (S)-MTPA esters of 3a.
A solution of 3a (1.31 mg) in dehydrated CH2Cl2 (2 mL) was treated with (R)-(+)−methoxy−z-(trifluoromethyl)phenylacetyl chloride [(R)-MTPA-Cl (10 mg)] in the presence of anhydrous pyridine, and the mixture was stirred at room temperature for 13 h. After cooling, the reaction mixture was poured into iced-water and extracted with EtOAc. The EtOAc extract was successively washed with 5% aqueous HCl, saturated aqueous NaHCO3, and brine, then dried over Na2SO4 and filtered. The solvent was removed from the filtrate under reduced pressure. The residue was purified by semi-preparative HPLC (C18 column, 3.0 mL min−1, UV 210 nm, 80% CH3CN–H2O) to yield (S)-MTPA ester derivative of 3a (compound 3aa 1.03 mg). (R)-MTPA ester derivative of 3a (compound 3ab 0.92 mg) was obtained from 3a (1.05 mg).

(−)-(1R,2R,6R,7R)-Clauselansine B (4a). White powder; [α]D20 +142.7 (c 0.1 MeOH); UV (MeOH) λ max (log ε) 201.6 (4.40), 241.8 (4.24), 294.8 (3.68) nm; ECD (MeOH) λ max (Δε) 335 (+0.90), 290 (−1.71), 244 (−15.40), 223 (−2.31) nm; IR (microscope) r max 3319, 2971, 1613, 1573, 1503, 1456, 1383, 1248, 1081, 1024, 745 cm−1; 1H NMR (DMSO-d6, 600 MHz) and 13C NMR (DMSO-d6, 150 MHz), see Table 3; HRESIMS m/z 326.1398 [M + Na]+ (calcd for C19H19NNaO5, 326.1387).

(−)-(1R,2R,6R)-Clauselansine D (5a). White powder; [α]D20 +52.0 (c 0.11 MeOH); UV (MeOH) λ max (log ε) 195.6 (3.71), 235.0 (3.87), 278.2 (4.11) nm; ECD (MeOH) λ max (Δε) 335 (+0.28), 261 (−1.94), 243 (+1.89), 225 (−1.50) nm; IR (microscope) r max 3319, 2971, 1613, 1573, 1503, 1456, 1383, 1248, 1024, 745 cm−1; 1H NMR (DMSO-d6, 600 MHz) and 13C NMR (DMSO-d6, 150 MHz), see Table 3; HRESIMS m/z 364.1163 [M + Na]+ (calcd for C21H20NNaO5, 364.1155).

Preparation of (S)-Clauselansine B (1a).
To determine the relative configuration of 1b, compound 1b (3 mg) was treated with 2,2-dimethoxypropane (1 mL) and pyridinium p-toluene sulfonate (1 mg), then stirred at 30 °C for 5 h under N2 circumstance. The reaction solution was evaporated in vacuo and purified by reversed-phase preparative HPLC using CH3OH/H2O (60 : 40 v/v) to yield the acetonide (1c) as a white amorphous powder. [α]D20 +12.0 (c 0.2 MeOH); UV (MeOH) λ max (log ε) 206 (6.24), 225 (6.40), 277 (5.75) nm; ECD (MeOH) λ max (Δε) 267 (−1.28), 251 (−0.78), 234 (−2.14) nm; MoO2(OAc)3-induced CD (DMSO) 323 (Δε +0.26) nm; IR (microscope) r max 3307, 2923, 2854, 1645, 1542, 1452, 1400, 1240, 1099, 660 cm−1; 1H NMR (DMSO-d6, 600 MHz) and 13C NMR (DMSO-d6, 150 MHz), see Table 1; HRESIMS m/z 382.1993 [M + Na]+ (calcd for C21H20NNaO5, 382.1989).

Neuroprotection bioassays.
Phaeochromocytoma (PC12) cells were incubated in DMEM supplemented with 5% fetal bovine serum and 5% equine serum as basic medium. PC12 cells in logarithmic phase were cultured at a density of 5000 cells per well in a 96-well microtiter plate. After 24 h incubation, the medium of the model group was changed to DMEM or basic medium with 15 μM Ab25-35 for 48 h or basic medium with 50 nM OKA for 24 h. Test compounds dissolved in dimethyl sulfoxide (DMSO) were added to each well for >1000-fold dilution in the model medium at the same time. Each sample was tested in triplicate. After the incubation at 37 °C in 5% CO2 for 24 h, 10 μL of MTT (5 mg mL−1) was added to each well and incubated for another 4 h; then liquid in the wells was removed. DMSO (100 μL) was added to each well. The absorbance was recorded on a microplate reader (Bio-Rad model 550) at a wavelength of 570 nm.37 Analysis of variance (ANOVA) followed by Newman–Keuls post hoc test were performed to assess the differences between the relevant control and each experimental group. p-Values of <0.05, <0.01, and <0.001 were regarded as statistically significant. Data were expressed as mean ± SEM as indicated.

Conflict of interest.
There are no conflicts of interest to declare.
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Notes and references