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

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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); (+)-(3S,4R,5S,6S)-clauselansine C (3a) and (−)-(3R,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_{26}NO_{4} based on the ^{13}C 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 ^{1}H NMR (Table 1) spectrum showed a set of signals for 1,2,3-

Introduction

*Clausena* is a small genus within the family Rutaceae, which is distributed throughout the south of the Yangtze River in China, such as the Yunnan, Guangxi and Guangdong regions. Previous phytochemical studies have revealed that the *Clausena* genus contains a wide variety of carbazole alkaloids, amide alkaloids, coumarins, and limonoids, many of which possess various pharmacological properties such as antibacterial, anti-inflammatory, anti-HIV, cytotoxic, hepatoprotective and neuroprotective effects. The leaves and fruits of *Clausena lansium*, *Clausena excavata*, *Clausena emarginata*, and *Clausena anisomoles* are not only eaten as food but also used for traditional medicine.

*Clausena lansium* (Lour.) Skeels (Rutaceae), a fruit tree, was widely distributed in southern China. In traditional Chinese medicine, the leaves and roots of *C. lansium* were used for cough, asthma, dermatological disease, viral hepatitis, and gastro-intestinal diseases; the seeds are used to treat acute and chronic gastro-intestinal inflammation, ulcers, etc. Various bioactive constituents including coumarins, carbazole alkaloids and amide alkaloids have been isolated and identified from this plant. Previously, twenty new natural products including thirteen new carbazole alkaloids, eight alkaldoid glycosides, four new coumarins, a new amide and a new megastigmene glucoside from the leaves and skedes of *C. lansium* were reported by our research group, and some of these alkaloids showed selective neuroprotective effects. In order to investigate the potential neuroprotective constituents from different parts of *C. lansium*, n-BuOH extract of the stems of this plant were selected for investigation. This paper reported further investigation of n-BuOH extract from the stems of *C. lansium* which led to the isolation and characterization of four new indole alkaloids (1a, 1b, 2a, 2b), two new amide alkaloids (3a, 3b), and two carbazole alkaloids (4a, 5a) along with twelve known compounds (4b, 5b, 6a, 6b, 7a, 7b, 8–13) from the stems of *C. lansium*. The neuroprotective activities of 1–13 were also evaluated. We present herein the isolation and structural characterization of 1–13, as well as their bioactivities (Fig. 1).
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 5.66 (1H, t, J = 7.3 Hz, H-2″), a methylene at 3.55 (2H, d, J = 7.3 Hz, H-1″) and two methyli 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 hexalobines19 suggested that their structures are closely related, except for 1,3,4-trihydroxy-3-methylbut-2-y1 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-y1 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]D 20 +18.7 (c 0.1 MeOH); 1b: [α]D 20 −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 p-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.20 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 (TD-DFT) method at the B3LYP/6-31G (d) level.21,22 The calculated ECD spectrum for 2′R,3′S- 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.23,24 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*. 

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Table 1  $^1$H and $^{13}$C NMR spectroscopic data of compounds 1, 1c, and 2 ($\delta$ in ppm, J in Hz)

| Position | $\delta_{H}^{a}$ | $\delta_{C}^{b}$ | $\delta_{H}^{a}$ | $\delta_{C}^{b}$ | $\delta_{H}^{a}$ | $\delta_{C}^{b}$ |
|----------|------------------|------------------|------------------|------------------|------------------|------------------|
| 2        | 7.18, d (2.4)    | 123.0 d          | 7.18, d (2.4)    | 123.0 d          | 7.23, d (2.4)    | 123.9 d          |
| 3        | 114.2 s          | 106.1            | 114.2 s          | 106.1            | 118.7            | 115.6 d          |
| 3a       | 128.3 s          | 127.3            | 128.3 s          | 127.3            | 118.7            | 115.6 d          |
| 4        | 7.43, d (7.4)    | 116.8 d          | 7.43, d (7.4)    | 116.8 d          | 7.42, d (7.4)    | 116.5 d          |
| 5        | 6.90, t (7.4)    | 118.3 d          | 6.90, t (7.4)    | 118.3 d          | 6.90, t (7.4)    | 118.5 d          |
| 6        | 6.85, d (7.4)    | 119.7 d          | 6.85, d (7.4)    | 119.7 d          | 6.85, d (7.4)    | 119.9 d          |
| 7        | 123.7 s          | 123.4            | 123.7 s          | 123.4            | 123.5 s          | 123.5 s          |
| 7a       | 134.4 s          | 134.4            | 134.4 s          | 134.4            | 134.7 s          | 134.7 s          |
| 1'       | 4.02, m; 3.76, m | 62.6 t           | 4.28, d (11.6); 3.68, dd (11.6, 3.6) | 61.0 t           | 4.02, m          | 71.8 t          |
| 2'       | 3.21, m          | 43.6 d           | 2.87, d (3.6)    | 38.9 d           | 3.42, t (9.7)    | 46.3 d          |
| 3'       | 75.0 s           | 48.6 t           | 2.12, s          | 22.0 q           | 1.18, s          | 22.9 q          |
| 4'       | 3.19, m          | 68.4 t           | 3.41, m; 3.26, m | 68.4 t           | 3.79, s          | 80.5 t          |
| 5'       | 1.00, s          | 22.0 q           | 1.02, s          | 22.0 q           | 1.18, s          | 22.9 q          |
| 1''      | 3.55, d (7.3)    | 28.6 t           | 3.57, d (7.4)    | 28.7 t           | 3.56, d (7.4)    | 28.5 t          |
| 2''      | 5.66, t (7.3)    | 121.2 d          | 5.67, t (7.4)    | 121.2 d          | 5.65, t (7.4)    | 121.1 d         |
| 3''      | 136.3 s          | 136.3 s          | 136.3 s          | 136.3 s          | 136.3 s          | 136.3 s         |
| 4''      | 3.84, d (5.6)    | 66.3 t           | 3.85, s          | 66.2 t           | 3.83, s          | 66.2 t          |
| 5''      | 1.70, s          | 13.7 q           | 1.70, s          | 13.7 q           | 1.69, s          | 13.6 q          |
| NH       | 10.80, br s      | 10.60, s         | 10.60, s         | 10.60, s         | 10.90, s         | 10.90, s         |
| 1''      | 1.56, s          | 28.3             | 1.56, s          | 28.3             | 1.56, s          | 28.3            |
| 2''      | 71.7             | 26.0             | 71.7             | 26.0             | 71.7             | 26.0            |

$a$ In DMSO-$d_6$ (600 MHz). $b$ In DMSO-$d_6$ (150 MHz). Coupling constants (J) in Hz are given in parentheses. The assignments were based on HSQC and HMBC experiments.

The isolation of 1a was supported further by the HMBC correlations between $H$-1' ($\delta_{H}^{1}$, 4.02) and $C$-4' ($\delta_{C}^{4}$, 80.5) and $H$-4' ($\delta_{H}^{4}$, 3.79) and $C$-1' ($\delta_{C}^{1}$, 71.8). 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 $\{2a: [\alpha]_{D}^{20} +28.0 (c 0.1$ MeOH)$; 2b: [\alpha]_{D}^{20} -32.6 (c 0.1$ 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. 4 Calculated ECD spectra of (2'R,3'S)-1c, (2'R,3'R)-1c, (2'S,3'R)-1c, (2'S,3'S)-1c and the experimental ECD spectrum of (1c) in MeOH.

Fig. 5 CD spectrum of compound 1c in a DMSO of dimolybdenum tetracacetate (the inherent CD of the diol was subtracted).

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 C18H17NO2 based on the 13C NMR spectroscopic data and HRESIMS (m/z 280.1333 [M + Na]+, calcld for C18H17NO2Na, 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 1H NMR spectrum showed nine aromatic protons [ring A: δH 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: δH 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 δH 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 δH 5.97 (1H, s). The 13C NMR spectrum exhibited twelve aromatic carbons, one carbonyl δC 174.1, one oxymethine δC 75.0, three methines (δC 52.3, 53.3, 65.2), and one methyl (δC 29.6). The 1H, 1H-COSY correlations (Fig. 7) suggested one OCH–CH–CH–CH fragment. The 1H and 13C 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' (δC 144.7) and C-6' (δC 124.3); H-5 with C-2' (δC 144.7) and C-1' (δC 140.7); H-6 with C-3' (δC 125.8) and C-2' (δC 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.25,26 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-CH3 and H-6 with H-5, H-6', H-3 with H-2' showed that H-4, H-5 and H-6 were β orientation, while H-3 was α orientation. Thus, compound 3 (clauselansine C) was fully identified.

![Fig. 7](image-url)  
**Fig. 7** Key 1H, 1H-COSY, HMBC and NOESY correlations of compound 3.

### Table 2 1H and 13C NMR spectroscopic data of compounds 3 (δ in ppm, J in Hz)

| Position | δH a | δC b | Position | δH a | δC b |
|----------|------|------|----------|------|------|
| 2        | 4.11 (8.3) | 75.0 | 6'        | 7.45 (7.6) | 124.3 |
| 3        | 4.66 (8.3) | 65.2 | 2'        | 6.98 (7.1) | 129.6 |
| 4        | 4.77 (8.3) | 53.3 | 3', 5'    | 7.27, m | 128.2 |
| 1'       | 142.8 s | 4'   | 7.23 c    | 126.9 |
| 2'       | 144.7 s | N-CH3 | 2.10, s | 29.6 |
| 3'       | 6.92 (7.6) | 125.8 | OH       | 5.97, s | 128.0 |
| 4'       | 7.23  | 128.0 |          |      |

a In DMSO-d6 (600 MHz). b In DMSO-d6 (150 MHz). c Signal overlapped. Coupling constants (J) in Hz are given in parentheses. The assignments were based on HSQC and HMBC experiments.
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 \(3a: [\alpha]_D^{20} +17.9 \text{ (c 0.1 MeOH)}; 3b: [\alpha]_D^{20} -22.0 \text{ (c 0.1 MeOH)}\).

Compound 3a was treated with (R)- and (S)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA-Cl) in anhydrous CH2Cl2 to afford the 3a-(S)-MTPA ester (3aa) and 3a-(R)-MTPA ester (3ab), respectively. The \(\Delta [\alpha]_D\) values were calculated as shown in Fig. 8. Application of Mosher’s rule\(^{21}\) 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.\(^{21,22}\) 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)-clauleansine C and (−)-(3R,4S,5R,6R)-clauleansine C, respectively.

**Table 3** \(^{1}H\) and \(^{13}C\) NMR spectroscopic data of compounds 4a and 5a

| Position | \(\delta_{\text{H}}\) | \(\delta_{\text{C}}\) | \(\delta_{\text{H}}\) | \(\delta_{\text{C}}\) |
|----------|------------------|-----------------|------------------|------------------|
| 1        | 144.3            | 138.6           | 143.0            | 134.3           |
| 1A       | 132.6 s          | 136.3           |                   |                  |
| 2        | 123.2 s          | 135.1           | 123.6            | 136.3           |
| 3        | 129.1 s          | 118.8           | 123.6            | 136.3           |
| 4        | 7.62, s          | 111.2 s         | 8.37, s          | 112.7           |
| 4A       | 122.7 d          | 122.6           | 122.7 d          | 122.6           |
| 5        | 8.04, d (7.5)    | 120.1 d         | 8.27, d (7.5)    | 121.0           |
| 5A       | 123.6            | 126.3           | 123.6            | 126.3           |
| 6        | 7.14, t (7.5)    | 118.8           | 7.22, t (7.5)    | 119.6           |
| 7        | 7.38, t (7.5)    | 125.8           | 7.46, t (7.5)    | 126.7           |
| 8        | 7.50, t (7.5)    | 111.4           | 7.55, d (7.5)    | 111.7           |
| 8A       | 140.1            | 140.8           | 140.1            | 140.8           |
| 1\(^{1}\) | 4.77, d (7.5)    | 61.2            | 6.16, s          | 77.6            |
| 2\(^{1}\) | 4.30, s          | 86.0            | 3.89, d (7.5)    | 75.8            |
| 3\(^{1}\) | 76.9             | 71.6            |                  |                  |
| 4\(^{1}\) | 1.27, s          | 29.7            | 1.22, s          | 28.5            |
| 5\(^{1}\) | 1.04, s          | 23.2            | 1.26, s          | 24.7            |
| 6\(^{1}\) | 6.09, s          | 100.3           |                  | 170.8           |
| 1-OCH\(_{3}\) | 3.91, s | 61.0            | 4.06, s          | 60.3            |
| 1′-OH    | 5.34, d (7.5)    | 11.80 br s      |                  |                  |
| 2′-OH    | 5.71, d (6.0)    | 7.60            |                  |                  |

\(^{a}\) In DMSO-\(d_6\) 600 MHz, \(^{b}\) In DMSO-\(d_6\) 150 MHz. Coupling constants (\(J\)) in Hz are given in parentheses. The assignments were based on HSQC and HMBC experiments.

**Fig. 8** The Mosher’s method of 3a.

**Fig. 9** Calculated ECD spectra of (3S,4R,5S,6S)-3 and (3R,4S,5R,6R)-3 and the experimental ECD spectra of (+)- and (−)-clauleansine C (3a/3b) in MeOH.

**Fig. 10** Calculated ECD spectra of (1′R,2′R,6′R)-4 and (1′S,2′S,6′S)-4 and the experimental ECD spectra of (+)- and (−)-clauleansine B (4a/4b) in 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 (+)-(1′R,2′R)-clauselansine D, and 5b was identified as the known compound (−)-(1′S,2′S)-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 clauselansine B (4b), clauselansine D (5b), (−)-(1′S,3′S)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (6a), (+)-(1′R,3′S)-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), dictamine (11), N-(2-hydroxy-2-phenylethyl)-cinnamamide (12), N-phenethylbenzamide (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 several compounds isolated from Clausena lancea (Lour) Skeels. 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 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. lancea 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. lancea 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 others biological activities such as anti-inflammatory, hepatoprotective, cytotoxicity which indicating that the alkaloid compounds and their biological activities of C. lancea 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 MeOH-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

![Fig. 11 Calculated ECD spectra of (1′R,2′R)-5 and (1′S,2′S)-5 and the experimental ECD spectra of (+)- and (−)-clauselansine D (5a/5b) in MeOH.](image-url)
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), Aprotinin (28000 U/mL), and 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 Engineer Guangri Lou, 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 CHCl3 (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 H2O, 30% EtOH, 60% EtOH, and 95% EtOH to yield four different fractions A–D.

Fraction B (180.6 g) was fractionated via silica gel column chromatography, eluting with CHCl3–MeOH–H2O (10 : 1 : 0.05, 9 : 1 : 0.1, 8 : 2 : 0.2, 7 : 3 : 0.3, 6 : 4 : 0.4) to afford twelve fractions B1–B12, on the basis of TLC analysis. Fraction B4 (5.9 g) was further separated by reversed-phase silica MPLC with MeOH–H2O (20–50%, 50 mL min−1, 6 h) to afford B4-1–B4-47. Fractions B4-18–B4-42 were successively separated using preparative HPLC (detection at 210 nm, 18% CH3CN, 8 mL min−1) to yield 3 (0.7 mg, tR 53.75 min), 4 (9.2 mg, tR 39.05 min), 10 (3.1 mg, tR 52.10 min), 12 (2.4 mg, tR 49.16 min) and 13 (3.2 mg, tR 65.64 min). Fraction 3 was further separated by semipreparative chiral HPLC (n-hexane-2-propanol, 9 : 1, 3 mL min−1) to give 2a (2.8 mg, tR 25.81 min) and 2b (2.1 mg, tR 34.17 min). Compound 3 was further separated by reversed-phase silica MPLC with MeOH–H2O (35–55%, 50 mL min−1, 6 h) to afford C1-5-1–C1-5-8 fractions. Fraction C1-5-6 was successively separated via silica gel column chromatography and then using preparative HPLC (detection at 210 nm, 32% CH3CN, 8 mL min−1) to yield 4 (2.3 mg, tR 53.75 min) and 5 (3.6 mg, tR 49.16 min). Fraction C1-5-7 was further separated by reversed-phase silica MPLC with MeOH–H2O (35–55%, 50 mL min−1, 6 h) to afford C1-6-1–C1-6-10 fractions. Fraction C1-6-3 was successively separated via silica gel column chromatography and then using preparative HPLC (detection at 210 nm, 30% CH3CN, 8 mL min−1) to yield 5 (2.8 mg, tR 23.17 min) and 11 (7.1 mg, tR 23.17 min).

(+)-(2'S,3'R)-Clauselansine A (1a). Colourless oil; [α]D20 +18.7 (c 0.1 MeOH); UV (MeOH) λmax (log ε) 202.8 (4.29), 222.6 (4.38), 281.8 (3.48) nm; ECD (MeOH) λmax (Δε) 268 (+0.75), 226 (+4.01) nm; IR (microscope) νmax 3313, 2925, 1671, 1612, 1439, 1379, 1109, 1040, 749 cm−1; 1H NMR (DMSO-d6, 400 MHz) and 13C NMR (DMSO-d6, 100 MHz), see Table 1; HRESIMS m/z 320.1854 [M + H]+” (calcd for C18H24NO3, 320.1854).

(+)-(2'R,3'S)-Clauselansine A (1b). Colourless oil; [α]D20 −17.0 (c 0.1 MeOH); ECD (MeOH) λmax (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]D20 +28.0 (c 0.1 MeOH); UV (MeOH) λmax (log ε) 203.0 (4.37), 220.0 (4.37), 281.6 (3.53) nm; ECD (MeOH) λmax (Δε) 276 (−0.18), 233 (+1.03) nm; IR (microscope) νmax 3358, 2917, 2851, 1615, 1377, 1039, 1002, 739 cm−1; 1H NMR (DMSO-d6, 600 MHz) and 13C NMR (DMSO-d6, 150 MHz), see Table 1; HRESIMS m/z 302.1758 [M + H]+” (calcd for C18H24NO3, 302.1751).

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**Paper**

**RSC Advances**

RSC Adv. 2017, 7, 35417–35425 | 35423
(−)-[2'R,3'S]-Clauselansine B (2b). White powder; [α]D20 −32.6 (c 0.1 MeOH); ECD (MeOH) λmax (Δ) 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) λmax (log ε) 203.8 (4.28) nm; ECD (MeOH) λmax (Δ) 272 (−0.53), 234 (+1.27), 215 (−2.27) nm; IR (microscope) rmax 3320, 2923, 1678, 1483, 1454, 1403, 1202, 1132, 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 C18H12NaNO2, 280.1332).

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

(+)-(1'R,2'R,6'R)-Clauselansine B (4a). White powder; [α]D20 +142.7 (c 0.1 MeOH); UV (MeOH) λmax (log ε) 201.6 (4.40), 241.8 (4.24), 294.8 (3.68) nm; ECD (MeOH) λmax (Δ) 335 (+0.90), 290 (−1.71), 244 (+15.40), 223 (−2.31) nm; IR (microscope) rmax 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 C19H16NO4S, 326.1387).

(−)-(1'R,2'R)-Clauselansine D (5a). White powder; [α]D20 +62.0 (c 0.11 MeOH); UV (MeOH) λmax (log ε) 195.8 (3.71), 231.0 (3.87), 278.2 (4.11) nm; ECD (MeOH) λmax (Δ) 335 (+0.28), 261 (−1.94), 243 (+1.89), 225 (−1.50) nm; IR (microscope) rmax 3379, 2319, 2850, 1737, 1613, 1462, 1358, 1242, 1099, 729 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 C12H16NO4S, 364.1155).

Preparation of acetamide 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 circulation. The reaction solution was evaporated in vacuo and purified by reversed-phase preparative HPLC using CH3OH/H2O (60% 40 % v/v) to yield the acetamide (1c) (2.47 mg).

Compound 1c. White amorphous powder; [α]D20 +12.0 (c 0.2 MeOH); UV (MeOH) λmax (log ε) 206 (6.24), 225 (6.40), 277 (5.75) nm; ECD (MeOH) λmax (Δ) 267 (−1.28), 251 (−0.78), 234 (−2.14) nm; MoO2(OAc)3-induced CD (DMSO) 323 (Δ +0.26) nm; IR (microscope) rmax 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 C21H25NO6A4, 382.1989).

 Determination of absolute configurations of the 3′,4′-diol unit in 1c

According to the published procedure, a 1 : 1.2 mixture of diol/MoO2(OAc)3 for 1c was subjected to CD measurements at a concentration of 0.1 mg mL−1 in anhydrous DMSO. The first CD spectrum was recorded immediately after mixing, and its time evolution was monitored until stationary (about 10 min after mixing). The inherent CD was subtracted. The observed sign of the diagnostic band at around 310 nm in the induced CD spectrum was correlated to the absolute configuration of the 3′,4′-diol unit.

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-trifluoromethylphenylacetyl 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 ice-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 to afford a residue. 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).

Compound 3aa. Colourless oil; 1H NMR (600 MHz, CDCl3) δH 5.73 (1H, d, J = 4.2 Hz, H-3), 3.83 (1H, dd, J = 8.1, 4.2 Hz, H-4), 4.62 (1H, d, J = 8.1 Hz, H-5), 4.69 (1H, d, J = 8.1 Hz, H-6), 2.17 (3H, s, N-CH3), 3.69 (3H, s, OCH3), 6.99–7.67 (1H, Ar−H).

Compound 3ab. Colourless oil; 1H NMR (600 MHz, CDCl3) δH 5.52 (1H, d, J = 4.2 Hz, H-3), 4.07 (1H, dd, J = 8.1, 4.2 Hz, H-4), 4.75 (1H, m, H-5), 4.75 (1H, overlapped, H-6), 2.19 (3H, s, N-CH3), 3.59 (3H, s, OCH3), 7.00–7.70 (1H, Ar−H).

Neuroprotection bioassays

Pheochromocytoma (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 in basic medium. PC12 cells in logarithmic phase were cultured in basic medium. PC12 cells in logarithmic phase were cultured in basic medium. PC12 cells in logarithmic phase were cultured in basic medium. PC12 cells in logarithmic phase were cultured in basic medium. PC12 cells in logarithmic phase were cultured in basic medium.

Conflict of interest

There are no conflicts of interest to declare.
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