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Geranylated carbazole alkaloids with potential neuroprotective activities from the stems and leaves of *Clausena lansium*

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ABSTRACT

Clausena lansium (Lour.) Skeels is an evergreen small tree or shrub with great economic value, which belongs to the genus *Clausena* of the Rutaceae family. *C. lansium* is indigenous to Southern China, while currently widely cultivated in subtropical and tropical regions not only for the nutritional value and pharmacological uses of its fruits but also as a medicinal and ornamental plant. In this study, a systematic phytochemical study on the stems and leaves of *C. lansium* caused the separation and identification of two new geranylated carbazole alkaloids, clauselansiumines A (**1**) and B (**2**), as well as 10 known geranylated carbazole alkaloids (**3–12**). The chemical structures of these isolated geranylated carbazole alkaloids (**1–12**) were unambiguously determined based on comprehensive spectral data analyses. All these isolated geranylated carbazole alkaloids were tested for their neuroprotective effects against 6-hydroxydopamine induced cell death in human neuroblastoma SH-SY5Y cells *in vitro*. Compounds **1–12** displayed remarkable neuroprotective effects holding the EC_{50} values ranging from 0.48 ± 0.04 to 12.36 ± 0.16 μM. These research results disclosed that the separation and purification of these geranylated carbazole alkaloids possessing remarkable neuroprotective effects separated from *C. lansium* could be extremely important to the discovery of new agents for the treatment and prevention for Parkinson's disease.

1. Introduction

Parkinson's disease (PD) is a common progressive neurodegenerative disease of the central nervous system closely related to aging, which is characterized by the accumulation of cytoplasmic inclusions named Lewy bodies as well as the selective death of dopaminergic neurons in substania nigra, causing a clinical syndrome characterized by slowness of movement, stiffness, tremor and postural instability [1]. Due to the complex etiology of PD, the exact pathogenic factors for PD has not been fully illuminated, an effective therapeutic strategy for this disease has not yet been developed. The treatment with levodopa currently is the most effective therapeutic option for PD, however, it is only effective for symptomatic relief in the later stages of this disease. So, there is a particularly urgent medicinal need for new therapy for PD. Though there is no completely understood about the mechanisms accountable for the death of dopaminergic cell, more and more evidence obtained from human post-mortem studies and animal investigations has revealed that oxidative stress played an important role in initiating this process of Parkinson's disease [2–4]. Therefore, the inhibition against oxidative stress could be an effective and feasible treatment strategy for PD. A large number of studies have confirmed that lots of chemical neurotoxins have been associated with the pathological development of PD [5,6]. Based on these studies, lots of PD models have been established to assist in the evaluating neuroprotective agents for PD. Most of these experimental models are usually developed using the experimental neurotoxins, including 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) as well as 6-hydroxydopamine (6-OHDA). Among these neurotoxins, 6-hydroxydopamine is commonly applied as a dopaminergic neuron degenerative agent to study the neurotoxicity in the experimental models for PD for that 6-hydroxydopamine could selectively damage dopaminergic neurons in *vivo* and *in vitro* and could result in oxidative stress. Therefore, 6-hydroxydopamine is a effective useful tool in establishing experimental models of PD, applied for investigating the effectiveness and mechanisms of action of potential

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therapeutic agents for curing and/or alleviating PD [5,6].

The genus *Clausena* (Rutaceae) is composed of approximately 30 species which are distributed throughout the subtropical and tropical regions. There are approximately 10 species together with 2 varieties occurring in Southern China [7]. Several species of the genus *Clausena* are famous for their fruits, which are usually very popular tropical health-promoting fruits, while, their roots, stems, leaves and seeds have also been extensively applied in traditional Chinese medicine or folk medicine for the therapies of cold, malaria, dermatopathy and abdominal pain [8]. Among them, *Clausena lansium* (Lour.) Skeels, also known as wampee or wampi, is an evergreen small tree or shrub with great economic value, which belongs to the genus *Clausena* of the Rutaceae family. *C. lansium* is indigenous to Southern China and is currently widely cultivated in China, Malaysia, Vietnam, Indonesia, and the Philippines, less frequently grown in India, Queensland and Sri Lanka, occasionally cultivated even in Hawaii and Florida, not only for the nutritional value and pharmacological uses of its fruits but also as a medicinal and ornamental plant [8]. In China, the fresh ripe fruits of *C. lansium* are consumed as a very popular tropical fruit, whereas its leaves, stems, roots and seeds have been applied as folk medicine or as materials for Chinese medicine for the therapy of cold, fever, abdominal pain and stomach pain [7]. Previous phytochemical studies of *C. lansium* have already led to the isolation of a range of biologically active natural products, such as carbazole alkaloids, sesquiterpenes, glycosides and coumarins, which usually displayed a diverse variety of biological activities containing anti-inflammatory, neuroprotective, antitumor, hepatoprotective, anti-obesity, anti-microbial, anti-fungal, hypoglycemic and nematicidal activities [9–23]. The 90% EtOH extract of the stems and leaves of *C. lansium* was tested *in vitro* and displayed extremely pronounced neuroprotective effects against 6-hydroxydopamine induced cell death in human neuroblastoma SH-SY5Y cells holding the EC_{50} value of 7.36 ± 0.04 μg/mL in our preliminary study. In the course of our continuing study on biologically active and structurally diverse natural products from the tropical medicinal plants [24–28], a systematic chemical study on the stems and leaves of *C. lansium* was accordingly carried out and resulted in the separation and identification of two new geranylated carbazole alkaloids, clauselansiumines A (**1**) and B (**2**), as well as 10 known geranylated carbazole alkaloids, namely, mahanimbine (**3**) [29–31], mahanine (**4**) [32], murrayamine B (**5**) [32], murrastinine A (**11**) [33], pyrayafoline D (**6**) [34], *O*-methylpyrayafoline D (**7**) [34], murrayamine C (**8**) [33], murrayamine J (**9**) [35], murrayamine N (**10**) [35] and murrayakonine C (**12**) [36]. The chemical structures of **1** and **2** were unambiguously established by means of comprehensive spectral data analyses. The known geranylated carbazole alkaloids (**3–12**) were determined by comparing their experimental spectral data with those data described in the corresponding literature. In addition, all these isolated geranylated carbazole alkaloids (**1–12**) were measured for their neuroprotective effects against 6-hydroxydopamine induced cell death in human neuroblastoma SH-SY5Y cells *in vitro*. In our current paper, the extraction, separation, structure elucidation, neuroprotective effects of these isolated geranylated carbazole alkaloids will be described in detail.

2. Materials and methods

2.1. Apparatus and chemicals

A JASCO P-1020 digital polarimeter was applied to measure the optical rotations of geranylated carbazole alkaloids **1–12**. An Applied Photophysics Chirascan spectrometer was used for record the CD spectra of geranylated carbazole alkaloids **1–12**. A Beckman DU 640 spectrophotometer was used to acquire the UV spectra of new geranylated carbazole alkaloids, clauselansiumines A (**1**) and B (**2**); A Nicolet 6700 spectrophotometer was applied to obtain the IR spectra of new geranylated carbazole alkaloids, clauselansiumines A (**1**) and B (**2**); A Bruker 400 MHz spectrometer was applied to acquire the 1D and 2D NMR spectra of carbazole alkaloids **1–12**; A Thermo Scientific Q Exactive mass spectrometer was applied to record the HRESIMS spectra of geranylated carbazole alkaloids **1–12**; A Dionex UltiMate 3000 LC series with a MWD detector was applied to perform semi-preparative HPLC, with an Waters XBridge C_{18} column (5 μm, 250 × 10 mm); Precoated silica gel plates (G60, F-254) were the product of Yan Tai Zi Fu Chemical Group Co., and were applied for thin-layer chromatography; Silica gel (200–300 mesh) was the product of Yan Tai Zi Fu Chemical Group Co., and was applied for open column chromatography (CC); RP-18 gel (50 μm) was the product of YMC, and was applied for medium-pressure CC.

2.2. Plant material

The stems and leaves of *C. lansium* were collected from Bawangling Nature Reserve, Changjiang County, Hainan Province, China, in April 2017, which were identified by Yan-Hui Fu, Key Laboratory of Tropical Medicinal Resource Chemistry of Hainan Province. A voucher specimen (No. CLEM20170408) has been deposited at the Key Laboratory of Southern Medicinal Plants Resources of Haikou City.

2.3. Extraction, isolation, and purification procedures

The extraction of the powdered air-dried stems and leaves of *C. lansium* (18.8 kg) were performed using 48 L of 90% ethanol for four time under reflux at 55–60°C, each time for approximately 5 days. The extraction solution was combined and condensed on a rotary evaporator under reduced pressure to afford a crude residue, which was further dissolved in 20 L of distilled water, and then subjected to liquid-liquid extracting successively using petroleum ether (PE) for six times (each time for 20.0 L) and EtOAc for six times (each time for 20.0 L) to yield a PE extract as well as an EtOAc extract. The PE extract (1238.7 g) was chromatographed on a silica gel CC and eluted using PE/acetone (95:5–30:70, *v/v*) to give 10 fractions (Fr. 1–Fr. 10). Fr. 5 (24.9 g) was further separated on a RP-18 gel medium-pressure CC using the eluent of CH_3OH/H_2O (55:45–95:5, *v/v*) to yield seven fractions (Fr. 5A–Fr. 5G). Compounds **1** (12.3 mg), **5** (7.9 mg), **6** (87.2 mg), **10** (32.5 mg) and **12** (42.6 mg) were separated from Fr. 5B (6.3 g) *via* being purified by a Sephadex LH-20 gel CC and then were prepared by means of a semi-preparative HPLC (Waters XBridge C_{18} column, 5 μm, i.d. 10 × 250 mm, 33% CH_3CN, 2.8 mL/min, t_R 18.3, 28.7, 35.8, 43.1 and 47.9 min), respectively. Compounds **2** (6.3 mg), **7** (10.6 mg) and **8** (25.9 mg) were obtained from Fr. 5C (7.8 g) *via* being purified by a Sephadex LH-20 gel CC and then were prepared using a semi-preparative HPLC (Waters XBridge C_{18} column, 5 μm, i.d. 10 × 250 mm, 48% CH_3CN, 2.8 mL/min, t_R 30.2, 35.9 and 39.8 min), respectively. Compounds **4** (10.3 mg), **3** (11.2 mg), **9** (53.1 mg) and **11** (25.3 mg) were acquired from Fr. 5D (1.5 g) *via* being purified by a Sephadex LH-20 gel CC and then were obtained by means of a semi-preparative HPLC (Waters XBridge C_{18} column, 5 μm, i.d. 10 × 250 mm, 60% CH_3OH, 2.8 mL/min, t_R 15.3, 30.9, 39.4 and 46.9 min), respectively.

Clauselansiumine A (**1**): Yellowish amorphous powder; ESI-MS *m/z* 348 $[M+H]^+$; HR-ESI-MS *m/z* 348.1960 (M + H; calcd for $C_{23}H_{26}NO_2$, 348.1958); ^1H and ^{13}C NMR data (as shown in Table 1); [α] + 57.2 (c 0.10, CH_3OH); CD (0.00018 M, CH_3OH) λ_{max} (Δε) 271 (+18.6), 302 (−1.6) nm; UV (CH_3OH) λ_{max} (log ε) 228 (4.62), 279 (4.30), 301 (3.68) and 340 (3.38) nm; IR (KBr) ν_{max} 3392, 2977, 1616, 1506, 1470, 1379, 1243, 1161, 1055 and 793 cm^{-1}.

Clauselansiumine B (**2**): Yellowish amorphous powder; ESI-MS *m/z* 366 $[M+H]^+$; HR-ESI-MS *m/z* 366.2068 (M + H; calcd for $C_{23}H_{28}NO_3$, 366.2064); ^1H and ^{13}C NMR data (as shown in Table 1); [α] + 48.7 (c 0.13, CH_3OH); CD (0.00021 M, CH_3OH) λ_{max} (Δε) 270 (+22.8), 302 (−1.8) nm; UV (CH_3OH) λ_{max} (log ε) 232 (4.67), 282 (4.32), 303 (3.72) and 339 (3.36) nm; IR (KBr) ν_{max} 3388, 2982, 1618, 1507, 1468, 1381, 1239, 1159, 1049 and 788 cm^{-1}.

Table 1
^{1}H and ^{13}C NMR data of clauselansiumines A (**1**) and B (**2**).

Position	Clauselansiumine A (**1**)		Clauselansiumine B (**2**)	
	δ_H[a]	δ_C[b]	δ_H[c]	δ_C[d]
1		104.1 s		104.3 s
2		149.8 s		149.0 s
3		118.3 s		116.5 s
4	7.68 (1H, s)	121.2 d	7.69 (1H, s)	120.9 d
5	7.92 (1H, d, *J* = 8.6 Hz)	119.3 d	7.91 (1H, d, *J* = 7.8 Hz)	119.1 d
6	7.19 (1H, dd, *J* = 8.6, 7.8 Hz)	119.5 d	7.08 (1H, dd, *J* = 7.8, 7.6 Hz)	118.5 d
7	7.32 (1H, dd, *J* = 8.0, 7.8 Hz)	124.2 d	7.26 (1H, dd, *J* = 8.0, 7.6 Hz)	122.8 d
8	7.38 (1H, d, *J* = 8.0)	110.4 d	7.40 (1H, d, *J* = 8.0)	110.5 d
1a		134.9 s		135.1 s
4a		116.7 s		115.8 s
5a		123.9 s		124.0 s
8a		139.5 s		139.7 s
2′		78.1 s		78.0 s
3′	5.66 (1H, d, *J* = 9.8 Hz)	128.2 d	5.78 (1H, d, *J* = 10.0 Hz)	128.7 d
4′	6.67 (1H, d, *J* = 9.8 Hz)	117.7 d	6.94 (1H, d, *J* = 10.0 Hz)	118.0 d
2′a	1.47 (3H, s)	26.0 q	1.04 (3H, s)	26.3 q
5′*α*	1.81 (2H, t, *J* = 8.0 Hz)	40.5 t	1.97 (1H, m)	37.7 t
5′*β*			1.62 (1H, m)	
6′*α*	2.24 (2H, td, *J* = 8.0, 7.2 Hz)	22.4 t	1.72 (1H, m)	25.2 t
6′*β*			1.33 (1H, m)	
7′	5.43 (1H, t, *J* = 7.2 Hz)	125.9 d	3.02 (1H, d, *J* = 9.9 Hz)	77.8 d
8′		134.9 s		71.6 s
9′	1.64 (3H, s)	13.5 q	1.40 (3H, s)	25.2 q
10′	3.97 (2H, s)	68.9 t	0.99 (3H, s)	24.7 q
3-CH_3	2.35 (3H, s)	16.0 q	2.25 (3H, s)	15.8 q
7′-OH			4.36 (1H, s)	
8′-OH			4.08 (1H, s)	
10′-OH	5.31 (1H, s)			
NH	7.94 (1H, s)		11.20 (1H, s)	

[a] Measured in $CDCl_3$ at 400 MHz.
[b] Measured in $CDCl_3$ at 100 MHz.
[c] Measured in DMSO-d_6 at 400 MHz.
[d] Measured in DMSO-d_6 at 100 MHz.

2.4. Neuroprotective activities bioassays

In this bioassay, the human neuroblastoma SH-SY5Y cell line was used and acquired from the American Type Culture Collection. The cells were routinely cultured in complete DMEM supplemented with 10% FBS, 10 μg/mL streptomycin, 100 U/mL penicillin and maintained at 37 °C with 5% CO_2 in a humidified atmosphere. The neuroprotective activity assay was carried out in 96-well microplates using the MTT method. Briefly, the SH-SY5Y cells were cultured at a density of 2×10^4 cells/well in 200 μL for 24 h in 96-well plates. The cells were treated using the positive control (6-hydroxydopamine) at a concentration of 100 μM, and were treated by the PE extract and the EtOAc extract of the stems and leaves of *C. lansium* as well as geranylated carbazole alkaloids of various concentrations (0.0625, 0.32, 1.6, 8.0 and 40.0 μM) for an additional 24 h. The aell viability was assessed by treatment using MTT dissolved in 0.5 mg/mL of phosphate-buffered saline (PBS) for 4 hat 37 °C. Then, the PBS was carefully removed, and then the formazan crystals were dissolved in dimethyl sulfoxide. The absorbance of this solution was then measured at the wavelength of 540 nm by a microplate reader. Neuroprotective effect against 6-OHDA induced cell death was calculated *via* a semilogarithmic graph depicting the relationship between at least four different concentrations of geranylated carbazole alkaloids and their percentage effects of these concentrations on cell viability. All the samples were tested in triplicate, and the results were reported as the mean with standard deviation. All experimental results were typically expressed as the EC_{50} value using curcumin used as a positive control.

3. Results and discussion

3.1. Separation and structural elucidation of the isolated geranylated carbazole alkaloids

The stems and leaves of *C. lansium* were collected, dried, powdered and extracted with 90% ethanol. The extraction solution was combined and condensed on a rotary evaporator to afford a crude residue, which was further dissolved in distilled water and then partitioned successively with PE and EtOAc to yield a PE extract as well as an EtOAc extract, respectively. The PE extract was successively chromatographed over silica gel, RP-18 gel CC, Sephadex LH-20 together with semi-preparative HPLC to obtain two new geranylated carbazole alkaloids, clauselansiumines A (**1**) and B (**2**), as well as 10 known geranylated carbazole alkaloids, as depicted in Fig. 1.

Clauselansiumine A (**1**) was isolated as a yellowish amorphous powder holding a specific rotation of [*α*] + 57.2 (c 0.10, CH_3OH). The molecular formula of **1**, $C_{23}H_{25}NO_2$, was assigned by means of its HR-ESI-MS (*m/z* 348.1960 $[M+H]^+$, calcd 348.1958), requiring 12 indices

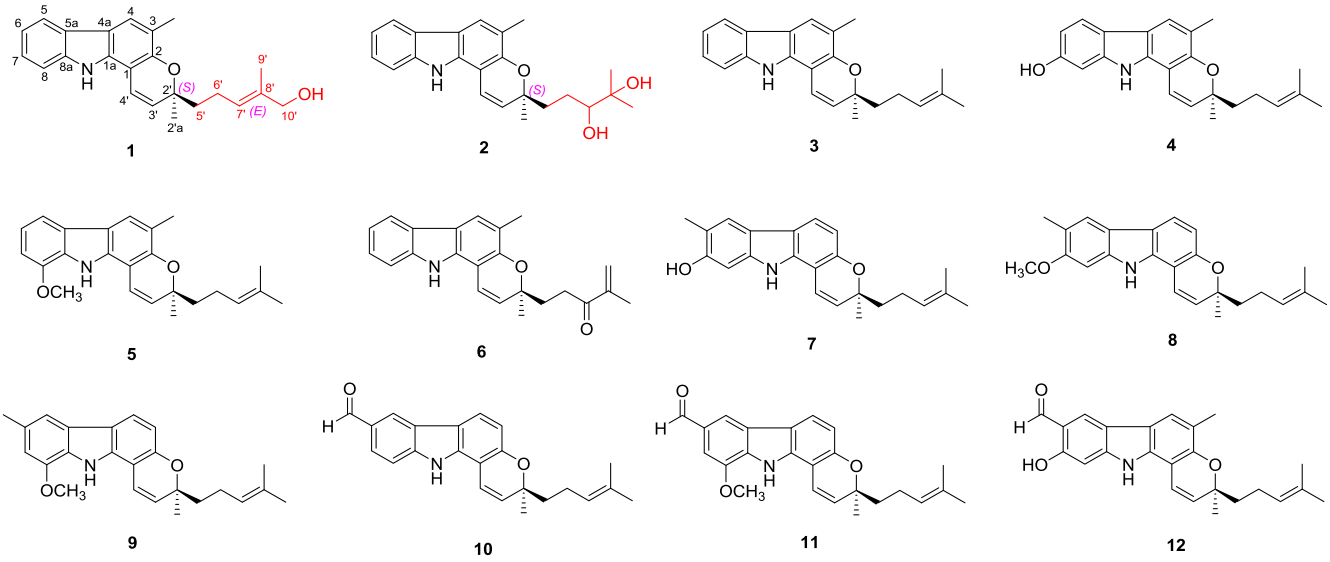

Fig. 1. Molecular structures of compounds **1–12** isolated from *C. lansium*.

of hydrogen deficiency. The IR spectrum of **1** gave the characteristic absorptions at 3392, 2977, 1616, 1506 and 1470 cm^{-1} revealing the presence of benzene ring, hydroxyl and double bond functionalities. The UV absorptions in the UV spectrum of **1** at 228, 279, 301 and 340 nm were characteristic absorption peaks of carbazole alkaloids [25]. Its ^{13}C NMR data combined with DEPT data (as shown in Table 1) suggested the presence of 23 carbons, which were due to 16 sp^2 carbons, one sp^3 quaternary carbon, three sp^3 methylenes and three methyls. Furthermore, the 16 sp^2 carbons were assigned to two double bonds and one carbazole ring. Since two double bonds together with one carbazole ring accounted for 11 out of 12 indices of hydrogen deficiency, the remaining one index of hydrogen deficiency was assigned to the presence of another one ring system in the molecular structure of **1**. The above data suggested that the chemical structure of **1** was extremely resemble to that of mahanimbine (**3**), apart from that the methyl group of C-10′ in mahanimbine (**3**) was replaced with a hydroxymethyl group in **1**, that was verified based on the HMBC correlations of H-7′ as well as H_3-9′ to C-10′ (δ_C 68.9), together with H_2-10′ to C-7′ (δ_C 125.9), C-8′ (δ_C 134.9) as well as C-9′ (δ_C 13.5) [28–31]. All these differences between **1** and mahanimbine (**3**) suggested that **1** was a 10′-hydroxyl derivative of mahanimbine (**3**), that was further verified by means of detailed analysis of the ^1H-^1H COSY, HSQC, HMBC as well as ROESY spectra, as depicted in Fig. 2. Furthermore, the ROESY correlation between H-7′ and H_2-10′ in the ROESY spectrum of **1** confirmed the assignment of the orientation of the double bond between H-7′ and H-8′ as *E*. From the biogenetic and structural point of view, some chemical structural analogues of **1** such as mahanimbine (**3**), mahanine (**4**), murrayamine B (**5**) and murrayakonine C (**8**), also separated from *C. lansium* this time, whose chemical structures and absolute configurations had been determined on the basis of comprehensive spectral data spectroscopic methods, possessing the same chiral centre at C-2′ with **1**, could be used as the model compounds for the assignment of the absolute configuration of **1** by comparing their rotation values and CD spectra. Therefore, the absolute configuration of **1** at C-2′ could be determined as 2′*S*, the same as that of mahanimbine (**3**), mahanine (**4**), murrayamine B (**5**) and murrayakonine C (**8**), in consideration of their resemble rotation values and similar patterns of Cotton effects in the CD spectra [29–31,33]. Therefore, the chemical structure of **1** was elucidated as depicted in Fig. 1.

Clauselansiumine B (**2**) possessed a molecular formula of $C_{23}H_{27}NO_3$ determined by means of its HR-ESI-MS (*m/z* 366.2068, $[M+H]^+$; calcd: 366.2064), which was larger than that of mahanimbine (**3**) by 34 mass units. Its ^{13}C NMR data combined with DEPT data (as shown in Table 1) suggested that **2** held 23 carbons in its molecular structure which display extremely resemble features with those of mahanimbine (**3**), apart from that the 3-methylbut-2-ene group located at C-5′ in mahanimbine (**3**) was replaced with a 3-methylbutane-2,3-diol group in **2** [28–31]. The above inference was verified based on the HMBC correlations of H-6′α, H-6′β, H_3-9′ together with H_3-10′ to C-7′ (δ_C 77.8) as well as C-8′ (δ_C 71.6), H-6′α and H-6′β to C-2′ (δ_C 78.0) as well as C-5′ (δ_C 37.7), together with H-7′ to C-5′ [28–31]. The planar structure of **2** was further confirmed on the basis of the detailed analyses of the HSQC, HMBC, ^1H-^1H COSY as well as ROESY spectra, as depicted in Fig. 3. Similar to **1**, geranylated carbazole alkaloid **2** possessed the same chiral centre at C-2′ with **1**. Therefore, the absolute configuration of **2** at C-2′ could be also determined as 2′*S*, the same as that of **1**, in consideration of their resemble rotation values and similar patterns of Cotton effects in the CD spectra. However, the relative or the absolute configuration of 2 at C-7′ cannot be determined on the basis of the available spectral data due to the free rotational feature of this type of compounds. Accordingly, the chemical structure of **2** was elucidated as depicted in Fig. 1.

3.2. Neuroprotective effect

All these separated geranylated carbazole alkaloids were tested for their neuroprotective effects against 6-hydroxydopamine induced cell death in human neuroblastoma SH-SY5Y cells using the MTT method *in vitro*, with curcumin as a positive control. As a result, all isolated geranylated carbazole alkaloids **1–12** displayed pronounced neuroprotective effects with the EC_{50} values ranging from 0.48 ± 0.04 to 12.36 ± 0.16 μM (as shown in Table 2), which are equivalent to that of the positive control (curcumin), even more remarkable neuroprotective effects than that of the positive control (curcumin). The neuroprotective effects of all these isolated geranylated carbazole alkaloids **1–12** were found to show some relationships with their chemical structures. Among these isolates, compounds **1**, **2**, **6** and **8** displayed more significant neuroprotective effects with the EC_{50} values ranging from 0.48 ± 0.04 to 1.57 ± 0.09 μM below that of curcumin, compounds **3**, **4**, **9** and **11** showed comparable neuroprotective effects with the EC_{50} values ranging from 2.59 ± 0.12 to 5.29 ± 0.12 μM, while the other compounds **7**, **10** and **12** showed relatively weaker neuroprotective effects, holding the EC_{50} values ranging from 6.85 ± 0.13 to 12.36 ± 0.16 μM above that of curcumin. It is worth mentioning that **1** displayed the most remarkable neuroprotective effect holding an EC_{50} value of 0.48 ± 0.04 μM, while the positive control, curcumin, possessed an EC_{50} value of 6.03 ± 0.10 μM. Compared with **1**, its structural analogues **2** and **6** also showed significant and comparable neuroprotective effects with **1**. Detailed analysis of the structures and the neuroprotective effects of **1**, **2** and **6** revealed that these compounds with a substituted isopentenyl group at C-2′ were more likely to possess significant neuroprotective effects. It was noteworthy that compound **8** displayed more remarkable neuroprotective effect than curcumin holding an EC_{50} value of 1.57 ± 0.09 μM. Compared with **8**, it structural analogue **7** showed the weakest neuroprotective effect holding an EC_{50} value of 12.36 ± 0.16 μM among all tested compounds. Detailed analysis of the chemical structures and the neuroprotective effects of **7** and **8** revealed that the methoxy group located at C-7 in the molecular structures of these compounds might be play an important role in their neuroprotective effects. In addition, compound **11** possessed pronounced neuroprotective effect holding an EC_{50} value of 2.59 ± 0.12 μM, while their structural analogues **10** and **12** displayed the moderate neuroprotective effects holding the EC_{50} values of 7.36 ± 0.15 as well as 6.85 ± 0.13 μM, respectively. Detailed analysis of the structures and the neuroprotective effects of **11–12** suggested that holding a methoxy group at C-8 in the molecular structures of these

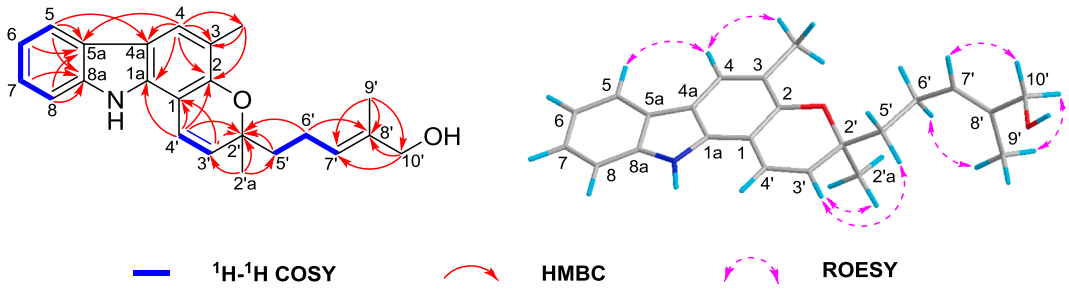

Fig. 2. Selected 2D NMR correlations for clauselansiumine A (**1**).

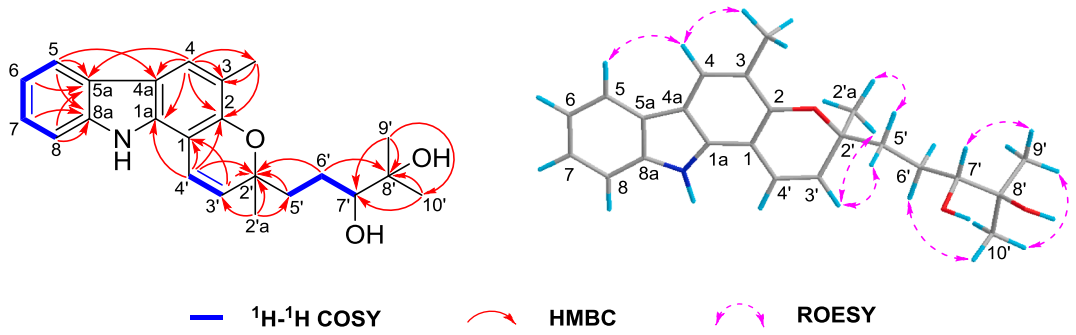

Fig. 3. Selected 2D NMR correlations for clauselansiumine B (**2**).

Table 2
Neuroprotective activities of compounds **1–12**.

Compound	EC_{50} (μM)[a]	Compound	EC_{50} (μM)[a]
1	0.48 ± 0.04	**7**	12.36 ± 0.16
2	0.98 ± 0.08	**8**	1.57 ± 0.09
3	5.29 ± 0.12	**9**	3.85 ± 0.11
4	3.25 ± 0.07	**10**	7.36 ± 0.15
5	6.46 ± 0.13	**11**	2.59 ± 0.12
6	1.28 ± 0.06	**12**	6.85 ± 0.13
Curcumin[b]	6.03 ± 0.10		

[a] EC_{50} was defined as the concentration giving half maximal protection against 6-OHDA induced cell death cell death in human neuroblastoma SH-SY5Y cells and expressed as the mean ± SD of triplicate determinations.
[b] Positive control.

compounds might be more important in their neuroprotective effects. The structure-activity relationships and molecular mechanisms of action of these isolated geranylated carbazole alkaloids with potential neuroprotective effects need to be further investigated. The present findings could provide a basis for further exploration of the stems and leaves of *C. lansium* as a new source of natural neuroprotective agents.

4. Conclusions

In conclusion, a systematic phytochemical study on the stems and leaves of *C. lansium* was implemented and caused the separation and identification of two new geranylated carbazole alkaloids, clauselansiumines A (**1**) and B (**2**), along with 10 known geranylated carbazole alkaloids (**3–12**). In addition, the neuroprotective effects of all these isolated geranylated carbazole alkaloids against 6-hydroxydopamine induced cell death in human neuroblastoma SH-SY5Y cells were also assessed and verified to be extremely remarkable. The pronounced neuroprotective effects of geranylated carbazole alkaloids **1–12** against 6-hydroxydopamine induced cell death in human neuroblastoma SH-SY5Y cells may account for the medical application of *C. lansium* in folk medicine or traditional Chinese medicine, which is usually considered to play an important role in preventing or reducing the occurrence of Parkinson's disease. All these research results also reveal that all these isolated geranylated carbazole alkaloids possessing remarkable neuroprotective effects separated from *C. lansium* could be considered as candidates for further research for therapeutic purposes in neural degenerative disease, especially Parkinson's disease.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.103278.

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