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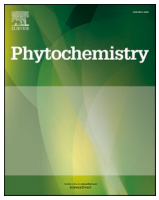

Gelselegine-gelsedine type bisindoles as well as the units from *Gelsemium elegans* with promoting proliferation of oral mucosa fibroblast cells

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ABSTRACT

Two undescribed bisindole alkaloids, gelseginedine A (**1**) and its rearranged gelseginedine B (**2**), and seven unreported gelselegine-type oxindole alkaloids (**3**–**9**) were isolated from the stems and leaves of *Gelsemium elegans*, together with five known alkaloids (**10**–**14**). Compounds **1** and **2** represented the first examples of gelselegine-gelsedine type alkaloids which bridged two units by a double bond. Their structures with absolute configurations were elucidated by means of HRESIMS, NMR and calculational chemistry. The performed bioassay revealed that **14** could promote the proliferation of human oral mucosa fibroblast cells.

1. Introduction

As a poisonous evergreen vine growing in Southeast Asia and southern China, *Gelsemium elegans* (Gardner. & Champ.) Benth. has been attracting numerous scholars at home and abroad to study *G. elegans* which was mainly applied externally in Chinese folk medicine, with the therapeutic efficacy of subduing swellings and pulling out toxins, killing worms and stopping itching, and dispelling wind. Up to now, more than 120 monoterpene indole alkaloids have been reported in the seeds, roots, flowers, stems and leaves of *G. elegans*, in addition to a number of compounds with variations in the substituent groups, and a number of dimers that are polymerized by different units and ways. For example, gelsekoumidines A and B, feature a 20,21-*seco*-koumine scaffold fused with a gelsedine framework via a double bond (Zhang et al., 2017). gelsecorydine B, composed of a gelsedine-type alkaloid and a modified corynanthe-type one through a pyridine ring (Li et al., 2018). Gelegenidines B and C are constructed from two monomeric units that are bridged by azo and carbonyl groups (Zhang et al., 2015). Through molecular network analysis of the *G. elegans* extracts in the literature, it is revealed that there are a large number of different dimers waiting for researchers to discover (Liu et al., 2022). In our study, we isolated nine undescribed alkaloids, along with five known analogues from the stems and leaves of *G. elegans* harvested in Yunnan, China. Recent years, with the in-depth research on the chemical composition and pharmacological effects of *G. elegans*, it is revealed that *G. elegans* also exhibits anti-tumor (Zhao et al., 2010), anti-inflammatory (Qu, et al., 2013; Li et al., 2021), and analgesic effects (Jin et al., 2021). No more than 10 gelselegine-type compounds have been reported in the literature. Studies on their activities have shown that gelselegine and 11-methoxygelselegine have immunoregulatory effects (Xu et al., 2012). There are two dimers containing gelselegine units, seven compounds with aldehyde groups attached to the N_4 position from *G. elegans* for the first time and investigated their proliferative activity of hOMF. In this paper, in addition to these pharmacological findings on Gelsemium alkaloids, the aim is to provide theoretical support for the subsequent isolation, structure elucidation and other new potential applications of this undescribed class of alkaloids.

2. Results and discussion

Alklaloid **1** was obtained as colorless oil. The molecular formula of **1** was determined to be $C_{40}H_{46}N_4O_9$ by its HR-ESI-MS data (*m/z* 727.3342 $[M+H]^+$, calcd for $C_{40}H_{47}N_4O_9$, 727.3343). The ^{1}H NMR spectrum of **1** exhibited signals for seven aromatic protons [δ_H 7.76 (1H, d, $J = 7.5$ Hz), 7.45 (1H, d, $J = 8.0$ Hz), 7.28 (1H, t, $J = 7.5$ Hz), 7.12 (1H, t, $J = 7.5$ Hz), 6.94 (1H, d, $J = 7.5$ Hz), 6.79 (1H, d, $J = 2.0$ Hz), 6.77 (1H, dd, $J = 8.0$, 2.0 Hz)], three methoxy groups [δ_H 3.56 (3H, s), 3.84 (3H, s), 3.94 (3H, s)], four oxymethylenes [δ_H 4.69 (1H, d, $J = 10.1$ Hz), 4.40 (1H, d, $J =$

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10.1 Hz), 4.25 (1H, d, $J = 10.6$ Hz), 4.13 (1H, dd, $J = 10.6$, 3.8 Hz)] and four oxymethines [δ_H 5.07 (1H, q, $J = 6.1$ Hz), 5.01 (1H, s), 4.11 (1H, s), 3.56 (1H, d, $J = 6.5$ Hz)]. Forty carbons, including five methyl, five methylenes, 18 methines, and 12 quaternary carbons were indicated in the ^{13}C NMR and DEPT spectra of **1**. The above spectral data implied that **1** could be a monoterpenoid bis-indole alkaloid combination with its characteristic UV spectrum (213.5 and 278.5 nm) of oxindole nucleus (Lin et al., 1990; Kitajima et al., 2003). A comparison of the NMR data of **1** with those of known alkaloids revealed that the **1** had 14-hydroxygelsenicine (Ponglux et al., 1988) and 11-methoxygelselegine (Xu et al., 2012) units. Proton at δ_H 5.07 (1H, q, $J = 6.1$ Hz) and carbon signals (δ_C 69.2) in the low-field region, combined with HMBC correlation between H-15 [δ_H 2.81 (1H, m)] with C-19, H-19 with C-21 (δ_C 140.9) indicated that a hydroxyl group was substituted on C-19 in the 11-methoxygelselegine unit (Fig. 2). Combined with the HMBC cross-peak between H-21 [δ_H 6.96 (1H, s)] and C-19′ (δ_C 136.6), the dimer was formed by connecting C-21 and C-19′ of the two units via carbon-carbon double bonds. The distribution of the ^{1}H and ^{13}C NMR signals of **1** could be obtained by ^{1}H–^{1}H COSY, HSQC, HMBC and NOESY experiments as shown Fig. 1.

The relative configuration of **1** was elucidated by the NOESY experiment, proton coupling constant and DP4+ analysis. The NOESY correlations between H-9 with H-17α, H-5 with H-16, H-15 with H-16, H-9′ with H-17α′, H-5′ with H-16′ as well as H-15′ with H-16′ indicated that the relative configurations of C-5, C-7, C-15, C-16, C-5′, C-7′, C-15′ and C-16′ in **1** were identical to those of 14-hydroxygelsenicine and 11-methoxygelselegine (Fig. 3). In addition, the NOESY correlations between H-21 with H-16/H-15′/H-16′ revealed the *E* configuration of C-21/19′ double bond. The configuration of the hydroxyl group at C-14′ was inferred to be β from the small coupling constant of the H-14′ (δ_H 5.01, s) (Kitajima et al., 2003).

Since both units were caged, so the absolute configurations of C-19 and C-20 were tried to elucidated by DP4+ analysis on four possible configurations, 19*S**20*S**–**1** (**1a**),19*S**20*R**–**1** (**1b**),19*R**20*S**–**1** (**1c**) and 19*R**20*R**–**1** (**1d**) (Fig. S1). The chemical shifts of isomers **1a**, **1b**, **1c** and **1d** were predicted with the polarized continuum model (PCM) in pyridine at the mPW1PW91/6-31+G(d,p)//M062X/def2svp level using the gauge-independent atomic orbitals (GIAO) method, and the chemical shifts of the isomers in pyridine were calculated using density functional theory (DFT). On this basis, the experimental and calculated chemical shifts were analyzed statistically DP4+ probability. Though possibility of isomer **1b** was the better than other configurations (Fig. S6), however, key data of them are not very agree with experimental data. Further, the calculated ECD were used to determine the configuration. Result showed CD cottons of the isomers 19*S*,20*R*-**1** and 19*R*20*R*-**1** were fitted with experimental data (Fig. 4). Consideration of simultaneously isolated 11-methoxy-19*R*-hydroxygelselegine (**14**), unit of **1**, hence, the absolute configuration of **1** was determined as 3*R*,5*S*,7*S*,15*R*,16*S*,19*R*,20*R*,3′*S*,5′*S*,7′*S*,14′*R*,15′*R*,16′*S* and named as gelseginedine A.

Compound **2** have the same molecular formula of $C_{40}H_{46}N_4O_9$ as **1** by its HRESIMS spectrum. Compared to the ^{1}H and ^{13}C spectra, both alkaloids had the same 14-hydroxygelsenicine unit. However, the chemical shifts of C-5, C-16, C-19 and C-20 of the gelselegine unit in compound **1** did not identically appear in compound **2**. According to the HMBC spectrum (Fig. 2), correlations of δ_H 3.39 (1H, dd, $J = 15.0$, 8.1 Hz), 2.65 (1H, d, $J = 15.0$ Hz) with δ_C 75.6, of δ_H 1.51 (3H, d, $J = 6.2$ Hz) with δ_C 75.6, and of δ_H 4.18 (1H, q, $J = 6.2$ Hz) with δ_C 141.4/56.0. Therefore, it could be inferred that pyrrolidine was rearranged to generate piperidine, and its planar structure is shown in Fig. 1. The relative configuration of **2** were identical to those of **1** except for new chiral centers. In addition, the NOESY spectrum of **2** exhibited obvious NOESY correlations between H-19 with H-6β/H-14β, suggesting that the hydroxyl at C-19 was α oriented.

For the determination of the configuration of the quaternary carbon C-20, the same DP4+ approach was used for the analyses. The ^{1}H and ^{13}C NMR chemical shifts of 19*S**20*R**–**2** (**2a**) and 19*S**20*S**–**2** (**2b**) (Fig. S7) at the mPW1PW91/6-31+G(d,p)//M062X/def2svp level with the PCM in pyridine were calculated, respectively. The results of DP4+ analysis showed that isomer **2a** was the most plausible configuration, combining ^{1}H and ^{13}C data with a probability of 100% (Fig. S10). The absolute configurations of isomers 19*S*,20*R*–**2** and their enantiomers (ent–**2**) were established by matching the calculated and experimental ECD curves (Fig. 4). Hence, the absolute configuration of **2** was determined as 3*R*,5*S*,7*S*,15*R*,16*S*,19*S*,20*R*,3′*S*,5′*S*,7′*S*,14′*R*,15′*R*,16′*S* and subsequently named as gelseginedine B.

Compound **3** was assigned the molecular formula $C_{21}H_{26}N_2O_5$ on the basis of the HRESIMS peak at *m/z* 409.1731 [M + Na] $^+$, showing 28 mass units more than that of simultaneously isolated gelselegine (Lin et al., 1990). Additionally, the ^{1}H and ^{13}C NMR data (Table 2) of **3** were similar to those of another one **10** except for an additional aldehyde group [δ_H 8.23 (1H, s); δ_C 163.3]. The HMBC cross-peak between this aldehyde group with C-5 (δ_C 59.3) supported presence of N_4–CHO. An extensive analysis of the ^{1}H–^{1}H COSY, HSQC, HMBC spectra of **3** could confirm it as N_4-aldehydegelsegine.

The molecular formula of compound **4** was established to be $C_{22}H_{28}N_2O_6$ from the HRESIMS peak at *m/z* 439.1843 [M + Na] $^+$. The ^{1}H and ^{13}C NMR spectra of **4** indicated an extra methoxy group (δ_C 56.0) compared to those of **3**. The HMBC spectrum showed correlations between a methoxy groups [δ_H 3.81 (3H, s)] with the carbonyl at δ_C161.9 supported presence of 11-OCH$_3$. The structure of **4** was thus established as 11-methoxy-N_4-aldehydegelsegine.

The molecular formula of compound **5** was determined to be $C_{21}H_{26}N_2O_6$ from the HRESIMS peak at *m/z* 403.1864 [M + H] $^+$, which indicated that **5** had an extra hydroxyl group compared to **3**. Similar to NMR pattern of **4**, **5** had 11-hydroxyl rather than 11-OMe. The structure of **5** was thus determined as 11-hydroxy-N_4-aldehydegelsegine.

Compound **6** had the molecular formula $C_{21}H_{26}N_2O_6$ from HRESIMS 425.1687 [M + Na] $^+$, an additional oxygen than **3**. In addition, a low-field methine proton signal at δ_H 4.68 (1H, s) and an oxygenated methine carbon signal at δ_C 65.1 were observed, suggesting the existence of an additional hydroxyl group. The HMBC correlations between H-3 with C-16/C-20 indicated that the hydroxyl group at C-14. From these

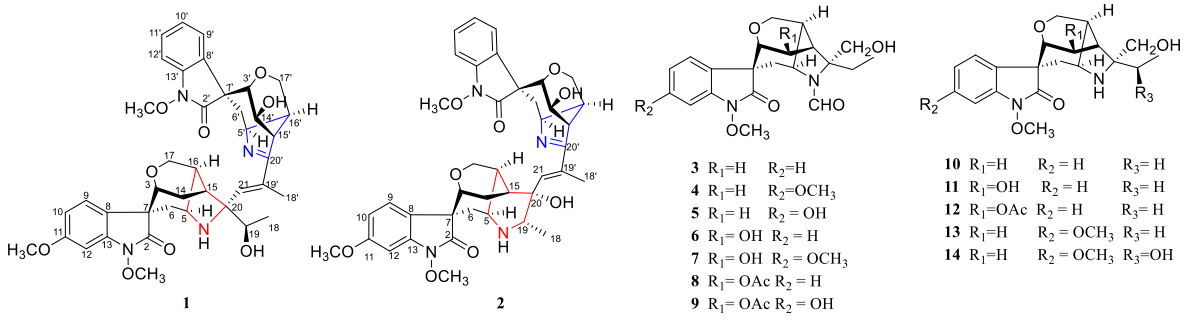

Fig. 1. Structures of alkaloids **1**–**14**.

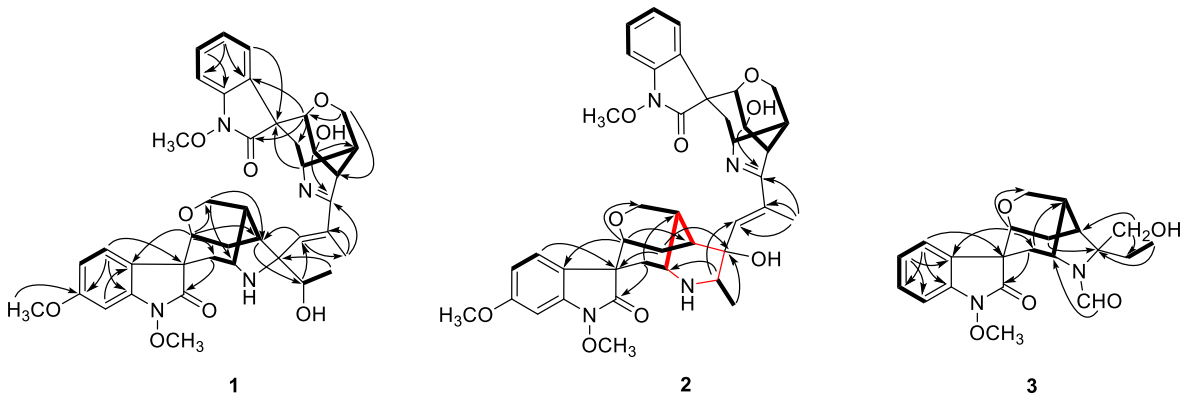

Fig. 2. Key ^{1}H–^{1}H COSY and HMBC correlations of alkaloids **1**–**3**.

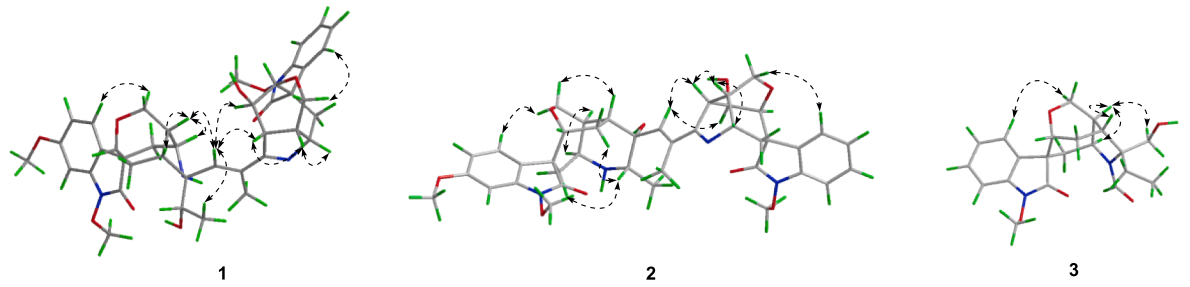

Fig. 3. Key ROESY correlations of alkaloids **1**–**3**.

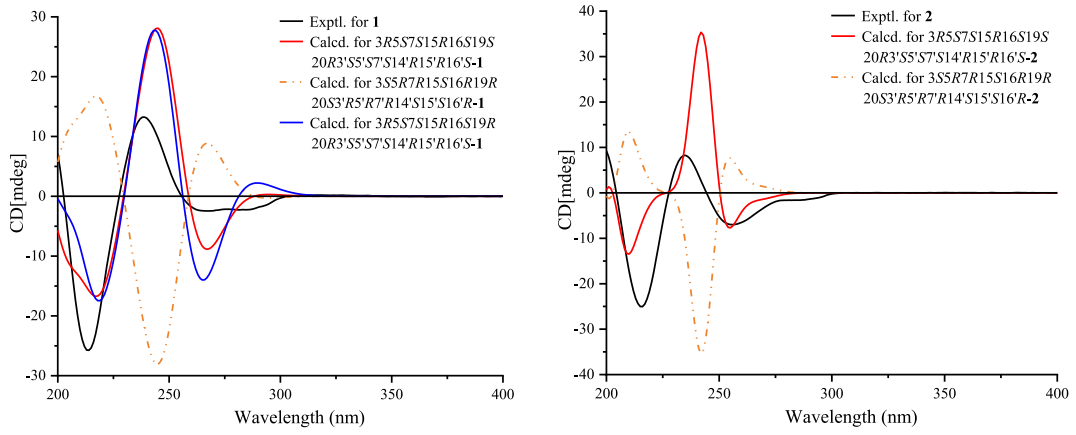

Fig. 4. Calculated and experimental CD spectra of alkaloids **1** and **2**.

data, compound **6** was deduced to be 14-hydroxy-N_4-aldehydegelsegine.

The HRESIMS data established the molecular formula of compound **7** as $C_{22}H_{28}N_2O_7$, which displayed an extra methoxy group (δ_C 56.1) compared to **6**. The HMBC spectrum showed correlations between a methoxy groups [δ_H 3.82 (1H, s)] with the carbonyl at δ_C 162.0 supported presence of 11-OCH_3. The structure of **7** was thus established as 11,14-dimethoxy-N_4-aldehydegelsegine.

The molecular formula $C_{23}H_{28}N_2O_7$ of the compound **8** from the HRESIMS *m/z* 467.1781 $[M + Na]^+$ indicated an extra acetoxy group compared to that of **3** in combination with the NMR spectra [δ_H 2.00 (3H, s); δ_C 172.1 and 20.9] and a downfield methine [δ_H 5.92 (1H, t, J = 1.6 Hz); δ_C 68.7] were observed. The HMBC correlations between δ_H 5.92 (1H, t, J = 1.6 Hz) with δ_C 172.1 indicated the acetoxy group at C-14. Like to **1**, the configuration of the acetoxy group at C-14 was inferred to be β from the small coupling constant between H-14 and H-3/H-15. Therefore, compound **8** was established as 14-acetoxy-N_4-aldehydegelsegine.

Based on the HRESIMS and NMR data analysis, the structure of compound **9** was found to be close to that of **8**, except for the presence of a hydroxyl group. C-11 was shifted to low field δ_C 161.7, suggesting hydroxyl substitution at C-11. Therefore, the structure of **9** was defined as 11-hydroxy-14-acetoxy-N_4-aldehydegelsegine.

The 3D-conformation was described from NOESY correlations of compounds **3**–**9** as in Fig. 3. And the ECD spectra of compounds **3**–**9** (Fig. S91) was similar to that of 14-hydroxygelselegine (Zhang et al., 2014) whose absolute configuration was already determined indicating that **3**–**9** possessed absolute configuration as depicted in Fig. 1.

To determine whether these compounds were naturally occurring *in vivo*, UPLC-MS/MS analyses were performed. The dimeric alkaloid **2** was detected as a trace compound in the crude extract of *G.elegans*. This implies that these alkaloids may be natural products (Fig. S92).

The known compounds were identified as gelselegine (**10**)

(Takayama et al., 1994a), 14-hydroxygelselegine (**11**) (Zhang et al., 2014), 14-acetoxygelselegine (**12**) (Kitajima et al., 2006), 11-methoxygelselegine (**13**) (Xu et al., 2012), 11-methoxy-19*R*-hydroxygelselegine (**14**) (Takayama et al., 1994b) by NMR and MS data analysis.

The biogenetic pathways of **1** and **2** were proposed from gelselegine and gelsedine units as shown in Fig. 5. Firstly, as one of substrates, hydroxymethyl of 11-methoxy-19*R*-hydroxygelselegine (**14**) was oxidize to aldehyde. On the same time, the deprotonation of the H-19 neighbouring imine of 14-hydroxygelsenicine, the other substrate, produced negative ions under alkaline environment. Subsequently, a nucleophilic addition between both units constructed **1** after degration of a molecule of H_2O. 19-OH in **1** was protonated and degrated a molecule of H_2O, giving an intermediate with positive charge at the C-19. Finally, N_4 rearrangement from C-20 to C-19 of this intermediate and attack from a hydroxyl group to C-20 produced alkaloid **2**. This rearrangement gave birth to an undescribed *Gelsemium* alkaloid with new piperidine ring.

As far part of continuously exploring undescribed activity of *Gelsemium* alkaloids, cytotoxicity against tumor cells were screened as we previous reported (Chen et al., 2021), however, compounds **1**–**14** could not indicate cytotoxicity at 20 μM against cancer cells SMMC-7721, HT-29 and A549. However, the bioactive screening disclosed **14** with the protective property on hOMF cells at concentrations (25−100 μM) in 48h according to reported method (Yin et al., 2021). Alkaloid **14** promoted the proliferation of human oral mucosa fibroblast cells with values of 108.17 ± 2.96%, 120.48 ± 2.51%, and 106.27 ± 1.14% at the concentrations of 25, 50, 100 μM, respectively, indicating a certain effect on the healing of oral soft tissue defects (Fig. 6).

3. Conclusions

In conclusion, two undescribed natural dimeric alkaloids, gelseginedine A (**1**) and gelseginedine B (**2**), and seven undescribed gelselegine-type oxindole alkaloids (**3**–**9**) were isolated from the stems and leaves of *G. elegans*. Gelseginedine A (**1**) and B (**2**) represent an undescribed class of bisindole alkaloids which composed of a gelselegine and a gelsedine units by C–C double bonds. 11-Methoxy-19*R*-hydroxygelselegine could promote the proliferation of hOMF cells. This finding suggested new potential pharmaceutical value of *Gelsemium* alkaloids.

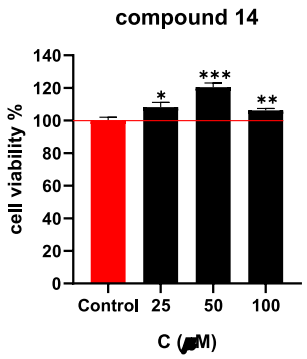

Fig. 6. Dose-response of compound **14** affected hOMF cells proliferation. The significance was determined by Student' s test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs the control group).

4. Experimental section

4.1. General experimental procedures

Optical rotations were measured on a Jasco p-1020 digital polarimeter (Jasco International Co., Tokyo, Japan). UV spectra were recorded on a Shimadzu 2401A spectrophotometer (Shimadzu Corp., Kyoto, Japan). X-ray crystallographic analysis using Cu Kα radiation was performed on a Bruker D8 QUEST instrument (Bruker, Karlsruher, Germany). CD spectra were obtained on a Chirascan V100 circular dichroism spectrometer (Applied Photophysics, Surrey, UK). ^{1}H, ^{13}C and 2D NMR spectra were obtained on Bruker AVANCE III-500, 600 and 800 MHz spectrometers (Bruker BioSpin GmBH, Rheinstetten, Germany) with $SiMe_4$ as an internal standard. HRESIMS data were recorded on an Agilent G6230 TOF MS (Applied Biosystems, Ltd., Warrington, UK). Column chromatography (CC) (200 × 1500, 26 × 254, 20 × 203, and 13.4 × 203 mm, respectively) was performed on either silica gel (200–300 mesh, Qing-dao Haiyang Chemical Co., Ltd., Qingdao, China) or RP-18 silica gel (20–45 μm, Fuji Silysia Chemical Ltd., Japan). Fractions were monitored by TLC on silica gel plates (GF254, Qingdao Haiyang Chemical Co., Ltd., Qingdao, China), and spots were visualized with Dragendorff's reagent spray. Medium pressure liquid chromatography (MPLC) was performed using a Buchi pump system coupled with RP-18 silica gel-packed glass columns (15 × 230, 26 × 460, and 70 × 460 mm, respectively). High performance liquid chromatography (HPLC) was performed using Waters 1525E pumps (Waters Corp.,

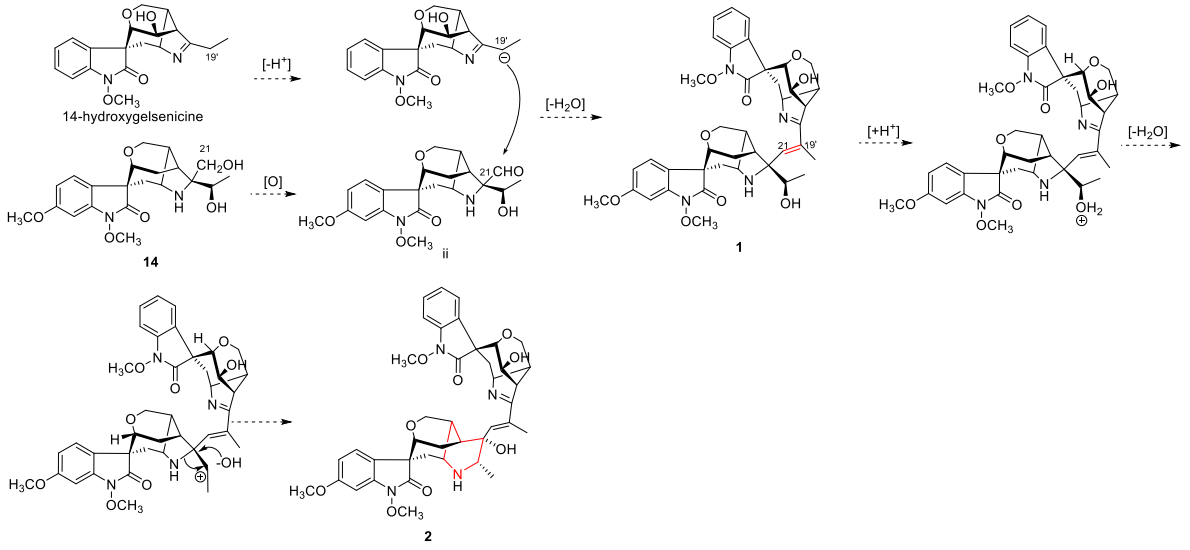

Fig. 5. Plausible biosynthetic pathways of alkaloids **1** and **2**.

Milford, MA, USA) coupled with analytical semi-preparative or preparative XBridge C_{18} columns (4.6 × 150, 10 × 150, and 19 × 250 mm, respectively). The HPLC system employed a Waters 2998 photodiode array detector and a Waters fraction collector III (Waters Corp., Milford, MA, USA).

4.2. Plant material

The stems and leaves of *Gelsemium elegans* (Gardner. & Champ.) Benth. were collected in Feb. 2018 in Wenshan County, Yunnan Province, P. R. China, and identified by Dr. Chunxia Zeng. A voucher specimen (Cai 20180215) was deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

4.3. Extraction and isolation

The air-dried samples (18 kg) were pulverized and extracted with 90% MeOH (3 × 4d) at room temperature. The MeOH extract was suspended in 0.5% HCl solution and EtOAc. The acidic water layer was adjusted to pH 8–9 with 15% ammonia solution and subsequently extracted with EtOAc, yielding fraction of EtOAc layer. The concentrated EtOAc fraction (370 g) was subjected to silica gel column chromatography (CC), using gradient elution of $CHCl_3$–MeOH (10:0, 9:1, 8:2, 7:3, 5:5), to yield six major fractions (Fr.1∼Fr.6) by their thin layer chromatograph profiles.

Fr.5 (95.4 g) was further fractionated using silica gel CC eluted with a $CHCl_3$–MeOH step gradient (100:0 → 1:1) to afford eight fractions (Fr.5.1∼ Fr.5.8), Fr.5.2, Fr.5.3 and Fr.5.7 were submitted to a C18 MPLC column with gradient elution of MeOH–H_2O (10:90–100:0), respectively, followed by a Sephadex LH-20 CC eluted with MeOH, to afford fraction Fr.5.2.3.2, Fr.5.2.3.3, Fr.5.2.3.4, Fr.5.2.4.2, Fr.5.3.2.3, Fr.5.3.3.7, Fr.5.3.4.2. and Fr.5.7.10.2. Fr.5.2.3.2 was purified by HPLC column (CH_3CN/H_2O, 25:75–40:60) to give compound **12** (3.1 mg, t_R = 49.2 min). Fr.5.2.3.3 was purified by HPLC column (CH_3CN/H_2O, 30:70–45:55) to give compound **2** (1.9 mg, t_R = 33.3 min). Fr.5.2.3.4 was purified by HPLC column (CH_3CN/H_2O, 35:65–50:50) to give compound **1** (2.1 mg, t_R = 31.2 min). Fr.5.2.4.2 was purified by HPLC column (CH_3CN/H_2O, 20:80–30:70) to give compound **8** (4.4 mg, t_R = 55.2 min). Fr.5.3.2.3 was purified by HPLC column (CH_3CN/H_2O, 25:75–35:65) to give compounds **3** (5.4 mg, t_R = 41.1 min) and **4** (7.2 mg, t_R = 44.8 min). Fr.5.3.3.7 was purified by HPLC column (CH_3CN/H_2O, 25:75–40:60) to give compound **10** (2.6 mg, t_R = 40.0 min). Fr.5.3.4.2 was purified by HPLC column (CH_3CN/H_2O, 25:75–40:60) to give compound **13** (3.7 mg, t_R = 33.2 min). Fr.5.7.10.2. was purified by same HPLC column (CH_3CN/H_2O, 15:70–30:70) to give compound **11** (2.3 mg, t_R = 44.0 min).

Fr.6 (46.7 g) was subjected to silica gel CC using $CHCl_3$–MeOH (50:1 → 1:1) as gradient eluent to afford nine fractions (Fr.6.1∼ Fr.6.9), Fr.6.6 and Fr.6.7 were submitted to a C18 MPLC column with gradient elution of MeOH–H_2O (10:90–100:0), respectively, followed by a Sephadex LH-20 CC eluted with MeOH, to afford fraction Fr.6.6.5.2, Fr.6.7.5.2, Fr.6.7.6.2 and Fr.6.7.7.3. Fr.6.6.5.2 was purified by same HPLC column (CH_3CN/H_2O, 15:85–30:70) to give compound **6** (8.0 mg, t_R = 35.1 min). Fr.6.7.5.2 was purified by same HPLC column (CH_3CN/H_2O, 15:85–30:70) to give compounds **7** (1.7 mg, t_R = 28.9 min) and **9** (2.3 mg, t_R = 36.0 min). Fr.6.7.6.2 was purified by same HPLC column (CH_3CN/H_2O, 15:85–30:70) to give compound **5** (5.1 mg, t_R = 20.0 min). Compound **14** (2.8 mg, t_R = 57.0 min) was obtained from Fr.6.7.7.3 by same HPLC column using CH_3CN–H_2O (15:85–30:70) as eluents.

4.3.1. Gelseginedine A (1)

Colorless oil; $[\alpha]_D^{21.6}$-131.20 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε): 213.5 (4.63), 278.5 (3.73); ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 727.3342 [M + H] $^+$ (calcd for $C_{40}H_{47}N_4O_9$, 727.3343).

Table 1
^{1}H (800 MHz) and ^{13}C NMR (200 MHz) data of alkaloid **1** and **2** in pyridine-d_5 (δ in ppm, *J* in Hz).

No.	δ_H (1)	δ_C (1)	δ_H (2)	δ_C (2)
2		176.1 s		176.5 s
3	3.56, d (6.5)	75.8 d	4.09, d (8.1)	74.3 d
5	3.95, d (3.8)	60.4 d	3.84, dd (9.8, 6.7)	56.0 d
6	2.08, d (10.6)	34.4 t	3.09, dd (15.7, 6.7)	33.9 t
	2.06, dd (10.6, 3.8)		2.11, dd (15.7, 9.8)	
7		57.8 s		56.2 s
8		124.6 s		123.1 s
9	7.45, d (8.0)	127.3 d	7.60, d (8.3)	127.9 d
10	6.77, dd (8.0, 2.0)	108.8 d	6.80, dd (8.3, 2.4)	108.4 d
11		161.1 s		160.9 s
12	6.79, d (2.0)	95.2 d	6.84, d (2.4)	95.3 d
13		140.1 s		141.3 s
14	2.81, m	23.8 t	3.39, dd (15.0, 8.1)	24.9 t
	2.18, m		2.65, d (15.0)	
15	2.81, m	41.7 d	2.94, m	40.1d
16	3.03, d (3.8)	41.3 d	2.67, d (5.8)	38.4 d
17	4.25, d (10.6)	64.4 t	4.37, d (10.6)	67.8 t
	4.13, dd (10.6, 3.8)		4.05, dd (10.6, 5.8)	
18	1.81, d (6.1)	21.1 q	1.51, d (6.2)	16.2 d
19	5.07, q (6.1)	69.2 d	4.18, q (6.2)	50.2 d
20		76.0 s		75.6 s
21	6.96, s	140.9 d	7.58, s	141.4 d
2′		172.1 s		171.9 s
3′	4.11, s	81.2 d	4.07, s	81.4 d
5′	4.69, d (4.7)	73.5 d	4.69, ddd (8.4, 5.0, 2.0)	73.7 d
6′	2.61, dd (15.4, 4.7)	38.2 t	2.61, dd (15.5, 5.0)	38.1 t
	2.46, d (15.4)		2.48, dd (15.5, 2.0)	
7′		55.1 s		55.0 s
8′		133.4 s		133.3 s
9′	7.76, d (7.5)	125.8 d	7.76, d (7.5)	125.9 d
10′	7.12, t (7.5)	123.8 d	7.13, td (7.5, 0.9)	123.9 d
11′	7.28, t (7.5)	128.8 d	7.28, td (7.5, 0.9)	128.9 d
12′	6.94, d (7.5)	107.3 d	6.93, d (7.5)	107.3 d
13′		139.1 d		139.1 s
14′	5.01, s	68.1 d	5.05, s	68.1 d
15′	3.73, m	50.4 d	3.93, d (8.4)	50.2 d
16′	2.46, m	39.7 d	2.57, td (8.4, 3.5)	39.7 d
17′	4.69, d (10.1)	62.2 t	4.73, dd (10.6, 3.5)	62.3 t
	4.40, d (10.1)		4.44, d (10.6)	
18′	3.17, s	13.0 q	3.05, s	15.4 q
19′		136.6 s		135.6 s
20′		178.8 s		178.9 s
11-OCH_3	3.56, s	56.0 q	3.77, s	56.0 q
N–OCH_3	3.84, s	63.5 q	3.75, s	63.3 q
N′-OCH_3	3.94, s	63.7 q	3.91, s	63.8 q

4.3.2. Gelseginedine B (2)

Colorless oil; $[\alpha]_D^{21.6}$-180.06 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε): 215.0 (4.61), 237.5 (4.32), 277.0 (3.76); ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 727.3341 [M + H] $^+$ (calcd for $C_{40}H_{47}N_4O_9$, 727.3343).

4.3.3. N_4-aldehydegelsegine (3)

Colorless oil; $[\alpha]_D^{21.7}$-76.63 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε): 208.2 (4.49), 257.6 (3.80), 280.4 (3.38), 310.2 (2.79); ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m/z* 409.1731 [M + Na] $^+$ (calcd for $C_{21}H_{26}N_2O_5Na$, 409.1734).

4.3.4. 11-Methoxy-N_4-aldehydegelsegine (4)

Colorless oil; $[\alpha]_D^{21.8}$-92.37 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε): 209.6 (4.42), 255.4 (3.69), 281.8 (3.47); ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m/z* 439.1843 [M + Na] $^+$ (calcd for $C_{22}H_{28}N_2O_6Na$, 439.1840).

4.3.5. 11-Hydroxy-N_4-aldehydegelsegine (5)

Colorless oil; $[\alpha]_D^{22.5}$-23.72 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε):

Table 2
^{1}H NMR spectral data for alkaloids **3**–**9** in methanol-d_4 (δ in ppm, J in Hz).

No.	δ_H (3)[a]	δ_H (4)[b]	δ_H (5)[b]	δ_H (6)[b]	δ_H (7)[b]	δ_H (8)[b]	δ_H (9)[c]
3	3.60, d (6.1)	3.52, d (6.4)	3.52, d (6.3)	3.43, s	3.42, s	3.44, d (1.6)	3.41, d (1.6)
5	4.47, ddd (9.3, 4.1, 2.6)	4.45, ddd (9.8, 4.2, 3.0)	4.44, ddd (10.0, 4.1, 2.7)	4.41, ddd (9.8, 4.2, 2.7)	4.39, ddd (9.8, 4.1, 2.7)	4.45, ddd (10.0, 4.2, 2.6)	4.41, ddd (9.7, 4.2, 2.6)
6	2.98, dd (16.1, 2.6)	2.80, dd (16.0, 3.0)	2.79, dd (16.0, 2.7)	2.81, dd (16.1, 2.7)	2.79, dd (16.2, 2.7)	2.83, dd (16.1, 2.6)	2.78, dd (16.2, 2.6)
	1.96, dd (16.1, 4.1)	2.03, dd (16.0, 4.2)	2.01, dd (16.0, 4.1)	2.14, dd (16.1, 4.2)	2.11, dd (16.2, 4.1)	2.16, dd (16.1, 4.2)	2.09, dd (16.2, 4.2)
9	7.40, dd (7.6, 1.1)	7.34, d (8.3)	7.24, d (8.2)	7.49, dd (7.7, 1.2)	7.37, d (8.3)	7.44, dd (7.6, 1.1)	7.18, d (8.2)
10	7.08, td (7.6, 1.1)	6.64, dd (8.3, 2.4)	6.50, dd (8.2, 2.3)	7.13, td (7.7, 1.2)	6.66, dd (8.3, 2.4)	7.12, td (7.6, 1.1)	6.47, dd (8.2, 2.3)
11	7.26, td (7.6, 1.1)			7.33, td (7.7, 1.2)		7.33, td (7.6, 1.1)	
12	6.90, dd (7.6, 1.1)	6.56, d (2.4)	6.42, d (2.3)	6.99, dd (7.7, 1.2)	6.57, d (2.4)	6.99, dd (7.6, 1.1)	6.39, d (2.3)
14	3.60, dd (15.0, 6.1)	2.51, dd (15.0, 6.4)	2.14, dd (15.0, 6.3)	4.68, s	4.66, s	5.92, t (1.6)	5.90, t (1.6)
	3.60, d (6.1)	2.21, d (15.0)	2.14, d (15.0)				
15	2.17, d (4.4)	2.22, m	2.22, m	2.08, d (6.4)	2.07, d (6.6)	2.33, dd (5.7, 1.6)	2.32, dd (5.9, 1.6)
16	2.74, dt (9.3, 4.4)	2.78, dd (9.8, 3.9)	2.78, dd (10.0, 3.9)	2.84, ddd (9.8, 6.4, 4.1)	2.82, ddd (9.8, 6.6, 4.0)	2.93, ddd (10.0, 5.7, 4.2)	2.89, ddd (9.7, 5.9, 4.2)
17	4.26, d (11.3)	4.32, d (11.4)	4.31, d (11.3)	4.38, d (11.0)	4.36, d (11.0)	4.43, d (11.3)	4.38, d (11.2)
	4.20, dd (11.3, 4.4)	4.18, dd (11.4, 3.9)	4.18, dd (11.3, 3.9)	4.31, dd (11.0, 4.1)	4.29, dd (11.0, 4.0)	4.29, dd (11.3, 4.2)	4.26, dd (11.2, 4.2)
18	0.96, t (7.4)	0.99, t (7.5)	0.98, t (7.5)	1.14, t (7.4)	1.13, t (7.7)	0.93, t (7.5)	0.92, t (7.5)
19	2.91, dqd (14.0, 7.4, 1.4)	2.51, dqd (15.2, 7.5, 1.1)	2.52, dqd (14.5, 7.5, 1.6)	2.59, dqd (13.7, 7.4, 1.2)	2.58, dqd (13.7, 7.7, 1.6)	2.56, dqd (14.9, 7.5, 1.7)	2.55, dqd (14.0, 7.5, 1.1)
	2.26, dq (14.0, 7.4)	2.17, dq (15.2, 7.5)	2.23, dq (14.5, 7.5)	2.30, dq (13.7, 7.4)	2.29, dq (13.7, 7.7)	2.33, dq (14.9, 7.5)	2.31, dq (14.9, 7.5)
21	3.66, d (11.4)	3.65, d (11.6)	3.65, d (11.5)	3.71, d (11.5)	3.70, d (11.5)	3.71, d (11.5)	3.70, d (11.5)
	3.53, dd (11.4, 1.4)	3.45, dd (11.6, 1.1)	3.45, dd (11.5, 1.6)	3.46, dd (11.5, 1.2)	3.45, dd (11.5, 1.6)	3.50, dd (11.5, 1.7)	3.48, dd (11.5, 1.1)
N_1–OCH_3	3.95, s	3.92, s	3.91, s	3.95, s	3.94, s	3.98, s	3.94, s
N_4–CHO	8.23, s	8.14, s	8.14, s	8.16, s	8.15, s	8.19, s	8.17, s
11-OCH_3		3.81, s			3.82, s		
14-$COCH_3$						2.00, s	2.00, s

[a] Measured at 500 MHz in chloroform-*d*.
[b] at 500 MHz.
[c] at 600 MHz.

Table 3
^{13}C NMR spectral data for alkaloids **3**–**9** in methanol-d_4 (δ in ppm, J in Hz).

No.	δ_C (3)[a]	δ_C (4)[b]	δ_C (5)[b]	δ_C (6)[b]	δ_C (7)[b]	δ_C (8)[b]	δ_C (9)[c]
2	172.3 s	174.7 s	174.8 s	173.9 s	174.4 s	173.5 s	174.2 s
3	73.7 d	75.3 d	75.4 d	81.6 d	82.0 d	77.9 d	78.5 d
5	59.3 d	60.7d	60.7 d	59.8 d	59.8 d	59.8 d	59.7 d
6	30.6 t	31.5 t	31.5 t	31.8 t	32.0 t	31.1 t	31.5 t
7	55.7 s	56.8 s	56.7 s	55.2 s	54.8 s	55.3 s	54.8 s
8	131.7 s	124.8 d	123.4 d	132.7 d	124.4 d	132.0 d	121.1 d
9	125.3 d	127.4 d	127.4 d	126.6 d	127.4 d	126.4 d	127.2 d
10	123.4 d	109.1 d	110.8 d	124.8 d	109.3 d	124.7 d	111.7 d
11	128.3 d	161.9 s	159.4 s	129.6 d	162.0 s	129.8 d	161.7 s
12	106.9 d	95.2 d	96.2 d	108.1 d	95.3 d	108.1 d	96.9 d
13	138.2 s	140.4 s	140.2 s	139.2 s	140.3 s	139.4 s	140.2 s
14	22.8 t	23.7 t	23.8 t	65.1 t	65.1 t	68.7 t	68.8 t
15	36.1 d	36.9 d	36.9 d	47.6 d	47.6 d	44.4 d	44.4 d
16	35.8 d	37.0 d	37.0 d	35.6 d	35.6 d	35.7 d	35.2 d
17	62.6 t	63.3 t	63.3 t	62.8 t	62.8 t	63.0 t	62.9 t
18	9.7 q	9.7 q	9.7 q	9.1 q	9.1 q	9.1 q	9.1 q
19	22.5 t	23.3 t	23.3 t	23.6 t	23.6 t	23.6 t	23.5 t
20	70.8 s	72.0 s	72.0 s	71.7 s	71.7 s	71.9 s	71.8 s
21	64.6 t	64.1 t	64.2 t	64.3 t	64.3 t	64.3 t	64.3 t
N_1–OCH_3	63.4 q	64.0 q	63.8 q	64.0 q	64.0 q	64.2 q	64.1 q
N_4–CHO	163.3 d	164.8 d	164.8 d	164.6 d	164.6 d	164.5 d	164.5 d
11-OCH_3		56.0 q			56.1 q		
14-$COCH_3$						172.1 s	172.1 s
14-$COCH_3$						20.9 q	20.9 q

[a] Measured at 125 MHz in chloroform-*d*.
[b] at 125 MHz.
[c] at 150 MHz.

212.8 (4.36), 286.6 (3.53); ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m/z* 403.1864 [M + H] $^{+}$ (calcd for $C_{21}H_{27}N_2O_6$, 403.1864).

4.3.6. 14-Hydroxy-N_4-aldehydegelsegine (6)

Colorless oil; $[\alpha]_D^{22.3}$-15.04 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε): 208.2 (4.41), 258.8 (3.70), 279.8 (3.29); ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m/z* 425.1687 [M + Na] $^{+}$ (calcd for $C_{21}H_{26}N_2O_6Na$, 425.1683).

4.3.7. 11,14-Dimethoxy-N_4-aldehydegelsegine (7)

Colorless oil; $[\alpha]_D^{22.5}$-24.27 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε): 214.4 (4.42), 265.6 (3.49), 279.8 (3.46); ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m/z* 433.1974 [M + H] $^{+}$ (calcd for $C_{22}H_{29}N_2O_7$, 433.1969).

4.3.8. 14-Acetoxy-N_4-aldehydegelsegine (8)

Colorless oil; $[\alpha]_D^{22.0}$-2.20 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε): 209.0 (4.44), 258.2 (3.74), 278.4 (3.29); ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m/z* 467.1781 [M + Na] $^+$ (calcd for $C_{23}H_{28}N_2O_7Na$, 467.1789).

4.3.9. 11-Hydroxy-14-acetoxy-N_4-aldehydegelsegine (9)

Colorless oil; $[\alpha]_D^{20.0}$-16.23 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε): 205.0 (3.40), 286.0 (2.60); ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m/z* 461.1913 [M + H] $^+$ (calcd for $C_{23}H_{29}N_2O_8$, 461.1918).

4.4. Human oral mucosa fibroblasts proliferation assay

hOMF cells were seeded into 96-well tissue culture dishes at 4×10^3 cells/well and cultured overnight in cell culture medium supplemented with 10% FBS and 1% penicillin and streptomycin. Cells were then incubated in culture medium with each compound for 48 h (Yin et al., 2021). The MTS-reducing activity was evaluated by measuring the absorbance at 490 nm using the CellTiter 96 Aqueous One Solution Cell Proliferation Assay kit (Promega, USA) and an Infinite M200 Pro (Tecan, Austria) microplate reader.

CRediT authorship contribution statement

Jing Lin: Writing – original draft, Visualization, Software, Investigation, Formal analysis, Data curation. **Jing Wu:** Supervision, Methodology. **Mei-Fen Bao:** Resources, Methodology, Investigation. **Sumet Kongkiatpaiboon:** Supervision, Funding acquisition, Conceptualization. **Xiang-Hai Cai:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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