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•Original article•

Glasesterterpenoids A−C: three sesterterpenoids with 7-cyclohexyl-decahydronaphthalene carbon skeleton isolated from the root of *Lindera glauca*

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[ABSTRACT] Three novel sesterterpenoids glasesterterpenoids A−C (**1**−**3**), featuring an unprecedented 7-cyclohexyldecahydronaphthalene carbon skeleton, were isolated from the root of *Lindera glauca* (*L. glauca*). Their structures were elucidated by quantum chemical calculations and spectroscopic methods. The biogenetic pathway for **1**−**3** is proposed. In the bioassay, glasesterterpenoid C exhibited DNA topoisomerase 1 (Top1) inhibitory activity compared with the positive control, camptothecin. These findings represent the first examples of sesterterpenoids with a 7-cyclohexyldecahydronaphthalene carbon skeleton from the root of *L. glauca*.

[KEY WORDS] Sesterterpenoids; *Lindera glauca*
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Introduction

Sesterterpenoids (STDs) are significant components of terpenoids, composed of five isoprene units. Approximately 1300 STDs have been identified in nature, making them the rarest class of terpenoids, accounting for only 1.6% of all terpenoids. Until now, a total of 50 different basic carbon skeletons of STDs have been reported [1]. Some of these compounds exhibit remarkable biological activities, such as the immunosuppressive and adipogenesis-inhibiting sesterterpenoids eurysoloids A and B [2] and the antifeedant and antifungal activities of sesterterpenoids leucosceptroids A and B [3]. Consequently, chemists and pharmacologists have shown increasing interest in STDs due to their intriguing structures and diverse bioactivities.

Lindera glauca (*L. glauca*), a traditional Chinese medicine, contains terpenoids as its main components, including sesquiterpenoids and sesterterpenoids. As part of our ongoing investigation to discover potential anti-inflammatory sesquiterpenoids and sesterterpenoids from *L. glauca* [4-6], a chemical analysis of *L. glauca* were conducted, leading to the identification and isolation of three unprecedented sesterterpenoids: glasesterterpenoids A−C, featuring an unusual 7-cyclohexyldecahydronaphthalene carbon skeleton. Additionally, glasesterterpenoid C displayed significant DNA topoisomerase I (TOP1) inhibitory activity. Herein, we described the details of the isolation, structure elucidation, and bioactivity of these compounds (Fig. 1).

Results and Discussion

Glasesterterpenoid A (**1**) had a molecular formula of $C_{25}H_{38}O_2$, as established by the (−)-high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) at *m/z* 369.2802 $[M - H]^-$ (Calcd. for $C_{25}H_{37}O_2$, 369.2788). The nuclear magnetic resonance (NMR) features of **1** were similar to those of our previously reported linderasesterterpenoids A and B [6], all of which possessed 25 carbons in the ^{13}C NMR spectra. Detailed analyses of the ^1H NMR spectrum revealed only slight differences. For example, **1** exhibited five methyl groups with singlet signals, as opposed to linderasesterterpenoid A, which displayed four methyl groups with singlet signals and one methyl group with a doublet signal. These data suggested that the A/B fused ring in **1** might

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These authors have no conflict of interest to declare.

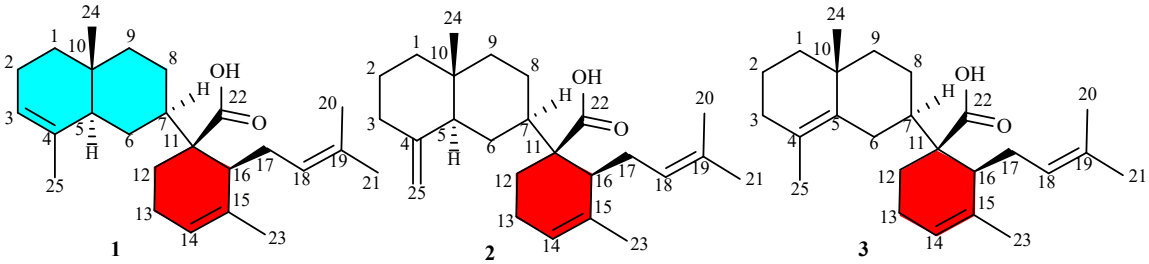


Fig. 1 Structures of 1–3.

have been altered to form a 6/6 ring system. This deduction was subsequently validated by 2D NMR data. In the ^{1}H-^{1}H correlation spectroscopy (COSY) spectrum, two spin systems, H_2-1/H_2-2/H-3 and H_2-9/H_2-8/H-7/H_2-6/H-5, were identified. The A/B (6/6) fused ring systems were further confirmed by the heteronuclear multiple bond correlations (HMBCs) from H_3-25 to C-3, C-4, and C-5, from H_2-1 to C-10 and C-5, from H_3-24 to C-1, C-10, C-9, and C-5. This information led to the determination of unit A. For unit B, compound **1** shared the same NMR data and HMBCs as linderasesterterpenoids A and B (Fig. 2), thus confirming unit B. The linkage between units A and B was evidenced by HMBCs from H-7 to C-11, C-12, and C-22. Consequently, the planar structure of **1** was determined.

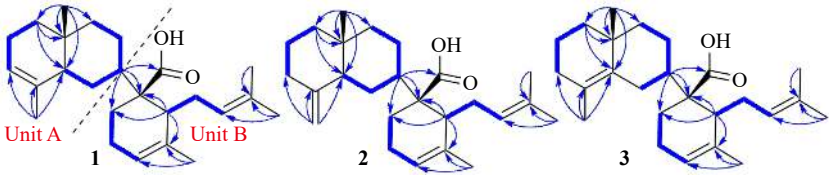


Fig. 2 ^{1}H−^{1}H COSY (blue lines) and key HMBC (blue arrows) correlations of 1–3.

The relative configuration of **1** was investigated by the nuclear Overhauser enhancement spectroscopy (NOESY) spectrum. The NOE correlation of H-5/H-7 could be observed, indicating that they were *α*-oriented protons (Fig. 3). Despite numerous attempts, single crystals of compound **1** could not be obtained from various solvents. Considering that **1**, costic acid [5], (+)-*α*-cyperone [5], and linderasesterterpenoid A [6] were all co-isolated from *L. glauca* and theoretically shared the same biosynthetic pathways, it is likely that they possessed the same absolute configuration. To further determine the absolute configuration, we used the electronic circular dichroism (ECD) calculation method. By comparing the calculated ECD curves with the experimental curves, the absolute configuration of the stereogenic centers in **1** was established (Fig. 4). Thus, compound **1** was assigned and named glasesterterpenoid A.

Glasesterterpenoid B (**2**) had the same molecular formula, $C_{25}H_{38}O_2$, as **1**. Meanwhile, the ^{1}H and ^{13}C NMR data of **2** (Table 1) were similar to those of **1**, with notable differences in the chemical shifts at C-3, C-4, and C-25. Structurally, a methyl group at C-25 (δ_C 21.3) in **1** was replaced by a terminal olefinic carbon (δ_C 105.7) in **2**, which was further determined by the HMBCs from H_2-25 to C-3, C-4, and C-5. Further ^{1}H-^{1}H COSY and HMBCs (Fig. 2) confirmed the planar structure (Fig. 1). The NOESY spectrum revealed the *α*-oriented protons of H-5 and H-7, evidenced by the NOE correlation from H-5 to H-7 (Fig. 3). Given the shared biosynthetic pathway of compounds **1** and **2**, the absolute config-

Fig. 3 Key NOESY correlations of compounds 1 and 2.

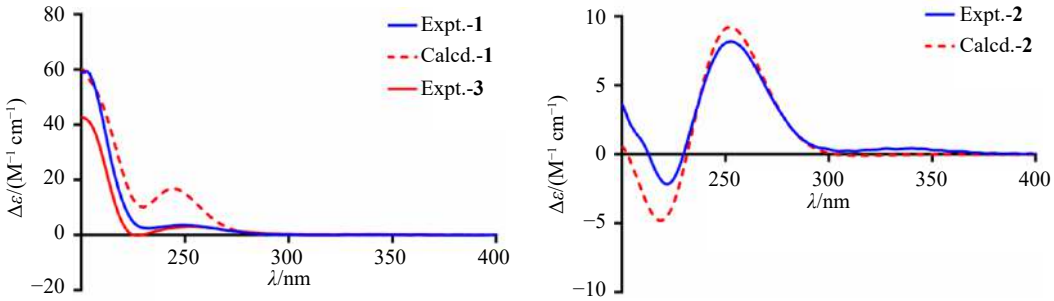


Fig. 4 Experimental and calculated ECD spectra of compounds 1–3 in methanol.

Table 1 ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data of compounds 1–3 in $CDCl_3$

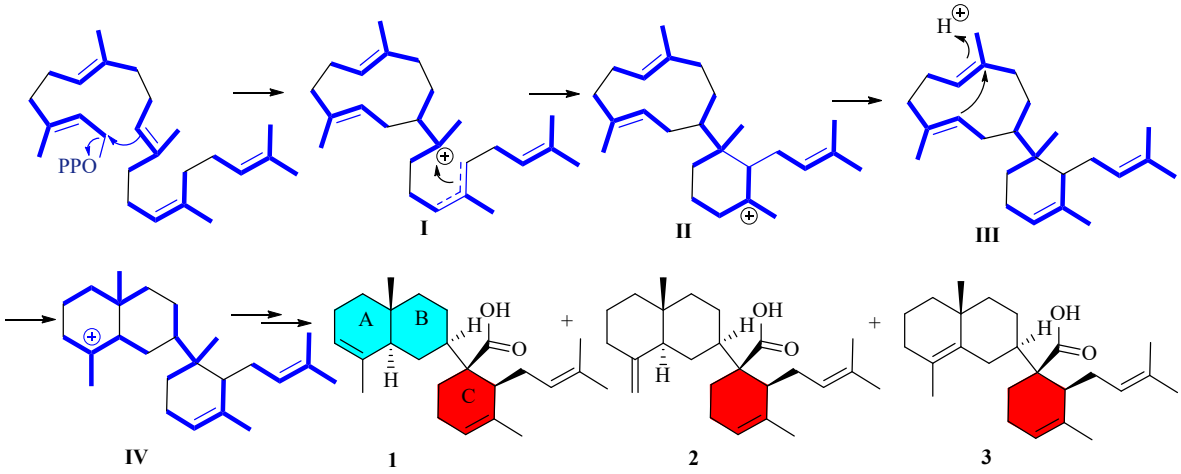
No.	1		2		3	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	1.30, m	37.9, CH_2	1.40, m	41.9, CH_2	1.45, m; 1.21, m	40.3, CH_2
2	2.05, m	23.1, CH_2	1.59, m	23.7, CH_2	1.53, m	19.3, CH_2
3	5.27, s	121.1, CH	2.27, d (12.7); 1.94, m	37.0, CH_2	1.85, m; 1.91, m	33.3, CH_2
4		135.1, C		150.9, C		124.3, C
5	1.79, s	47.3, CH	1.70. m	50.4, CH		135.2, C
6	1.75, m; 0.78, q (13.1)	25.6, CH_2	1.68, m; 0.98, q (12.5)	26.1, CH_2	2.54, d (12.1); 1.45, m	27.3, CH_2
7	1.60, m	39.6, CH	1.61, m	39.1, CH	1.45, m	40.4, CH
8	2.01, m; 1.38, m	22.2, CH_2	2.02, m; 1.40, m	22.4, CH_2	1.80, m; 1.45, m	23.2, CH_2
9	1.43, m; 1.06, td (13.2, 4.1)	40.6, CH_2	1.50, m; 1.14, m	41.7, CH_2	1.51, m; 1.12, m	42.8, CH_2
10		32.5, C		36.2, C		34.8, C
11		52.1, C		52.0, C		52.0, C
12	1.87, m; 1.94, m	20.7, CH_2	1.86, m; 1.94, m	20.7, CH_2	1.85, m; 1.96, m	20.9, CH_2
13	2.02, m; 1.94, m	22.5, CH_2	2.02, m	22.5, CH_2	2.03, m	22.5, CH_2
14	5.27, s	120.3, CH	5.26, s	120.3, CH	5.24, s	120.2, CH
15		136.3, C		136.3, C		136.3, C
16	2.40, m	42.6, CH	2.40, m	42.6, CH	2.40, m	42.6, CH
17	2.10, m	33.8, CH_2	2.10, m	33.9, CH_2	2.10, m	33.9, CH_2
18	5.20, m	124.5, CH	5.20, m	124.6, CH	5.20, m	124.5, CH
19		131.5, C		131.5, C		131.5, C
20	1.55, s	17.9, CH_3	1.56, s	17.9, CH_3	1.56, s	17.9, CH_3
21	1.66, s	25.9, CH_3	1.66, s	25.9, CH_3	1.66, s	25.9, CH_3
22		182.3, C		181.1, C		181.4, C
23	1.71, s	24.0, CH_3	1.71. s	24.0, CH_3	1.69, s	24.0, CH_3
24	0.69, s	15.7, CH_3	0.64, s	16.4, CH_3	0.94, s	24.5, CH_3
25	1.55, s	21.3, CH_3	4.66, s; 4.37, s	105.7, CH_2	1.56, s	19.3, CH_3

uration of stereogenic centers in compound **2** was determined by comparing the calculated ECD curves with the experimental ones (Fig. 4). Finally, compound **2** was named glasesterterp enoid B.

Glasesterterpenoid C (**3**) was isolated as the isomers of **1** and **2**, sharing the same molecular formula, $C_{25}H_{38}O_2$, as supported by the HR-ESI-MS data. The primary difference lies in the position of the double-bond carbon signal in the ^{13}C NMR spectrum. In **3**, the double bond was located at C-4 and C-5, supported by the HMBCs from H_3-25 to C-3, C-4, and C-5, from H_3-24 to C-10 and C-5. Subsequently, the complete structure of **3** was confirmed by the 2D NMR data (Fig. 2). Considering the same biosynthetic pathway and the similar ECD absorption curves between **3** and **1** (Fig. 4), the absolute configuration of stereogenic centers in **3** was assigned as the same as in **1**. Finally, compound **3** was named glasesterterpenoid C.

To the best of our knowledge, glasesterterpenoids A and B are the first reported examples of sesterterpenoids with an unprecedented 7-cyclohexyldecahydronaphthalene carbon skeleton. The proposed biosynthetic pathway for compounds **1**–**3** is illustrated in Scheme 1. Firstly, the key intermediates I and II were generated by the head-to-tail cyclization of geranylfarnesyl pyrophosphate (GFPP) [7]. Subsequently, the 6/6 ring system in unit A for the intermediate IV was built by the electrophilic addition reaction. Finally, compounds **1**–**3** were formed by a series of oxidation and reduction reactions catalyzed by enzymes. Meanwhile, a fresh methanol extract of the roots of *L. glauca* was also analyzed using UPLC-QTOF-MS to verify that compounds **1**–**3** are naturally occurring and not artifacts. The results confirmed the presence of these compounds, substantiating their natural occurrence.

In the bioassay, the topoisomerase 1 (Top1) inhibitory activities of the compounds **1**–**3** were determined by a Top1-mediated relaxation assay. As shown in Fig. 5, compound **3** exhibited inhibitory activity against DNA Top1 at the concentration of 100 μmol·L^{-1}, comparable to the positive control (camptothecin, CPT, 100 μmol·L^{-1}). However, com-



Scheme 1 Plausible biogenetic pathways of compounds 1–3.

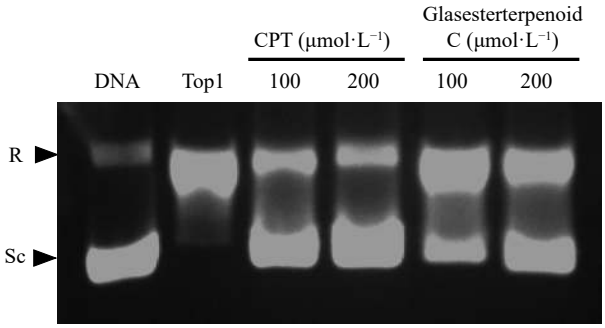


Fig. 5 Representative gels of the Top l mediated DNA relaxation assay. Lane 1 (DNA alone), lane 2 (DNA alone and Top 1), lane 3 (DNA and Top 1 with CPT, 100 μmol·L^{-1}), lane 4 (DNA and Top 1 with CPT, 200 μmol·L^{-1}), lane 5 (DNA and Top l with 3, 100 μmol·L^{-1}), lane 6 (DNA and Top 1 with 3, 200 μmol·L^{-1}), the supercoiled (Sc) and relaxed (R) DNA bands are shown in the gels.

pounds **1** and **2** showed no obvious inhibitory activity toward Topl-mediated DNA relaxation assay at the concentration of 200 μmol·L^{-1}. This study reports for the first time that compound **3**, with a novel 7-cyclohexyldecahydronaphthalene carbon skeleton, possessed inhibitory activity against DNA Top1. Consequently, compound **3** could be considered a lead compound with a new scaffold for drug discovery.

Conclusions

In summary, three unprecedented sesterterpenoids, glasesterterpenoids A−C (**1**−**3**), were isolated from the traditional Chinese medicine *L. glauca*. These compounds are the first reported examples of sesterterpenoids with an unprecedented 7-cyclohexyldecahydronaphthalene carbon skeleton. Notably, compound **3** exhibits inhibitory activity against DNA Top1, positioning it as a promising lead compound for new drug discovery targeting DNA Top1.

Experimental

General experimental procedures

The ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz), and 2D NMR spectra were obtained on a Bruker AVANCE-400 (Bruker BioSpin Corporation, Billerica, MA, USA) using TMS as an internal reference. Structural assignments were made with additional information from COSY, HSQC, and HMBC experiments. Optical rotations were measured on a Bellingham-Stanley ADP 440 + polarimeter at 25 °C (Bellingham and Stanley, Germany). ECD spectra were obtained on an Applied Photophysics Chirascan spectrometer (Applied Photophysics, UK). IR data were recorded on a Nicolet 5DX-FTIR (Thermo Fisher Scientific, Inc., Hudson, NH, USA) in KBr discs. UV data were recorded on a Shimadzu UV-240 spectrophotometer (Shimadzu, Kyoto, Japan). HR-ESI-MS spectra were acquired on an AB Sciex Triple-TOF 5600 + apparatus. Silica gel (200−300 mesh, Marine Chemical Ltd., Qingdao, China), and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Stockholm, Sweden) were used for column chromatography (CC). Preparative high-performance liquid chromatography (HPLC) separation was carried out on an LC-20AT Shimadzu liquid chromatography system with YMC-Pack ODS-A and Phenomenex Lux Cellulose-2 (10 mm × 250 mm, 5 μm) columns.

Materials

The roots of *L. glauca* were collected from Enshi Tujia and Miao Autonomous Prefecture in Hubei Province, China, in July 2021. The roots were authenticated by Dr. PENG Guangtian of Guangzhou University of Chinese Medicine, China. A voucher specimen was deposited in the School of Pharmaceutical Sciences, Guangzhou University of Chinese Medicine, China, with the access code 2021-GZU-lg.

Extraction and isolation

The air-dried roots (20 kg) were soaked in methanol at room temperature and then evaporated under reduced pressure to yield a crude extract (2.4 kg). Subsequently, the crude extract was dispersed in water and extracted with petroleum ether for three times (4 L) to obtain Fr. A (460 g). Fr. A was used for chromatographic separation in silica gel columns (500 g, 200−300 mesh, 15.0 cm × 100 cm), eluting with a step gradient of petroleum ether (PE)−ethyl acetate (EA)

(20 ∶ 1; 10 ∶ 1; 8 ∶ 2; 6 ∶ 4; 5 ∶ 5; 0 ∶ 100, *V*/*V*, each 5 L), resulting in six fractions (F1−F6). F4 (210 g) was separated by an ODS C_{18} column (500 g, 60 cm × 80 cm) with a step gradient of MeOH−H_2O (90 ∶ 10; 100 ∶ 0, *V*/*V*, each 10 L), and four sub-fractions were obtained (F401−F404). F403 (37.5 g) was further separated by silica gel columns (50 g, 200−300 mesh, 5.0 cm × 50 cm) eluting with a step gradient of petroleum ether (PE)−ethyl acetate (EA) (100 ∶ 0; 10 ∶ 1; 5 ∶ 5; 0 ∶ 100, *V*/*V*, each 1 L), resulting in six fractions (F403a−F403f). F403a (3 g) was further separated by HPLC (SHIMSEN Ankylo C_{18}, MeOH/H_2O 92 ∶ 8, flow rate = 5.0 mL·min^{-1}, l = 210 nm) to afford F403a-5 (1 g, t_R = 103.6 min) and F403a-6 (600 mg, t_R = 130.2 min). Finally, F403a-5 was further purified by HPLC (Phenomenex Lux 5 μm Cellulose-2, ACN/H_2O = 77/23, flow rate = 2.0 mL·min^{-1}, l = 225 nm) to yield **2** (18 mg, t_R = 34.4 min) and **3** (14 mg, t_R = 35.1 min). F403a-6 was further purified by HPLC (Phenomenex Lux 5 μm Cellulose-2, ACN/H_2O = 83/17, flow rate = 2.0 mL·min^{-1}, l = 225 nm) to yield **1** (58 mg, t_R = 42 min).

Glasesterterpenoid A (**1**), colorless oil; $[\alpha]_D^{20}$ +65.1 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 200 (3.8) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 203 (+58.3), 228 (+3.3), 250 (+4.0) nm; IR (KBr) ν_{max} 3367, 2961, 2850, 1694, 1443, 1260, 799, 736 cm^{-1}; (−)-ESI-MS *m/z* 369.2 [M − H]$^-$; (−)-HR-MS (ESI-TOF) *m/z* 369.2802 [M − H]$^-$ Calcd. for $C_{25}H_{37}O_2$ 369.2788.

Glasesterterpenoid B (**2**), colorless oil; $[\alpha]_D^{20}$ +43.5 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 200 (3.8) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 220 (−1.8), 254 (+7.8) nm; IR (KBr) ν_{max} 3436, 2962, 2927, 1715, 1378, 1259, 797, 735 cm^{-1}; (−)-ESI-MS *m/z* 369.2 [M − H]$^-$; (−)-HR-MS (ESI-TOF) *m/z* 369.2803 [M − H]$^-$ Calcd. for $C_{25}H_{37}O_2$ 369.2788.

Glasesterterpenoid C (**3**), colorless oil; $[\alpha]_D^{20}$ +66.2 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 200 (3.8) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 203 (+41.3), 228 (+0.3), 250 (+3.3) nm; IR (KBr) ν_{max} 3567, 2926, 2854, 1697, 1452, 1262, 801, 736 cm^{-1}; (−)-ESI-MS *m/z* 369.2 [M − H]$^-$; (−)-HR-MS (ESI-TOF) *m/z* 369.2804 [M − H]$^-$ Calcd. for $C_{25}H_{37}O_2$ 369.2788.

Inhibitory effects of Top1 relaxation activity assay [8]

One unit of Top1 (TaKaRa Biotechnology Co., Ltd., Beijing, China) and the test compounds were combined in an assay buffer (35 mmol·L^{-1} Tris-HCl, pH 8.0, 72 mmol·L^{-1} KCl, 5 mmol·L^{-1} $MgCl_2$, 5 mmol·L^{-1} dithiothreitol, 5 mmol·L^{-1} spermidine, and 0.1% BSA) [8]. The reaction was initiated by adding 1 μL of supercoiled pBR322 plasmid DNA (0.5 μg), and the total volume was adjusted to 20 μL with dd H_2O. The mixture was incubated at 37 °C for 30 min. The reactions were terminated by adding a pre-cooled 6 × DNA loading buffer. The products were then separated on a 1% agarose gel at 100 V for 70 min in TBE buffer (89 mmol·L^{-1} Tris, 89 mmol·L^{-1} boric acid, and 2 mmol·L^{-1} EDTA, pH 8.0). The gels were stained with GelRed (Beyotime, Shanghai, China) for 30 min and subsequently visualized with a UV transilluminator.

Supporting Information

Supporting information can be requested by sending an E-mail to the corresponding author.

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