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RESEARCH ARTICLE

Chemical constituents of the roots of *Codonopsis lanceolata*

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Abstract A new phenylpropanoid (**1**), a new alkaloid (**11**), and a new natural polyacetylene (**17**), together with nine phenolic compounds (**2**–**10**), five alkaloids (**12**–**16**), three polyacetylenes (**18**–**20**), three triterpenoidal saponins (**21**–**23**), one phenylethanoid glycoside (**24**), and three hexyl glycosides (**25**–**27**) with previous known structures, were isolated from the roots of *Codonopsis lanceolata*. All of the isolates **1**–**27** were evaluated for their inhibitory effects on LPS-induced nitric oxide (NO) production in RAW 264.7 macrophages and cell viability in A2780 human ovarian cancer cells. Among the isolates, lancemasides A and B have a significant inhibitory effect on the production of NO in RAW264.7 cells (IC_{50} values < 50 μM). In A2780 cells, lancemaside A exhibited the most potent inhibitory effect on cell viability. This is the first report on the pharmacological activities of lancemaside B (**22**).

Keywords *Codonopsis lanceolate* · Campanulaceae · Lancemasides · Nitric oxide · Ovarian cancer cells

Introduction

Codonopsis lanceolate Trautv. (Campanulaceae) is a perennial herb commonly known as the bonnet bell flower. It is distributed throughout East Asia in countries including Korea, Japan, and China. The roots of *C. lanceolata* have been widely used in Korean cuisines, where it is called deodeok and is one of the largest cultivated wild vegetables in Korea. The roots of *C. lanceolata* have also been widely used in medicines for treatment of bronchitis, cough, cancer, obesity, hyperlipidemia, asthma, tuberculosis, dyspepsia, and psychoneurosis (Wang et al. 1995; Lee et al. 2002; Ichikawa et al. 2009). Diverse secondary metabolites such as phenylpropanoids (Ren et al. 2013), polyacetylenes (Zhao et al. 2013), triterpenoids (Ushijima et al. 2008), alkaloids (Chang et al. 1986), and other compounds (Hu et al. 2012; He et al. 2015) have been isolated from the roots of *C. lanceolata*. Over the past few decades, there has been a growing interest in the roots of *C. lanceolata* because of the pharmacological effects including anti-inflammatory, anti-stress, antimicrobial, anti-oxidant, anti-cancer, and immunomodulatory activities (Byeon et al. 2009; Hossen et al. 2016). In the course of searching for bioactive secondary metabolites from food sources (Jeong et al. 2017), the roots of *C. lanceolata* were chosen for detailed study since it is one of the most popular medicinal foods in Korea.

In the present study, repeated chromatography of the 50% EtOH extract from the roots of *C. lanceolata* led to the isolation and characterization of a new phenylpropanoid (**1**), a new alkaloid (**11**), and a new natural polyacetylene (**17**) together with 24 known compounds (**2**–**10**, **12**–**16**, **18**–**27**). The structures of the new compounds were determined by spectroscopic data interpretation, particularly by 1D- and 2D-NMR studies. The structures of

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known compounds were identified by spectroscopic (^{1}H-NMR, ^{13}C-NMR, 2D-NMR, and MS) measurements and by comparing the data with published values. All of the isolates (**1**–**27**) were evaluated for their inhibitory effects on nitric oxide (NO) production in LPS-stimulated RAW264.7 cells and cell viability in A2780 human ovarian cancer cells in vitro, as the roots of *C. lanceolata* have been reported to exhibit anti-inflammatory and anti-cancer activities (Byeon et al. 2009; Hossen et al. 2016).

In this paper, we describe the isolation and identification of the isolates (**1**–**27**) from the roots of *C. lanceolata* and the structure elucidation of two new derivatives (**1** and **11**) and a new natural compound (**17**). In addition, the effects of the isolates on NO production in macrophages and cell viability in human ovarian cancer cells were examined.

Materials and methods

General experimental procedures

Melting points were carried out in MPA 100 (Stanford research systems, Sunnyvale, CA, USA) in open capillary tubes. Optical rotations were measured on a Jasco P-2000 polarimeter (JASCO, Tokyo, Japan), using a 10-cm microcell. UV spectra were obtained on Optizen pop (Mecasys, Daejeon, Korea). HR-Mass spectra were obtained using a Q-TOF micro mass spectrometer (Waters, Milford, MA, USA) and an AccuTOF-single-reflectron Tof mass spectrometer (Jeol Ltd, Tokyo, Japan) equipped with a DART ion source (IonSense, Saugus, MA, USA). NMR spectra were obtained using a JEOL 500 MHz and spectrometer using TMS as an internal standard and chemical shifts are expressed as δ values. IR spectra were obtained using an Agilent Cary 630 FTIR (Agilent Technologies, Santa Clara, CA, USA). TLC analyses were performed on Silica Gel 60 F_{254} (Merck) and RP-18 F_{254S} (Merck) plates. Compounds were visualized by dipping plates into 20% (v/v) H_2SO_4 reagent (Aldrich) and then heated at 110°C for 5–10 min. Silica gel (Merck 60A, 70–230 or 230–400 mesh ASTM), Sephadex LH-20 (Amersham Pharmacia Biotech), Diaion HP-20 (Mitsubishi), and reversed-phase silica gel (YMC Co., ODS-A 12 nm S-150 μm) were used for column chromatography. Flash chromatography was performed using the flash purification system (Combi Flash Rf, Teledyne Isco). Pre-packed cartridges, Redi Sep-Silica and Redi Sep-C18 (Teledyne Isco) were used for flash chromatography. HPLC was performed using the Gilson purification system (Gilson Inc, Middleton, WI, USA). All solvents used for the chromatographic separations were distilled before use. Roswell Park Memorial Institute (RPMI) 1640, fetal bovine serum (FBS), penicillin and streptomycin were purchased from Life Technologies Inc. (Grand Island, NY, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) powder was obtained from Molecular Probes Inc. (Eugene, OR, USA).

Plant material

The roots of *Codonopsis lanceolata* Trautv. (Campanulaceae) were obtained from a domestic Korean market (Kyungdong Crude Drugs Market, Seoul, South Korea), in November 2014. The origin of the herbal material was identified by Prof. Dae Sik Jang and a voucher specimen (COLA2-2014) has been deposited in the Lab. of Natural Product Medicine, College of Pharmacy, Kyung Hee University, Republic of Korea.

Extraction and isolation

The dried plant material (3.0 kg) was extracted twice with 18 L of 50% aqueous EtOH at 80–90 °C for 3 h. The 50% EtOH extract (1.6 kg) was chromatographed over Diaion HP-20 (ø 8.2 × 49 cm) as stationary phase eluting with a MeOH-H_2O gradient (from 0:1 to 1:0 v/v) to afford seven fractions (C1–C7).

Fraction C2 (38.68 g) was loaded to Diaion HP-20 (ø 5.1 × 34.0 cm) and eluted with an EtOH-H_2O gradient (from 1:4 to 1:0 v/v) to afford six subfractions (C2-1–C2-6). Fraction C2-2 was subjected to silica gel (70–230 mesh, ø 5.2 × 29.0 cm) column chromatography (CC) and eluted with EtOAc/EtOH/H_2O (5/4/1 v/v/v) yielding three fractions (C2-2-1–C2-2-3). Fraction C2-2-2 was chromatographed over Redi Sep-C18 cartridge (43 g, H_2O 100%), obtaining compounds **14** (75.5 mg) and **15** (5.0 mg).

Fraction C4 (15.65 g) was chromatographed over silica gel (70–230 mesh, ø 6.5 × 42.5 cm) with a CH_2Cl_2–MeOH–H_2O mixture (from 9:0.9:0.1 to 4:5.4:0.6 v/v/v) as mobile phase to afford six pooled fractions (C4-1–C4-6). Fraction C4-1 was further fractionated using silica gel CC (230–400 mesh, ø 3.6 × 25 cm, CH_2Cl_2–MeOH–H_2O = 8:1.8:0.2, v/v/v/) and produced six subfractions (C4-1-1–C4-1-6). Fraction C4-1-1 was purified by using a MPLC with Redi Sep-C18 cartridge (43 g, CH_3CN-H_2O, 1:9 to 8:2 v/v) to obtain compounds **5** (1.6 mg) and **8** (1.0 mg). Compounds **6** (20.0 mg), **7** (3.8 mg), **17** (2.0 mg), and **20** (3.0 mg) were isolated from fraction C4-1-2 by using reverse phase HPLC with a J´sphere ODS-M80 column (250 × 200 mm i.d., 4 μm). Fraction C4-1-3 was purified using HPLC with an YMC Pack ODS-A column (250 × 200 mm i.d., 5 μm), yielding compounds **2** (25.9 mg, t_R 20.8 min) and **3** (63.5 mg, t_R 19.5 min). Fraction C4-1-4 was fractionated by a flash chromatography system using a Redi Sep-C18 cartridge (26 g, CH_3CN-H_2O, 1.5:8.5 to 3.5:6.5 v/v) to yield compounds **12**

(4.0 mg) and **24** (3.3 mg). Fraction C4-2 was subjected to silica gel CC (230–400 mesh, ø 3.7 × 25 cm, CH_2Cl_2–MeOH–H_2O = 8:1.8:0.2 to 7:2.7:0.3 v/v/v) to produce nine fractions (C4-2-1–C4-2-9). Compounds **13** (5.0 mg) and **16** (19.5 mg) were purified by recrystallization in MeOH from fraction C4-2-5. Fraction C4-2-4 was fractionated further by flash chromatography system using a Redi Sep-C18 cartridge (130 g, MeOH-H_2O, 2:8 to 6:4 v/v) to give compounds **25** (31.3 mg), **26** (46.7 mg), and **27** (10.0 mg). Fraction C4-2-5 was purified with flash chromatography using a Redi Sep-C18 cartridge (43 g, MeOH–H_2O, 0:10 to 4:6 v/v), yielding compound **10** (5.1 mg). Compounds **11** (10.0 mg) and **19** (5.2 mg) were obtained from fraction C4-2-8 by flash chromatography (Redi Sep-C18 cartridge, 130 g, MeOH-H_2O, 2:8 to 6:4 v/v). Fraction C4-4 was subjected to a Lichroprep RP-18 column (40–63 µm, ø 4.9 × 45 cm) with a MeOH-H_2O-Formic acid mixture (3:7:0.1–4.5:5.5:0.1 v/v/v) to isolate compound **9** (34.2 mg).

Fraction C5 (5.46 g) was chromatographed over silica gel CC (70–230 mesh, ø 5.4 × 38 cm) with a CH_2Cl_2–MeOH–H_2O mixture (from 19:0.9:0.1 to 7:2.7:0.3 v/v/v) as mobile phase to afford 13 pooled fractions (C5-1–C5-13). Fraction C5-6 was fractionated using Sephadex LH-20 (ø 2.3 × 41 cm) with a CH_2Cl_2–MeOH mixture (1:1 v/v), obtaining compounds **1** (7.3 mg) and **4** (7.6 mg). Compound **18** (54.5 mg) was isolated from fraction C5-9 by using MPLC with a Redi Sep-C18 cartridge (43 g, CH_3-CN–H_2O, 2:8–3.5:6 5 v/v).

Fraction C6 (9.56 g) was chromatographed over silica gel CC (70–230 mesh, ø 5.2 × 31 cm) with a CH_2Cl_2–MeOH–H_2O mixture (from 7:2.7:0.3 to 4:5.4:0.6 v/v/v) as mobile phase to afford 14 pooled fractions (C6-1–C6-17), yielding compound **21** (760.8 mg). Fraction C6-6 was fractionated further by MPLC with a Redi Sep-C18 cartridge (43 g, MeOH-H_2O, 3.5:6.5–6:4 v/v) to give compound **23** (31.5 mg). Compound **22** (60.0 mg) was obtained from fraction C6-13 through silica gel CC (230–400 mesh, ø 4.7 × 38.5 cm, CH_2Cl_2–MeOH–H_2O = 7:2.7:0.3–5:4.5:0.5 v/v/v).

*7-O-Ethyltangshenoside II (**1**)*

White powder; m.p. 172.2 °C; $[\alpha]_D^{23}$: − 34.4° (*c* 0.1, MeOH); UV λ_{max} (MeOH) nm (log ε): 219 (4.07), 269 (3.38); IR (ATR) cm^{-1}: 2973, 1592, 1418, 1129, 1073; ^{1}H-NMR (pyridine-d_5, 500 MHz) δ: 6.91 (2H, s, H-2/6), 6.08 (1H, ddd, *J* = 17.0, 10.5, 6.0 Hz, H-8), 5.82 (1H, d, *J* = 7.0 Hz, Glc-1), 5.41 (1H, d, *J* = 17.0 Hz, H-9a), 5.20 (1H, d, *J* = 10.0 Hz, H-9b), 4.83 (1H, d, *J* = 6.0 Hz, H-7), 4.40–3.90 (5H, m, Glc-2–6), 3.76 (6H, s, OCH_3), 3.48 (1H, dq, *J* = 15.0 and 7.0 Hz, OCH_2CH_3), 1.27 (3H, t, *J* = 7.0 Hz, OCH_2CH_3); ^{13}C NMR (pyridine-d_5, 125 MHz) δ: 154.3 (C-3/5), 140.4 (C-1/8), 138.5 (C-4), 116.0 (C-9), 105.9 (C-2/6), 105.4 (Glc-1), 83.5 (C-7), 79.1 (Glc-3), 78.8 (Glc-5), 76.5 (Glc-2), 72.0 (Glc-4), 64.5 (OCH_2CH_3), 63.0 (Glc-6), 57.0 (OCH_3), 16.0 (OCH_2CH_3); HR-DART-MS (positive mode) *m/z* = 418.1997 $[M + NH_4]^+$ (calcd for $C_{19}H_{28}O_9NH_4$, 418.1992).

*Tatarine C-4′-O-β-D-glucopyranoside (**11**)*

Yellowish powder; m.p. 179.4 °C; $[\alpha]_D^{23}$: − 15.4° (*c* 0.1, MeOH); UV λ_{max} (MeOH) nm (log ε): 219 (4.14), 322 (3.43); IR (ATR) cm^{-1}: 3331, 2920, 1684, 1511, 1450; ^{1}H- and ^{13}C-NMR data, see Table 1. HR-ESI–MS (negative mode) *m/z* = 485.1773 $[M - H]^-$ (calcd for $C_{21}H_{29}N_2O_{11}$, 485.1771).

Table 1 ^{1}H-NMR and ^{13}C-NMR spectroscopic data of compound **11** and baimantuoluoamide B in DMSO-d_6

Position	**11**[a]		Baimantuoluoamide B[b]	
	δ_H Mult (*J* in Hz)	δ_C	δ_H Mult (*J* in Hz)	δ_C
2	–	155.8	–	155.6
3	–	153.8	–	153.7
5	7.29 s	116.6	7.32 s	116.5
6	–	131.3	–	131.2
7	–	130.9	–	130.8
8	6.93 s	116.1	6.93 s	115.9
9	–	124.9	–	124.8
10	–	123.4	–	123.2
11	2.19 s	18.8	2.19 s	20.6
12	2.23 s	19.4	2.23 s	21.2
1′	4.50 dd (13.5, 10.5)	44.3	4.46–4.52 m	44.5
	4.16 dd (13.5, 2.0)		4.02–4.06 m	
2′	4.01 m	67.3	4.02–4.06 m	67.7
3′	3.78 m	72.5	3.56–3.60 m	73.6
4′	3.78 m	80.8	3.76–3.78 m	70.6
4′	3.67 m	60.5	3.41–3.47 m	72.0
Glc-1	4.42 d (7.5)	102.6	4.17 d (7.6)	103.6
Glc-2	3.09 m	73.8	3.00 t (8.4)	73.6
Glc-3	3.20 m	76.5	3.06–3.19 m	76.2
Glc-4	3.06 m	70.3	3.06–3.19 m	69.8
Glc-5	3.17 m	77.1	3.06–3.19 m	76.8
Glc-6	3.60 dd (11.5, 5.0)	61.3	3.43 dd (11.2, 2.2)	60.8
	3.37 overlap		3.67 dd (11.2, 5.2)	

[a] ^{1}H- and ^{13}C-NMR spectroscopic data were acquired at 500 and 125 MHz, respectively

[b] ^{1}H- and ^{13}C-NMR spectroscopic data were acquired at 400 and 100 MHz, respectively (Yang et al. 2010)

*(4E,12Z)-threo-Tetradeca-4,12-diene-8,10-diyne-1,6,7,14-tetrol (**17**)*

Dark yellowish solid; $[\alpha]_D^{23}$: 8.8° (*c* 0.1, MeOH); UV λ_{max} (MeOH) nm (log ε): 219 nm (3.86), 268 nm (3.71); IR (ATR) cm^{-1}: 3339, 2928, 2128, 1668, 1058; ^{1}H- and ^{13}C-NMR data, see Table 2. HR-ESI–MS (positive mode) *m/z* = 273.1104 $[M + Na]^+$ (calcd for $C_{14}H_{18}O_4Na$, 273.1103).

Acid hydrolysis and determination of absolute configuration of the sugars

Acid hydrolysis

Compounds **1** and **11** (each 2.5 mg) was subjected to acid hydrolysis with 1 N HCl for 2 h. β-Glucose was identified by co-TLC (*Rf* 0.55, *n*-BuOH-acetic acid-H_2O, 2:1:1) with a standard compound, confirming they have a β-glucopyranosyl group.

Determination of absolute configuration of the sugars

The hydrolysate was dissolved in pyridine (0.5 mL) containing L-cystein methyl ester hydrochloride (0.5 mg) and heated to 60 °C for 1 h. One hundred microliters of *σ*-tolyl isothiocyanate (0.5 mg) solution in pyridine (0.5 mL) was added to the mixture then heated to 60 °C for 1 h. The reaction mixtures were directly analyzed by LC–MS (10–100% aqueous CH_3CN gradient with 0.1% formic acid over 50 min). The hydrolysate derivatives of compounds **1** and **11** were detected at 14.7 min. The retention time of authentic glucose samples were 14.3 (L-glucose) and 14.7 (D-glucose) min under the same LC/MS conditions. Therefore, the absolute configuration of the glucose unit in compounds **1** and **11** were established as D-configuration.

Table 2 ^{1}H-NMR and ^{13}C-NMR spectroscopic data of compound **17** and pilosulyne B in CD_3OD

Position	**17**[a]		Pilosulyne B[b]	
	δ_H Multi (*J* in Hz)	δ_C	δ_H Multi (*J* in Hz)	δ_C
1	3.58 t (7.0)	62.3	3.58 t (6.6)	62.3
2	1.64 quin (6.0)	33.0	1.65 quin (6.6)	33.1
3	2.16 dt (7.5, 7.0)	29.7	2.16 dt (6.9, 6.6)	29.7
4	5.82 dt (15.5, 7.5)	135.2	5.82 dt (15.4, 6.9)	135.2
5	5.57 dd (15.5, 6.5)	129.6	5.57 dd (15.4, 6.6)	129.6
6	4.00 t (6.5)	76.6	4.00 t (6.6)	76.6
7	4.23 d (6.5)	67.7	4.26 d (6.6)	67.8
8	–	82.2	–	83.8
9	–	71.0	–	70.8
10	–	74.2	–	74.7
11	–	77.4	–	79.4
12	5.81 br d (16.0)	108.5	5.64 br d (11.0)	109.1
13	6.41 dt (16.0, 4.5)	148.2	6.24 dt (11.0, 6.4)	147.8
14	4.14 dd (4.5, 2.5)	62.6	4.31 dd (6.4, 1.2)	61.1

[a] ^{1}H- and ^{13}C-NMR spectroscopic data were acquired at 500 and 125 MHz, respectively

[b] ^{1}H- and ^{13}C-NMR spectroscopic data were acquired at 400 and 100 MHz, respectively (Chao et al. 2015)

Measurement of cell viability

Human ovarian cancer cells A2780 were originally obtained from American Type Culture Collection (ATCC). A2780 cells were cultured in RPMI 1640 supplemented with 5% fetal bovine serum (FBS), penicillin (100 U/mL) and streptomycin sulfate (100 μg/mL). Cell viability was assessed by MTT [3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazoliumbromide] assays. Briefly, the cells (5×10^4) were seeded in each well containing 50 μL of RPMI medium in a 96-well plate. After 24 h, cisplatin and various concentrations of the compounds from the roots of *C. lanceolata* were added. After 48 h, 50 μL of MTT (5 mg/mL stock solution) was added, and the plates were incubated for an additional 4 h. The medium was discarded, and the formazan blue that formed in the cells was dissolved in 50 μL of DMSO. The optical density was measured at 540 nm by microplate spectrophotometer (SpectraMax; Molecular Devices, Sunnyvale, CA, USA).

Measurment of nitric oxide production

RAW264.7 cells were plated at 1.0×10^5 cells/well in 60-mm dishes and incubated with or without LPS (1 μg/mL) in the absence or presence of indicate concentration of the samples for 24 h. The nitrite which accumulated in culture medium was measured as an indicator of nitric oxide production according to the Griess reagent. The culture supernatant (100 μL) was mixed with 100 μL of Griess reagent [equal volumes of 1% (w/v) sulfanilamide in 5% (v/v) phosphoric acid and 0.1% (w/v) naphthyl ethylenediamine–HCl] for 10 min, and then the absorbance at 540 nm was measured in a microplate reader (SpectraMax; Molecular Devices, Sunnyvale, CA, USA). Fresh culture medium was used as the blank in all experiments. The amount of nitrite in the samples was determined with reference to a sodium nitrite standard curve.

Results

Structure elucidation of compounds 1, 11, and 17 from the roots of *C. lancerolata*

Compound **1** was isolated as a white powder and the molecular formula was established as $C_{19}H_{28}O_9$ by HR-DART-MS (*m/z* = 418.1997 $[M + NH_4]^+$ calcd for $C_{19}H_{28}O_9NH_4$, 418.1992). The UV spectrum showed absorption bands 219 and 269 nm, indicating **1** is a phenolic compound. The ^{1}H-NMR spectrum of **1** showed signals arising from two symmetrical methoxy protons at δ_H 3.76 (6H, s) and aromatic protons at δ 6.91 (2H, s, H-2/6), indicating that **1** has a syringyl group (Table 1). The anomeric proton signal δ_H 5.82 (1H, d, *J* = 7.0 Hz) and multiple peaks of δ_H 3.80–4.53 ppm revealed the presence of a sugar. The ^{1}H-NMR spectrum of **1** also showed the presence an ethoxy group [δ_H 3.48 (2H, dq, *J* = 15.0 and 7.0 Hz) and 1.27 (3H, t, *J* = 7.0 Hz)] and a vinyl group [δ_H 5.20 (d, *J* = 10.0 Hz), 5.41 (d, *J* = 17.0 Hz), and 6.08 (ddd, *J* = 17.0, 10.5, 6.0 Hz)]. The ^{13}C-NMR spectrum of **1** (Table 1) revealed 19 carbon signals, six aromatic signals [δ_C 154.3 (× 2), 140.4, 138.5, and 105.9 (× 2)], two methoxy signals [δ_C 57.0 (× 2)], an ethoxy signals (δ_C 64.5 and 16.0), two olefinic signals (δ_C 116.0 and 140.4), an oxygenated methine signal (δ_C 83.5), and a *β*-glucopyranosyl signals (δ_C 105.5, 79.1, 78.8, 76.5, 72.0, and 63.0), indicating that **1** is a phenylpropanoid glucoside with an ethoxy group. *β*-D-Glucopyranosyl group in **1** was determined by an acid hydrolysis and sugar analysis ("Materials and methods"). From these data, **1** was assumed to be a phenylpropanoid glucoside. The ^{1}H- and ^{13}C-NMR spectra of **1** were almost superimposable with those of tangshenoside II (**2**), which was also isolated from this study, except for the presence of an ethyl group. The HMBC correlation from the oxygenated methylene proton (δ_H 3.48) and C-7 (δ_C 83.5) indicated that the location of ethyl group is C-7. The chirality of the C-7 position of **1** was assigned as *S*-configuration by comparing the optical rotation of **1** ($[\alpha]_D^{23} = -34.4°$) with that of tangshenoside II ($[\alpha]_D^{23} = -29.0°$) (Mizutani et al. 1988). From all of the above data, the structure of the new **1** was elucidated as *7-O*-ethyltangshenoside II (Fig. 1).

Compound **11** was isolated as a yellowish powder. Its molecular formula was determined to be $C_{21}H_{30}N_2O_{11}$ based on the HR-ESI–MS (*m/z* = 485.1773 $[M - H]^-$ calcd for $C_{21}H_{29}N_2O_{11}$, 485.1771) and ^{13}C-NMR data. The ^{1}H-NMR spectrum of **11** showed some typical proton signals, including two *para* orientation benzene ring proton signals [7.36 (1H, s, H-8) and δ_H 6.94 (1H, s, H-5)], and two methyl proton signals in benzene ring [2.23 (3H, s, H-12) and δ_H 2.19 (3H, s, H-11)]. The ^{13}C-NMR spectrum of **11** revealed the presence of a benzene ring (δ_C 131.3, 130.9, 124.9, 123.4, 116.6, and 116.1), two amide carbonyl groups (δ_C 155.8 and 153.8), two methyl carbons (δ_C 19.4 and 18.8), and a ribityl group [$HOCH_2(CHOH)_3CH_2$] (δ_C 80.8, 72.5, 67.3, 60.5, and 44.3). In addition, ^{1}H- and ^{13}C-NMR data of **11** displayed a set of *β*-glucopyranosyl group [δ_H 4.42 (1H, d, *J* = 7.5 Hz, H-1″); δ_C 102.6 (C-1″), 73.8 (C-2″), 76.5 (C-3″), 70.3 (C-4″), 77.1 (C-5″), 61.3 (C-6″)] (Table 1). The ^{13}C-NMR data of **11** was very similar to an amide alkaloid baimantuoluoamide B (tatarine C-5′-*O*-glucoside) isolated from the flower of *Datura metel,* except for chemical shifts for C-4′ and C-5′ in the ribityl group (Yang et al. 2010). Thus, we could assume that the glucopyranosyl group in **11** is located in a different position from baimantuoluoamide B. The connection of the ribityl and glucopyranosyl groups in **11** were deduced through analysis of COSY and HMBC spectra of **11** (Fig. 2). *β*-D-Glucopyranosyl group in **11** was determined by an acid hydrolysis of **11** and sugar analysis ("Materials and methods"). The position of the ribityl group at N-1 was determined from HMBC correlations between δ_H 4.50 (1H, dd, *J* = 13.5 and 11.5, H-1′) and δ_C 155.8 (C-2) and 124.9 (C-9). The location of the β-D-glucopyranosyl group was determined at C-4′ by the long-range correlations from anomeric proton at δ_H 4.42 (1H, d, *J* = 7.5 Hz) to δ_C 80.8 (C-4′). On the basis of the above data, **11** was elucidated as tatarine C-4′-*O*-*β*-D-glucopyranoside.

Compound **17** was obtained as a dark yellowish solid, and the molecular formula was determined to be $C_{14}H_{18}O_4$ by HR-ESI–MS (*m/z* = 273.1104 $[M + Na]^+$ calcd for $C_{14}H_{18}O_4Na$, 273.1103). The ^{1}H-NMR spectrum of **17** revealed four CH_2 groups [δ_H 4.14 (2H, dd, *J* = 4.5, 2.5 Hz, H-14), 3.58 (2H, t, *J* = 7.0 Hz, H-1), 2.16 (2H, dt, *J* = 7.5, 7.0 Hz, H-3), and 1.64 (2H, quin, *J* = 6.0 Hz, H-2)] together with two O-bearing CH groups at [δ_H 4.23 (1H, d, *J* = 6.5 Hz, H-7) and 4.00 (1H, t, *J* = 6.5 Hz, H-6)], and two pairs of *trans*-olefinic protons at [δ_H 5.82 (1H, dt, *J* = 15.5, 7.5 Hz, H-4)/5.57 (1H, dd, *J* = 15.5, 6.5 Hz, H-5); 6.41 (1H, dt, *J* = 16.0, 4.5 Hz, H-13)/5.81 (1H, br d, *J* = 16.0 Hz, H-12)]. The ^{13}C-NMR spectrum of **17** revealed the presence of 14 carbons which were ascribed to four alkyne C atoms (δ_C 82.2, 77.4, 74.2, and 71.0), four oxygenated C atoms (δ_C 76.6, 67.7, 62.6, and 62.3), two high-field CH_2 groups (δ_C 33.0 and 29.7), and four olefinic C atoms (δ_C 148.2, 135.2, 129.6, and 108.5). On the basis of this observation it could be proposed that **17** is a C_{14} polyacetylene with two double bonds, two triple bonds and four hydroxyl groups. The ^{1}H- and ^{13}C-NMR spectra of **17** were almost the same as those of pilosulyne B, which was isolated from the roots of *Codonopsis pilosula*, except for a pair of olefinic protons at 6.41 (1H, dt, *J* = 16.0, 4.5 Hz, H-13)/5.81 (1H, br d, *J* = 16.0 Hz, H-12) (Chao et al. 2015). The *Z* geometry of the C-12/C-13

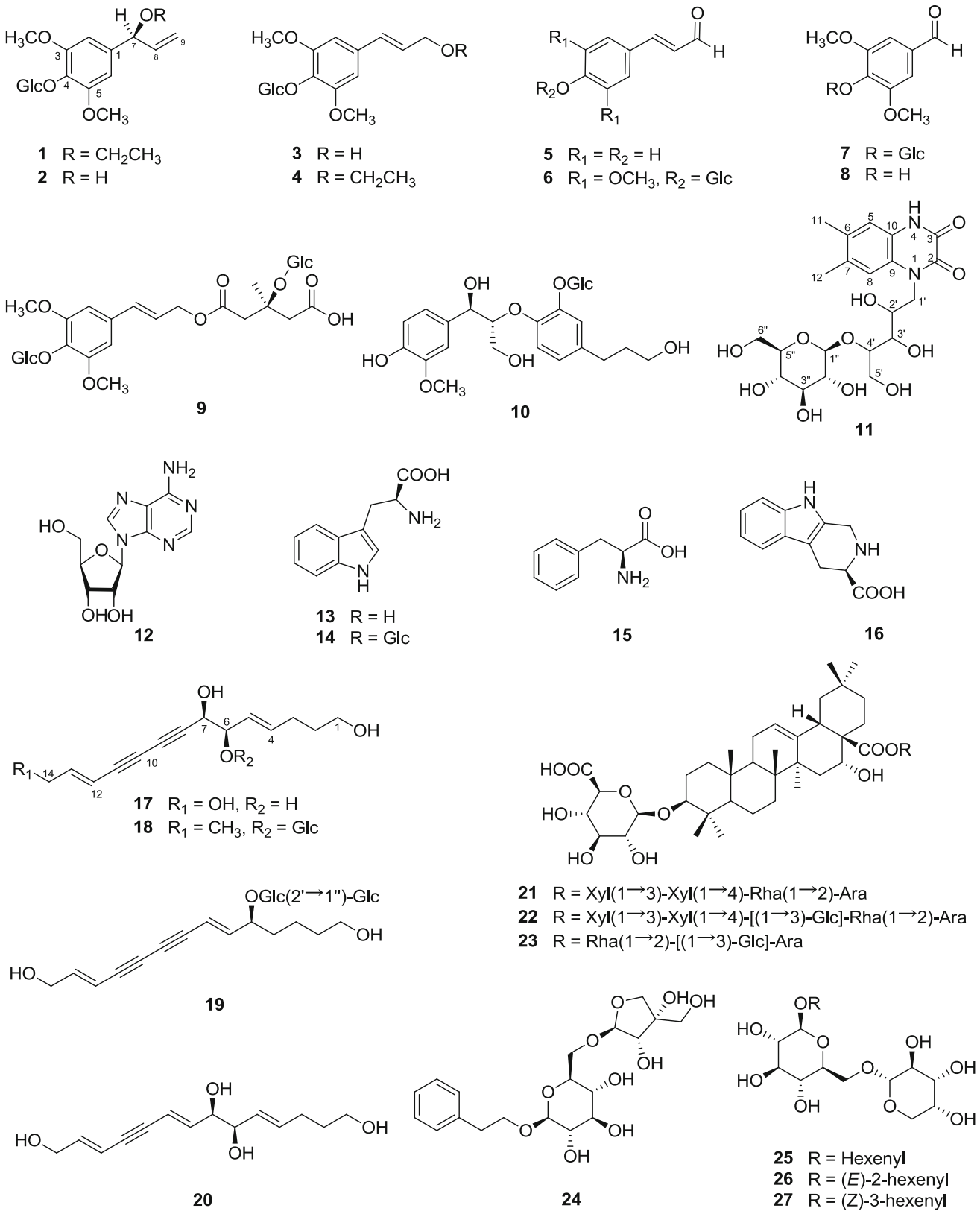

Fig. 1 Compounds **1**–**27** isolated from the roots of *C. lanceolata*

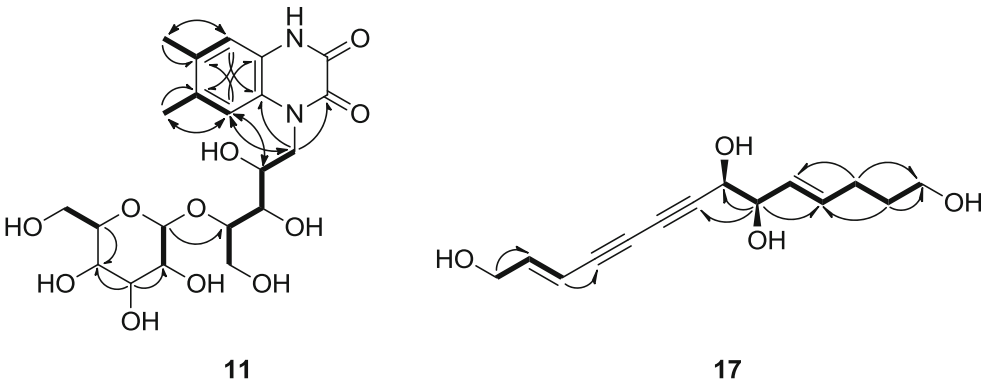

Fig. 2 Key correlations observed in the COSY (▬), HMBC (→), and NOESY (↔) NMR spectra of compounds **11** and **17**

double bond in pilosulyne B was identified from a coupling constant of $J_{12–13}$ = 11.0 Hz and an NOE correlation between these alkene protons. However, the geometric configuration at the C-12/C-13 double bond in **17** was determined to be *E* with observed coupling constant of $J_{12–13}$ = 16.0 Hz (Table 2). The *vicinal* diol group of **17** was determined to possess *threo*-configuration by the coupling constant (*J* = 6.5 Hz) between H-6 and H-7 (Chao et al. 2015). Therefore, the structure of **17** was elucidated as (4*E*,12*Z*)-*threo*-tetradeca-4,12-diene-8,10-diyne-1,6,7,14-tetraol. Previously, **17** has been reported in only one paper as a product from hydrolysis of cordifolioidyne A which is *O*-*β*-D-glucopyranoside of **17** (Mei et al. 2008). Thus, to the best of our knowledge, **17** was isolated from nature for the first time in this study.

Identification of the known compounds from the roots of *C. lanceolata*

The structures of the known compounds were identified to be tangshenoside II (**2**) (Mizutani et al. 1988), syringin (**3**) (Kiem et al. 2003), ethyl syringin (**4**) (Aga et al. 2012), *p*-hydroxycinnamaldehyde (**5**) (Leem et al. 1999), sinapaldehyde glucoside (**6**) (Slacanin et al. 1991), syringaldeyde-4-*O*-*β*-D-glucopyranoside (**7**) (Delay and Delmotte 1990), syringaldehyde (**8**) (Tripathi et al. 2010), tangshenoside I (**9**) (Mizutani et al. 1988), 7*R*,8*R*-*threo*-4,7,9,9′-tetrahydroxy-3-methoxy-8-*O*-4′-neolignan-3′-*O*-*β*-D-glucopyranoside (**10**) (Matsuda and Kikuchi 1996), adenosine (**12**) (Yu et al. 2005), tryptophan (**13**) (Bradbury and Norton 1973), tryptophan-*N*-glucoside (**14**) (Diem et al. 2000), phenylalanine (**15**) (Avison et al. 1990), 1,2,3,4-tetrahydro-*β*-carboline-3-carboxylic acid (**16**) (Wang et al. 2012), lobetyolin (**18**) (Ishimaru et al. 1991), pilosulyne E (**19**) (Chao et al. 2015), pilosulyne G (**20**) (Chao et al. 2015), lancemaside A (**21**) (Ushijima et al. 2008), lancemaside B (**22**) (Ushijima et al. 2008), lancemaside D (**23**) (Ushijima et al. 2008), icariside D_1 (**24**) (Miyase et al. 1987), creoside IV (**25**) (Al-Massarani et al. 2017), (*E*)-2-hexenyl α-L-arabinopyranosyl-(1 → 6)-*β*-D-glucopyranoside (**26**) (Yuda et al. 1990), and (*Z*)-3-hexenyl α-L-arabinopyranosyl-(1 → 6)-*β*-D-glucopyranoside (**27**) (Nishikitani et al. 1999) by spectroscopic measurements (^{1}H-NMR, ^{13}C-NMR, 2D-NMR, UV and IR), spectrometric methods (MS), comparison with published values, and comparison of TLC values with authentic samples (Fig. 1).

Inhibitory effects of compounds 1–27 on nitric oxide production in macrophages

We investigated the effects of the isolates **1**–**27** from the roots of *C. lanceolata* on the production of NO, a key inflammatory mediator, in LPS-stimulated RAW 264.7 macrophages. Based on MTT assay data, concentrations of the isolates that would not affect cell viability were chosen for the experiment. Among the twenty seven compounds, only *p*-hydroxycinnamaldehyde (**5**), lancemaside A (**21**), and lancemaside B (**22**) exhibited a significant inhibitory effect on the NO production with IC_{50} values of 63.86, 37.86, and 37.38 μM, respectively, without affecting cell viability (Table 3). Other isolates did not show any significant effect (IC_{50} > 100 μM) on nitric oxide (NO) production. Figure 3 shows dose-dependent effect of lancemaside A (**21**) and lancemaside B (**22**) on LPS-induced NO production in RAW 264.7 cells.

Inhibitory effects of compounds 1–27 on cell viability in ovarian cancer cells

To identify cytotoxic constituents in the 50% EtOH extract from the roots of *C. lanceolata*, we investigated the effects of compounds **1–27** on the cell viability of A2780 human ovarian cancer cells using MTT assays. The effects were assessed using IC_{50} values, and summarized in Table 3. Of the isolates, lancemaside A (**21**) exhibited the most potent inhibitory effect on viability of A2780 cells with observed IC_{50} value of 16.2 μM (Table 3). Adenosine (**12**) and lancemaside B (**22**) showed mild activity against A2780 cells with IC_{50} values of 35.19 and 77.75 μM, respectively. Other compounds did not show any significant effect

Table 3 Inhibitory effects of the compounds **5**, **12**, **21**, and **22** isolated from the roots of *C. lanceolata* on nitric oxide production in LPS-induced RAW 264.7 cells and cell viability in human ovarian cancer cells (A2780)

Compound	IC_{50} (mM)[a]	
	NO production in RAW264.7 cells	Cell viability in A2780 cells
5	63.86 ± 0.13	> 100
12	> 100	35.19 ± 0.86
21	37.86 ± 0.11	16.20 ± 0.32
22	37.38 ± 0.14	77.75 ± 1.82
L-NIL[b]	8.03 ± 0.03	–
Cisplatin[c]	–	10.64 ± 0.12

[a]IC_{50} values are defined as the concentration that results in a 50% decrease production of nitric oxide (NO assay) and a 50% decrease in the number of cells compared to that of the control cultures. The values represent the means of the results from three independent experiments with similar patterns. Other isolates did not show any significant effect (IC_{50} > 100 μM) on nitric oxide (NO) production in RAW264.7 cells and cell viability in A2780 cells

[b]L-NIL [L-N^6-(1-Iminoethyl)lysine]

[c]Cisplatin were used as a positive control for NO assay and for MTT assay, respectively

(IC_{50} > 100 μM) on cell viability in A2780 cells. Figure 3 shows dose-dependent effect of lancemasides A (**21**) and B (**22**) on cell viability in A2780 cells.

Discussion

Twenty seven compounds (**1**–**27**) including a new phenylpropanoid (**1**), a new alkaloid (**11**), and one new natural polyacetylene (**17**) were isolated from the roots of *C. lanceotata* in this study. It seems that compounds **1** and **4** are ethylated substitutions formed during the extraction procedure with aqueous EtOH. To the best of our knowledge, two phenylpropanoids, *p*-hydroxycinnamaldehyde (**5**) and sinapaldehyde glucoside (**6**), two phenolic compounds, syringaldeyde-4-*O*-*β*-D-glucopyranoside (**7**) and syringaldehyde (**8**), a lignin, 7*R*,8*R*-*threo*-4,7,9,9′-tetrahydroxy-3-methoxy-8-*O*-4′-neolignan-3′-*O*-*β*-D-glucopyranoside (**10**), three alkaloids, adenosine (**12**), tryptophan-*N*-glucoside (**14**) and phenylalanine (**15**), two polyacetylenes, pilosulyne E (**19**) and pilosulyne G (**20**) and three hexyl glycosides, creoside IV (**25**), (*E*)-2-hexenyl α-L-arabinopyranosyl-(1 → 6)-*β*-D-glucopyranoside (**26**), and (*Z*)-3-hexenyl α-L-arabinopyranosyl-(1 → 6)-*β*-D-glucopyranoside (**27**) were isolated from the roots of *C. lanceolata* for the first time in this study.

All the isolates **1**–**27** from the roots of *C. lanceolata* were evaluated for their inhibitory effects on LPS-induced nitric oxide (NO) production in RAW 264.7 cells and cell viability in A2780 human ovarian cancer cells. Of the compounds, lancemasides A (**21**) and B (**22**), major triterpenoidal saponins of *C. lanceolate*, exhibited significant effects in both bioactivity assays. There are several reports that show that lancemaside A possesses anti-inflammatory effects (Joh and Kim 2010; Kim et al. 2014), which are consistent with our data on lancemaside A in this study. Another report showed that lancemaside A effectively inhibited the growth of A549 human lung epithelial cancer cells (Joh et al. 2012). However, the inhibitory effect of lancemaside A on the cell growth in human ovarian cancer cells has not been reported to date. To the best of our knowledge, there is no report on any pharmacological activity of lancemaside B, an analogue of lancemaside A (**21**), to date. In this study, we demonstrated that lancemaside A markedly inhibited the viability of A2780 human ovarian cancer cells. In addition, we found that lancemaside B (**22**) has inhibitory effect on LPS-induced NO production in macrophages and cell viability in A2780 cells. Therefore, lancemaside B (**22**) is worthy of consideration as a potential anti-inflammatory and/or anti-cancer agent through additional biological evaluation.

In conclusion, a new phenylpropanoid (**1**), a new alkaloid (**11**), and a new natural polyacetylene (**17**), as well as 24 known compounds (**2**–**10**, **12**–**16**, **18**–**27**), were isolated from the roots of *C. lanceolata*. All the isolates were evaluated for their inhibitory effects on LPS-induced NO production in RAW264.7 macrophages and cell viability in A2780 human ovarian cancer cells. Lancemasides A (**21**) and B (**22**), major triterpenoidal saponins of *C. lanceolate,* were found to exhibit significant inhibitory effects on NO production in macrophages and cell viability in cancer

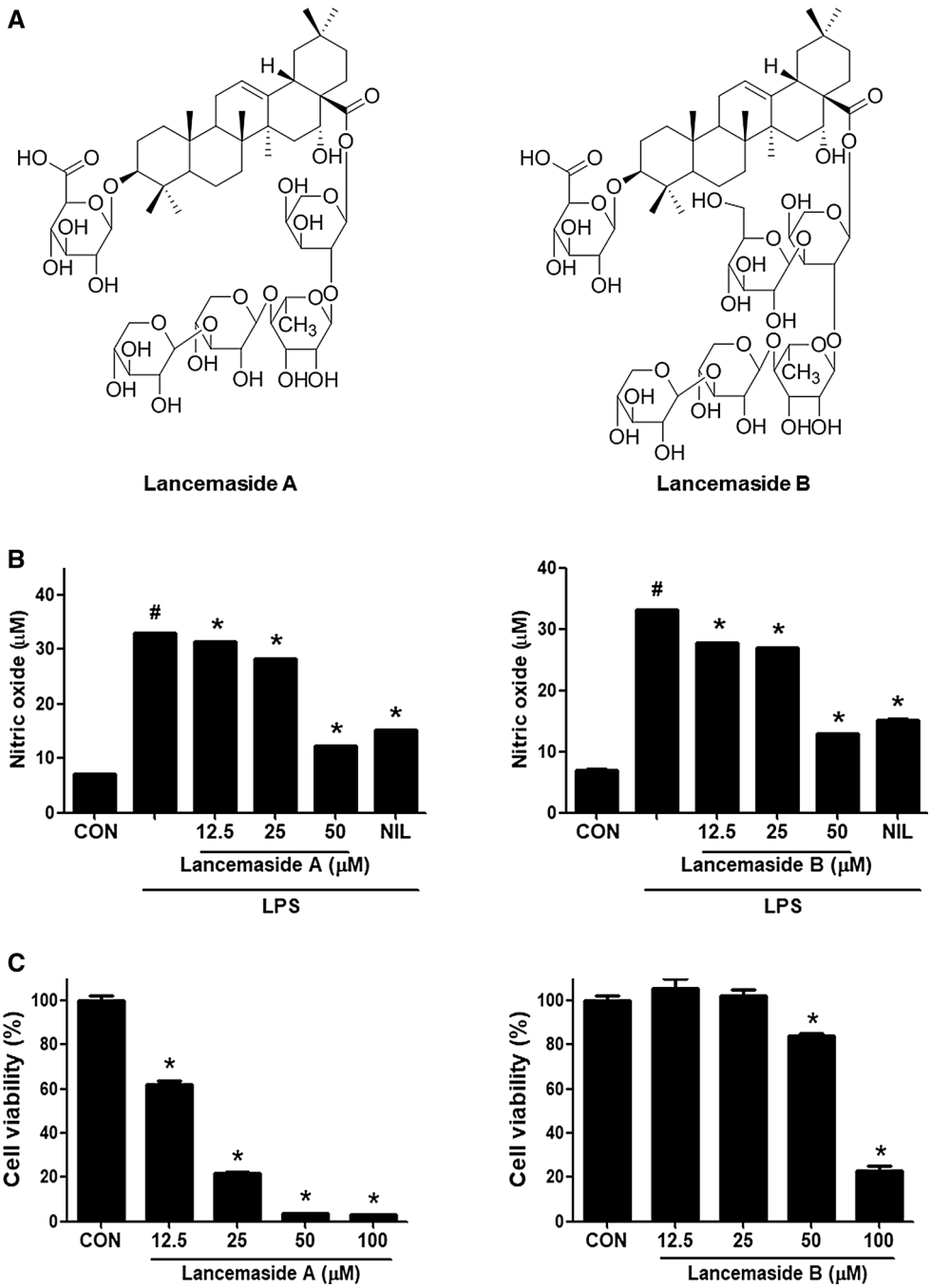

Fig. 3 Effects of lancemasides A (**21**) and B (**22**) on NO production. **a** Chemical structures of lancemasides A and B. **b** Following pretreatment with lancemasides A and B (12.5, 25, or 50 μM) for 1 h, cells were treated with LPS (1 μg/mL) for 24 h. Levels of NO in the culture medium were quantified using a Griess reaction assay. Controls were not treated with LPS nor compounds. l-N^6-(1-Iminoethyl)lysine (L-NIL) (10 μM) was used as a positive control for NO assay. Data are presented as the mean ± SD of three independent experiments. $^{\#}p < 0.05$ vs. the control group; $^{*}p < 0.05$ vs. LPS-stimulated cells. **c** Effect of lancemasides A and B on cell viability in human ovarian cancer A2780 cells was determined using MTT assay. Briefly, A2780 cells were seeded in a 96-well plate. After 24 h, the cells were treated with the indicated concentration of lancemasides A and B for 48 h. Data are presented as the mean ± SD of three independent experiments. $^{*}p < 0.05$ vs. the control group

cells. It is noteworthy that this is the first report on the pharmacological activities of lancemaside B.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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