Bioorganic Chemistry 85 (2019) 274–281

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Lanceoleins A–G, hydroxychalcones, from the flowers of *Coreopsis lanceolata* and their chemopreventive effects against human colon cancer cells

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ARTICLE INFO

Keywords:
Coreopsis lanceolata
Lanceoleins A – G
Chalcone
Cytotoxicity
Apoptosis
Western blot
Nitric oxide
RAW264.7 macrophages

ABSTRACT

Seven new chalcones, lanceolein A–G (compounds **5** and **7–12**), as well as five known chalcones (**1–4** and **6**), were isolated from the methanolic extract of *Coreopsis lanceolata* flowers. The chemical structures of **5** and **7–12** were determined on the basis of spectroscopic data interpretation. All compounds inhibited the production of nitrite oxide (NO) induced by LPS in RAW264.7 macrophage cells. Also, compounds **1–6** showed moderated cytotoxicity against human colon cancer cell lines, while compounds **7–12** hardly showed the cytotoxicity. Especially, compounds **2**, **5**, and **6** exhibited a little higher cytotoxicity on HCT15 cells, with IC_{50} values of 43.7 ± 2.17 μM, 35.6 ± 0.24 μM, and 47.9 ± 1.18 μM, respectively. In the Tali assay, compounds **2** and **5** increased the numeral of apoptotic cells. These compounds also significantly promoted the expression of apoptotic proteins including PARP and caspase-3.

1. Introduction

Coreopsis, a genus in the subfamily Asteroideae [1], is one of the most common Asteraceae phanerogams. *Coreopsis* species are widely distributed in continental America and Eastern Asia [2]. Typically, *C. drummondii*, *C. tinctoria*, and *C. lanceolata* are the most common *Coreopsis* plants distributed throughout Korea. *C. tinctoria* was reported to contain many flavonoids such as quercetin, luteolin, butin, and apigenin [3]. *C. tinctoria* and *C. lanceolata* have the same origin and almost the same shape and color. *C. lanceolata* is more commonly found in Korea, and has bigger flowers compared to *C. tinctoria* or *C. drummondii*. *C. tinctoria* also differs from *C. lanceolata* in that the central part of the flower is reddish purple, and the leaves are thinner. A recent report that *C. tinctoria* has antiproliferative activity against human colon cancer cells [4] led us to predict that *C. lanceolata* also contains various flavonoids that exhibit cytotoxicity to cancer cells (see Fig. 1).

C. lanceolata, a herbaceous perennial plant that originated in continental America, South Africa, and Eastern Asia, is easily found on the roadside. *C. lanceolata* has been mainly used as an ornamental plant [5]. However, these plants were also reported to have antioxidant [6], antiallergic [7], antibacterial [7], antileukemic [8], and nematicidal [9] effects. Despite several reported pharmacological activities of *C. lanceolata*, only a few flavonoids have been isolated from the flowers of *C. lanceolata* and identified [19]. Our previous study revealed that alcohol extracts of *C. lanceolata* flowers showed cytotoxicity to HCT15 human colon cancer cell lines with 34.97 ± 1.40% cell viability at 100 μg/ml. This study focused on identifying metabolites from the flowers of *C. lanceolata* with cytotoxic activity against human colon cancer cells.

Colorectal cancer is a terrible disease ranking third in cancer incidence and fourth in cancer mortality. The management of colorectal cancer mainly consists of surgical treatment, chemotherapy, and radiation therapy, all of which affect normal cells and cause many side effects [10]. In recent studies, cytokines that cause inflammation were revealed to promote invasion of cancer and contribute to maintaining sufficient blood supply for cancer cell growth [11–13]. The development of anticancer agents that react specifically with cancer cells to cause an anticancer effect yet do not have toxicity to normal cells has become an critical goal. In addition, an agent that reduces the excessive inflammatory cytokine expression in macrophages might be used as an adjuvant anticancer agent for colorectal cancer [13].

Natural plant resources have been reported to exhibit various bioactivities with high safety since ancient times. In particular, the flowers of plants contain a large amount of flavonoids, which have

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https://doi.org/10.1016/j.bioorg.2019.01.003
Received 28 November 2018; Received in revised form 28 December 2018; Accepted 1 January 2019
Available online 04 January 2019
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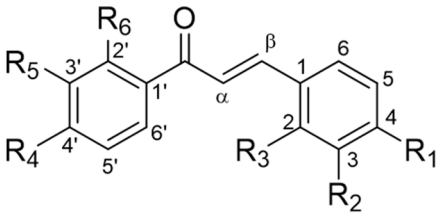

	R_1	R_2	R_3	R_4	R_5	R_6
1	OCH_3	H	OH	OCH_3	H	H
2	OH	H	H	OH	OCH_3	OH
3	OH	OH	H	OH	OCH_3	OH
4	OCH_3	OH	H	OH	OCH_3	OH
5	OCH_3	OCH_3	H	OH	OH	OCH_3
6	OCH_3	OCH_3	H	OH	H	OCH_3
7	OH	H	H	OH	OCH_3	-glc
8	OH	OH	H	OH	OH	-glc
9	OH	OCH_3	H	OH	OH	-glc
10	OH	OH	H	OH	OCH_3	-glc
11	OCH_3	OH	H	OH	OCH_3	-glc
12	OCH_3	OH	H	OH	OCH_3	6-rha-glc

rha: *α*-L-rhamnopyranosyl; glc: *β*-D-glucopyranosyl;
6-rha-glc: *O*-*α*-L-rhamnopyranosyl(1→6)-*O*-*β*-D-glucopyranosyl

Fig. 1. Structures of compounds **1–12** isolated from the flowers of *Coreopsis lanceolata*.

many biological activities such as antioxidant [14], anti-inflammatory [15], and antiproliferative [16] activity. Therefore, we hypothesized that flowers of *C. lanceolata* would contain cytotoxic materials with activity against human colon cancer cells.

In this study, seven new chalcones and five known ones were isolated and identified. With the exception of compounds **3** and **4,** these compounds were isolated for the first time from the flowers of *C. lanceolata.* The isolated compounds were evaluated for their inhibitory effects on LPS-induced nitric oxide (NO) production in RAW 264.7 macrophage cells and cytotoxic activity against HCT15 human colon cancer cells. This paper describes the procedure for isolation and identification of chalcones from *C. lanceolata* flowers and their inhibitory effects on LPS–induced NO production in RAW264.7 macrophages and cytotoxic activity against colon cancer cells.

2. Materials and methods

2.1. Plant materials

Dried flowers of *Coreopsis lanceolata* L. were collected at Kyung Hee University, Yongin, Korea in June 2015 and identified by Professor Dae-Keun Kim, Woosuk University, Jeonju, Korea. A voucher specimen (KHU2015-0624) is reserved at the Laboratory of Natural Products Chemistry, Kyung Hee University, Yongin, Korea.

2.2. Reagents and instruments

The silica gel and the octadecyl silica gel (ODS) resins used for column chromatography (CC) were Kiesel gel 60 (Merck, Darmstadt, Germany) and the Lichroprep RP-18 (40–60 μm, Merck) respectively. Sephadex LH-20 was purchased from Amersham Biosciences (Uppsala, Sweden). Thin layer chromatography (TLC) was carried out using Kiesel gel 60 F_{254} and RP-18 F_{254S} (Merck) TLC plates, and the spots were detected using a UV lamp Spectroline Model ENF-240 C/F (Spectronics Corporation, Westbury, NY, USA) and a 10% H_2SO_4 solution. Deuterium solvents were purchased from Merck Co. Ltd and Sigma Aldrich Co. Ltd (St. Louis, MO, USA). Nuclear magnetic resonance (NMR) spectra were recorded on a 400 MHz FT-NMR spectrometer (Varian Inova AS-400, Palo Alto, CA, USA). Infrared (IR) spectra were obtained using a Perkin Elmer Spectrum One FT-IR spectrometer (Buckinghamshire, England). Fast atom bombardment mass spectrometry (FABMS) spectra were recorded on a JEOL JMS-700 (Tokyo, Japan). Melting points were obtained using a Fisher-John's melting point apparatus (Fisher Scientific, Miami, FL, USA) with a microscope, and the values obtained were uncorrected.

2.3. Extraction and isolation of hydroxychalcones

The air-dried flowers of *C. lanceolata* (3.1 kg) were extracted with 80% aqueous MeOH (68 L × 4) at room temperature for 24 h. The extracts were filtered through filter paper and evaporated under reduced pressure at 43 °C to yield 1.1 kg of extract. The obtained MeOH extracts were suspended in H_2O (2 L) and then successively extracted with ethyl acetate (EtOAc, 2 L × 4) and *n*-butanol (*n*-BuOH, 2 L × 4). Each layer was concentrated under reduced pressure to obtain EtOAc (CLFE 194 g), *n*-butanol (CLFB 254 g), and H_2O (CLFW 652 g) fractions. Fraction CLFE (194 g) was subjected to a silica gel CC (φ 13 × 17 cm) and eluted with $CHCl_3$–MeOH–H_2O (32:3:1 → 12:3:1 → 9:3:1 → 6:4:1; 18 L of each) to yield 13 fractions (CLFE-1 to CLFE-13). Fraction CLFE-2 [5.2 g, elution volume/ total volume (V_e/V_t) 0.020–0.023] was subjected to a SiO_2 CC (φ 5 × 15 cm) and eluted with *n*-hexane–EtOAc (40:1 → 20:1; 27 L of both) to yield 26 fractions (CLFE-2-1 to CLFE-2-26). Fraction CLFE-2-25 (185.4 mg, V_e/V_t = 0.576–0.777) was subjected to an ODS CC (φ 2.5 × 5 cm) and eluted with acetone–H_2O (1:1 → 2:1; 6 L of both) to ultimately produce 11 fractions (CLFE-2-25-1 to CLFE-2-25-11) together with purified compound **6** [4′-hydroxy-3,4,2′-trimethoxychalcone, CLFE-2-25-3, 6.4 mg, V_e/V_t 0.006–0.056, TLC (SiO_2 F_{254}) R_f 0.60, *n*-hexane–EtOAc = 1:1]. Fraction CLFE-3 (13.9 g, V_e/V_t = 0.025–0.033) was subjected to a SiO_2 CC (φ 7 × 15 cm) and eluted with *n*-hexane–EtOAc (20:1, 9.4 L) to yield 21 fractions (CLFE-3-1 to CLFE-3-21). Fraction CLFE-3-16 (366.0 mg, V_e/V_t = 0.660–0.779) was subjected to a SiO_2 CC (φ 3 × 17 cm) and eluted with $CHCl_3$–EtOAc (40:1; 504 mL) to ultimately produce 10 fractions (CLFE-3-16-1 to CLFE-3-16-10) together with purified compound **1** [2′-hydroxy-4,4′-dimethoxychalcone, CLFE-3-16-2, 11.5 mg, V_e/V_t 0.071–0.250, TLC (SiO_2 F_{254}) R_f 0.80, $CHCl_3$–EtOAc = 10:1]. Fraction CLFE-5 (20.0 g, V_e/V_t = 0.048–0.101) was subjected to a SiO_2 CC (φ 7 × 15 cm) and eluted with $CHCl_3$–EtOAc (30:1, 10 L) to yield 16 fractions (CLFE-5-1 to CLFE-5-16). Fraction CLFE-5-6 (248.5 mg, V_e/V_t = 0.046–0.051) was subjected to an ODS CC (φ 3 × 5 cm) and eluted with MeOH–H_2O (3:1, 3 L) to ultimately produce nine fractions (CLFE-5-6-1 to CLFE-5-6-9) together with purified compound **5** [3′,4′-dihydroxy-3,4,2′-trimethoxychalcone, CLFE-5-6-5, 14.9 mg, V_e/V_t 0.068–0.130, TLC (SiO_2 F_{254}) R_f 0.80, $CHCl_3$–MeOH = 50:1]. Fraction CLFE-6 (1.0 g, V_e/V_t 0.103–0.116) was subjected to an ODS CC (φ 3.5 × 15 cm) and eluted with MeOH–H_2O (1:1; 12 L) to yield 10 fractions (CLFE-6-1 to CLFE-6-10). Fraction CLFE-6-4 (347.6 mg, V_e/V_t = 0.189–0.582) was subjected to an ODS CC (φ 3 × 5 cm) and eluted with MeOH–H_2O (1:1; 5.6 L) to yield nine fractions (CLFE-6-4-1 to CLFE-6-4-9). Fraction CLFE-6-4-4 (32.2 mg, V_e/V_t = 0.162–0.270) was subjected to an ODS CC (φ 3 × 5 cm) and eluted with MeOH–H_2O (3:2; 1 L) to yield six fractions (CLFE-6-4-4-1 to CLFE-6-4-4-6). Fraction CLFE-6-4-4-2 (24.1 mg, V_e/V_t = 0.274–0.374) was subjected to a SiO_2 CC (φ 1.5 × 15 cm) and eluted with $CHCl_3$–MeOH (37:1; 2.5 L) to ultimately produce 10 fractions (CLFE-6-4-4-2-1 to CLFE-6-4-4-2-10) together with purified compound **2** [kukulkanin B, CLFE-6-4-4-2-3, V_e/V_t 0.042–0.100, TLC (SiO_2 F_{254}) R_f 0.45, $CHCl_3$–MeOH–H_2O = 20:3:1]. Fraction CLFE-6-4-5 (45.7 mg, V_e/V_t = 0.274–0.374) was subjected to a

Table 1
^{1}H (400 MHz) NMR data for compounds **5** and **7–12**. δ_H in ppm, J in Hz (All proton positions were assigned by HSQC and HMBC data).

No.	5[a]	7[b]	8[b]	9[b]	10[b]	11[b]	12[b]
2	7.13, d, 1.2	7.61, d, 8.8	7.12, d, 1.2	7.33, d, 1.6	7.16, d, 2.0	7.24, d, 1.2	7.28, d, 1.2
3	–	6.84, d, 8.8	–	–	–	–	–
5	6.88, d, 8.0	6.84, d, 8.8	6.85, d, 8.4	6.83, d, 8.0	6.79, d, 8.4	6.97, d, 8.0	6.98, d, 8.0
6	7.22, dd, 8.0, 1.2	7.61, d, 8.8	7.10, dd, 8.4, 1.2	7.20, dd, 8.0, 1.6	7.05, dd, 8.4, 2.0	7.21, dd, 8.0, 1.2	7.22, dd, 8.0, 1.2
5′	6.46, d, 8.0	6.81, d, 9.2	6.83, d, 8.8	6.81, d, 9.6	6.75, d, 9.6	6.82, d, 9.2	6.82, d, 9.2
6′	7.83, d, 8.0	7.86, d, 9.2	7.58, d, 8.8	7.66, d, 9.6	7.73, d, 9.6	7.86, d, 9.2	7.89, d, 9.2
α	7.41, d, 15.6	7.60, d, 15.2	7.51, d, 15.2	7.62, d, 15.2	7.44, d, 15.2	7.61, d, 15.2	7.64, d, 15.6
β	7.82, d, 15.6	7.82, d, 15.2	7.73, d, 15.2	7.78, d, 15.2	7.69, d, 15.2	7.78, d, 15.2	7.79, d, 15.6
-OCH_3	3.93, s (3-OCH_3) 3.91, s (4-OCH_3) 3.83, s (2′-OCH_3)	3.87, s (3′-OCH_3)	–	3.93, s (3-OCH_3)	3.89, s (3′-OCH_3)	3.90, s (4-OCH_3) 3.87, s (3′-OCH_3)	3.90, s (4-OCH_3) 3.88, s (3′-OCH_3)
1″	–	5.07, d, 7.6	5.10, d, 7.0	4.97, d, 7.2	5.07, d, 7.2	5.07, d, 7.2	5.03, d, 7.2
2″	–	3.52, overlapped	3.58, overlapped	3.58, overlapped	3.56, overlapped	3.54, dd, 7.2, 7.2	3.54, overlapped
3″	–	3.47, overlapped	3.52, overlapped	3.52, overlapped	3.49, overlapped	3.47, overlapped	3.47, overlapped
4″	–	3.42, overlapped	3.43, overlapped	3.43, overlapped	3.44, overlapped	3.42, dd, 7.2, 7.2	3.42, overlapped
5″	–	3.46, overlapped	3.51, overlapped	3.51, overlapped	3.48, overlapped	3.46, overlapped	3.46, overlapped
6″	–	3.90, br. d, 12.0 3.71, dd, 12.0, 5.2	3.90, dd, 12.0, 1.2 3.72, dd, 12.0, 5.2	3.84, br. d, 12.0 3.72, dd, 12.0, 5.2	3.92, br.d, 12.0 3.73, dd, 12.0, 5.2	3.85, br.d, 12.0 3.70, dd, 12.0, 5.2	3.85, br. d, 12.0 3.70, dd, 12.0, 5.2
1‴	–	–	–	–	–	–	4.70, br.s
2‴	–	–	–	–	–	–	3.47, overlapped
3‴	–	–	–	–	–	–	4.03, br.d, 9.2
4‴	–	–	–	–	–	–	3.46, overlapped
5‴	–	–	–	–	–	–	3.45, overlapped
6‴	–	–	–	–	–	–	1.20, d, 6.0

[a] ^{1}H NMR data was measured in $CDCl_3$ at 400 MHz.
[b] ^{1}H NMR data were measured in CD_3OD at 400 MHz.

SiO_2 CC (φ 1.5 × 15 cm) and eluted with $CHCl_3$–EtOAc (20:1, 846 mL) to ultimately produce five fractions (CLFE-6-4-5-1 to CLFE-6-4-5-5) together with purified compound **4** [3,2′,4′-trihydroxy-4,3′-dimethoxychalcone, CLFE-6-4-5-1, 10.2 mg, V_e/V_t 0.000–0.088, TLC (SiO_2 F_{254}) R_f 0.53, $CHCl_3$–MeOH-H_2O = 20:3:1]. Fraction CLFE-8 (7.2 g V_e/V_t = 0.147–0.180) was subjected to a SiO_2 CC (φ 4 × 15 cm) and eluted with $CHCl_3$–MeOH–H_2O (32:3:1; 5.8 L) to yield 10 fractions (CLFE-8-1 to CLFE-8-10). Fraction CLFE-8-6 (4.7 g, V_e/V_t = 0.074–0.087) was subjected to an ODS CC (φ 4 × 9 cm) and eluted with MeOH–H_2O (1:2; 10 L) to yield nine fractions (CLFE-8-6-1 to CLFE-8-6-9). Fraction CLFE 8-6-5 (939.7 mg, V_e/V_t = 0.047–0.198) was subjected to an ODS CC (φ 4 × 12 cm) and eluted with MeOH–H_2O (1:2; 6.3 L) to ultimately produce 14 fractions (CLFE-8-6-5-1 to CLFE-8-6-5-14) together with purified compound **3** [lanceoletin, CLFE-8-6-5-13, 39.3 mg, V_e/V_t 0.927–0.997, TLC (SiO_2 F_{254}) R_f 0.55, $CHCl_3$–MeOH–H_2O = 14:3:1]. Fraction CLFE-11 (18 g, Ve/Vt = 0.590–0.856) was subjected to a SiO_2 CC (φ 7 × 15 cm) and eluted with $CHCl_3$–MeOH–H_2O (25:3:1, 26 L) to yield 13 fractions (CLFE-11-1 to CLFE-11-13). Fraction CLFE-11-10 (5.1 g, Ve/Vt = 0.217–0.721) was subjected to an ODS CC (φ 4.5 × 7 cm) and eluted with MeOH–H_2O (1:2 → 2:3; 6.6 L) to yield seven fractions (CLFE-11-10-1 to CLFE-11-10-7). Fraction CLFE 11-10-4 (422.1 mg, V_e/V_t = 0.047–0.198) was subjected to a SiO_2 CC (φ 3 × 15 cm) and eluted with $CHCl_3$–MeOH–H_2O (23:3:1; 1.5 L) to ultimately produce 18 fractions (CLFE-11-10-4-1 to CLFE-11-10-4-18) together with purified compound **7** [4,4′-dihydroxy-3′-methoxychalcone 2′-*O*-*β*-D-glucopyranoside, CLFE-11-10-4-13, 29.6 mg, V_e/V_t 0.553–0.580, TLC (SiO_2 F_{254}) R_f 0.65, $CHCl_3$–MeOH–H_2O = 65:35:10]. Fraction CLFE-11-11 (1.1 g, Ve/Vt = 0.722–0.997) was subjected to an ODS CC (φ 3.5 × 8 cm) and eluted with acetone–H_2O (1:3; 2.5 L) to yield 15 fractions (CLFE-11-11-1 to CLFE-11-11-15). Fraction CLFE 11-11-8 (113.0 mg, V_e/V_t = 0.063–0.122) was subjected to a SiO_2 CC (φ 2 × 15 cm) and eluted with EtOAc–*n*-BuOH–H_2O (55:3:1; 2.8 L) to ultimately produce 14 fractions (CLFE-11-11-8-1 to CLFE-11-11-8-14) together with purified compound **9** [4,3′,4′-trihydroxy-3-methoxychalcone 2′-*O*-*β*-D-glucopyranoside, CLFE-11-11-8-10, 49.0 mg, V_e/V_t 0.112–0.242, TLC (SiO_2 F_{254}) R_f 0.50, EtOAc–*n*-BuOH–H_2O = 15:3:1]. Fraction CLFB (254 g) was subjected to a SiO_2 CC (φ 13 × 15 cm) and eluted with $CHCl_3$–MeOH–H_2O (36:3:1 → 25:3:1 → 15:3:1; 15 L of each) to yield 14 fractions (CLFB-1 to CLFB-14). Fraction CLFB-13 (6.4 g, Ve/Vt = 0.852–0.916) was subjected to an ODS CC (φ 4.5 × 5 cm) and eluted with MeOH–H_2O (1:3; 6.5 L) to yield seven fractions (CLFB-13-1 to CLFB-13-7). Fraction CLFB-13-7 (615.6 mg, Ve/Vt = 0.770–1.000) was subjected to a SiO_2 CC (φ 3.5 × 16 cm) and eluted with EtOAc–*n*-BuOH–H_2O (35:3:1, 8.8 L) to yield 18 fractions (CLFB-13-7-1 to CLFB-13-7-18). Fraction CLFB-13-7-8 (32.5 mg, V_e/V_t = 0.099–0.128) was subjected to Sephadex LH–20 CC (φ 1.5 × 50 cm) and eluted with 100% MeOH (300 mL) to ultimately produce eight fractions (CLFB-13-7-8-1 to CLFB-13-7-8-8) together with purified compound **11** [3,4′-dihydroxy-4,3′-dimethoxychalcone 2′-*O*-*β*-D-glucopyranoside, CLFB-13-7-8-5, 8.8 mg, V_e/V_t 0.190–0.262, TLC (SiO_2 F_{254}) R_f 0.45, $CHCl_3$–MeOH–H_2O = 20:3:1]. Fraction CLFB-13-7-13 (32.8 mg, V_e/V_t = 0.238–0.360) was subjected to Sephadex LH–20 CC (φ 1.5 × 50 cm) and eluted with 100% MeOH (571 mL) to ultimately produce six fractions (CLFB-13-7-13-1 to CLFB-13-7-13-6) together with purified compound **12** [3,4′-dihydroxy-4,3′-dimethoxychalcone 2′-*O*-*α*-L-rhamnopyranosyl(1 → 6)-*O*-*β*-D-glucopyranoside, CLFB-13–7-13–6, 9.0 mg, V_e/V_t 0.119–1.000, TLC (SiO_2 F_{254}) R_f 0.56, EtOAc–*n*-BuOH–H_2O = 10:3:1]. Fraction CLFB-14 (5.8 g, Ve/Vt = 0.917–1.000) was subjected to an ODS CC (φ 5 × 9 cm) and eluted with acetone–H_2O (1:3; 5.5 L) to yield nine fractions (CLFB-14-1 to CLFB-14-9). Fraction CLFB-14-5 (522.3 mg, Ve/Vt = 0.068–0.132) was subjected to an ODS CC (φ 5 × 8 cm) and eluted with acetone–H_2O (1:3, 570 mL) to ultimately produce eight fractions (CLFB-14-5-1 to CLFB-14-5-8) together with purified compound **8** [3,4,3′,4′-tetrahydroxychalcone 2′-*O*-*β*-D-glucopyranoside, CLFB-14-5-6, 33.9 mg, V_e/V_t 0.433–0.467, TLC (SiO_2 F_{254}) R_f 0.40, $CHCl_3$–MeOH–H_2O = 10:3:1]. Fraction CLFB-14-7 (650.8 mg, Ve/Vt = 0.173–0.523) was subjected to the SiO_2 CC (φ 3.5 × 15 cm) and eluted with EtOAc–*n*-BuOH–H_2O (15:3:1; 1.4 L) to ultimately produce five fractions (CLFB-14-7-1 to CLFB-14-7-5) together with purified compound **10** [3,4,4′-trihydroxy-3′-methoxychalcone 2′-*O*-*β*-D-glucopyranoside, CLFB-14-7-2, 134.7 mg, V_e/V_t 0.035–0.050, TLC (SiO_2 F_{254}) R_f 0.54, EtOAc–*n*-BuOH–H_2O = 10:3:1].

Lanceolein A (***5***): Yellow amorphous powder; m.p. 180–181 °C; $[\alpha]_D^{25}$–185.2° (*c* 0.10, CH_3OH); IR (CaF_2 window) ν_{max} 3421, 3355, 1698, 1630, 1509 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) in Table 1; ^{13}C

Table 2
^{13}C NMR data of compounds **5** and **7–12**. δ_C in ppm (All carbon positions were assigned by HSQC and HMBC data).

No.	5[a]	7[b]	8[b]	9[b]	10[b]	11[b]	12[b]
1	129.1	132.1	128.2	128.2	128.3	129.6	130.0
2	114.1	127.6	116.6	116.6	116.1	115.5	115.4
3	150.0	117.0	149.8	149.4	146.8	148.2	147.9
4	151.4	161.9	150.1	151.2	150.2	152.2	151.7
5	111.1	117.0	113.7	112.3	106.7	112.7	112.5
6	123.2	127.6	122.5	122.8	124.1	124.0	124.1
α	117.9	118.1	118.1	118.5	116.7	119.2	119.2
β	144.5	146.7	146.7	146.8	147.4	146.8	147.1
7	191.7	194.4	194.5	194.6	194.3	194.5	194.3
1′	113.3	117.7	117.3	117.4	117.7	117.7	117.7
2′	166.0	157.3	153.6	153.9	157.2	157.6	157.1
3′	133.9	138.2	135.7	135.7	138.1	138.4	138.3
4′	166.6	159.1	151.8	151.7	159.2	159.3	158.8
5′	110.2	107.8	108.0	108.0	106.5	108.0	108.0
6′	127.7	127.4	125.2	125.2	127.7	127.6	127.5
1″	–	101.8	102.5	102.6	101.7	101.9	102.1
2″	–	74.8	74.7	74.7	74.8	74.9	74.8
3″	–	78.4	78.4	78.4	78.3	78.6	78.1
4″	–	71.2	71.3	71.3	71.3	71.4	71.5
5″	–	78.0	77.5	77.5	78.0	78.2	77.2
6″	–	62.5	62.4	62.4	62.2	62.6	70.0
1‴	–	–	–	–	–	–	104.2
2‴	–	–	–	–	–	–	72.3
3‴	–	–	–	–	–	–	72.5
4‴	–	–	–	–	–	–	74.0
5‴	–	–	–	–	–	–	70.9
6‴	–	–	–	–	–	–	18.1
3-OCH_3	56.0	–	–	56.5	–	–	–
4-OCH_3	56.0	–	–	–	–	56.6	56.4
2′-OCH_3	55.3	–	–	–	–	–	–
3′-OCH_3	–	61.3	–	–	61.5	61.5	61.4

[a] ^{13}C NMR data was measured in $CDCl_3$ at 100 MHz.
[b] ^{13}C NMR data were measured in CD_3OD at 100 MHz.

NMR (100 MHz, $CDCl_3$, δ_C) in Table 2; positive HRFABMS *m/z* 353.1005 $[M+Na]^+$ (calcd for $C_{18}H_{18}O_6Na$, 353.1001, $\Delta = +1.0$ ppm).

*Lanceolein B (**7**)*: Red amorphous powder; m.p. 175–176 °C; $[\alpha]_D^{25}$–14.4° (*c* 0.20, CH_3OH); IR (CaF_2 window) ν_{max} 3298, 1681, 1647, 1591, 1501 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD, δ_H) in Table 1; ^{13}C NMR (100 MHz, CD_3OD, δ_C) in Table 2; negative HRFABMS *m/z* 447.1289 $[M-H]^-$ (calcd for $C_{22}H_{23}O_{10}$, 447.1291, $\Delta = -0.5$ ppm).

*Lanceolein C (**8**)*: Red amorphous powder; m.p. 232–233 °C; $[\alpha]_D^{25}$–66.4° (*c* 0.10, CH_3OH); IR (CaF_2 window) ν_{max} 3298, 1680, 1654, 1592 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD, δ_H) in Table 1; ^{13}C NMR (100 MHz, CD_3OD, δ_C) in Table 2; negative HRFABMS *m/z* 449.1082 $[M-H]^-$ (calcd for $C_{21}H_{21}O_{11}$, 449.1083, $\Delta = -0.4$ ppm).

*Lanceolein D (**9**)*: Red amorphous powder; m.p. 210–211 °C; $[\alpha]_D^{25}$–66.5° (*c* 0.10, CH_3OH); IR (CaF_2 window) ν_{max} 3356, 1737, 1634, 1605, 1514 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD, δ_H) in Table 1; ^{13}C NMR (100 MHz, CD_3OD, δ_C) in Table 2; positive HRFABMS *m/z* 465.1400 $[M+H]^+$ (calcd for $C_{22}H_{25}O_{11}$, 465.1396, $\Delta = +0.7$ ppm).

*Lanceolein E (**10**)*: Red amorphous powder; m.p. 213–214 °C; $[\alpha]_D^{25}$–66.7° (*c* 0.10, CH_3OH); IR (CaF_2 window) ν_{max} 3356, 1700, 1631, 1600, 1514 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD, δ_H) in Table 1; ^{13}C NMR (100 MHz, CD_3OD, δ_C) in Table 2; negative HRFABMS *m/z* 463.1242 $[M-H]^-$ (calcd for $C_{22}H_{23}O_{11}$, 463.1240, $\Delta = +0.4$ ppm).

*Lanceolein F (**11**)*: Red amorphous powder; m.p. 207–208 °C; $[\alpha]_D^{25}$–66.6° (*c* 0.10, CH_3OH); IR (CaF_2 window) ν_{max} 3356, 1720, 1671, 1599, 1509 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD, δ_H) in Table 1; ^{13}C NMR (100 MHz, CD_3OD, δ_C) in Table 2; negative HRFABMS *m/z* 477.1393 $[M-H]^-$ (calcd for $C_{23}H_{25}O_{11}$, 477.1396, $\Delta = -0.9$ ppm).

*Lanceolein G (**12**)*: Red amorphous powder; m.p. 298–299 °C; $[\alpha]_D^{25}$–40.5° (*c* 0.20, CH_3OH); IR (CaF_2 window) ν_{max} 3355, 1728, 1632, 1591, 1508 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD, δ_H) in Table 1; ^{13}C NMR (100 MHz, CD_3OD, δ_C) in Table 2; positive HRFABMS *m/z* 647.1954 $[M+Na]^+$ (calcd for $C_{29}H_{36}O_{15}Na$, 647.1951, $\Delta = +0.4$ ppm).

*2.4. Acid hydrolysis of compounds **7–12** and determination of the absolute configuration of the monosaccharide components*

The absolute configuration of monosaccharides obtained by acid hydrolysis of **7–12** were identified by applying the method presented in literature [27]. The monosaccharide derivatives of D-glucose and L-rhamnose were appeared at 15.84 min 13.66 min in the GC/MS chromatogram, respectively.

2.5. Cell culture

The HCT15 (human colon cancer cell) cell line was obtained from the Korea Cell Line Bank (KCLB) and cultured in Dulbecco's modified essential medium (DMEM) and RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% penicillin-streptomycin. RAW 264.7 cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin. The cells were incubated at 37 °C in an atmosphere containing 5% CO_2.

2.6. Cell viability assay

The cytotoxic effect of the isolated chalcones was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma) assay. HCT15 cells were seeded into a 96-well microplate and treated with isolated chalcones (stock solutions dissolved in DMSO at 100 mM). The colon cancer cells were treated with various concentrations of the samples (25, 50, 100 μM) for 24 h. MTT solution (1 mg/ml) was added to the cells for 4 h and the formazan produced was dissolved with DMSO (dimethyl sulfoxide). The absorbance of the solutions was measured using a microplate reader (TECAN, Männedorf, Switzerland).

2.7. Cytometric analysis of apoptosis

The cytotoxicity of the chalcone on HCT15 cells was examined through an annexin V binding assay followed by propidium iodide (PI) staining. And cytometric analysis of apoptosis was performed using the method reported in literature [28] using Tali apoptosis assay kit.

2.8. Western blot

Western blot for the protein expression related to apotosis, PARP, caspase-3, and *β*-actin was carried out according to the established method previously reported in literature [28]. Briefly, protein extracts of HCT15 cells prepared, separated, and transferred to nitrocellulose (NC) membranes, which was blocked, washed, incubated with specific primary antibodies against PARP, caspase-3, and *β*-actin (1:1000), and successively with secondary antibodies. Target protein bands were visualized using EZ-Western Lumi Pico reagents.

2.9. Cell viability assay of RAW264.7 macrophage cells

RAW 264.7 cells were seeded at a density of 4×10^5 cells/mL in 96-well plates. The cells were co-treated with LPS and the chalcone samples for 24 h and then incubated with 10 μl of 5 mg/mL MTT for 4 h. The supernatant was removed and 100 μl DMSO was added to each well and shaken for 10 min to dissolve the formazan crystals. Optical density (OD) was measured at 570 nm with a Multi-Reader instrument (TECAN, Männedorf, Switzerland).

2.10. Determination of nitric oxide (NO) production

RAW 264.7 cells were seeded at a density of 4×10^5 cells/mL in 96-well plates. The cells were co-treated with the chalcone samples and

LPS for 24 h. Nitrite accumulation in the culture medium was assessed as an indicator of NO production. Briefly, 100 μl of the supernatant was transferred from each well into an empty 96-well plate. After addition of 100 μl of Griess reagent (1% sulfanilamide and 0.1% naphthylethylenediamine dihydrochloride in 5% phosphoric acid; Sigma–Aldrich) to each well, absorbance at 540 nm was measured with a microplate reader (TECAN). Nitrite concentration was extrapolated from the standard curve of sodium nitrite.

2.11. Statistical analysis

Data are represented as mean ± S.E.M (n = 3). Results were assessed using one-way analysis of the variance (ANOVA), which was performed by Graphpad Prism 5 (GraphPad Software, San Diego, CA, USA). Significant values are indicated by an asterisk ($^{*}P < 0.05$ compared with the NC group, $^{\#}P < 0.05$ compared with the LPS group).

3. Results and discussion

The flowers of *Coreopsis lanceolata* were extracted in aqueous MeOH and the concentrated extracts were successively partitioned into EtOAc, *n*-BuOH, and H_2O fractions. The EtOAc and *n*-BuOH fractions were used for isolation of cytotoxic metabolites because flavonoid spots were observed on a thin-layer chromatography (TLC) plate. Column chromatography of the fractions using SiO_2, ODS, and Sephadex LH-20 yielded twelve chalcones **1–12**.

Comparison of NMR and MS data with reported values led to identification of known compounds 2′-hydroxy-4,4′-dimethoxychalcone (**1**) [17], kukulkanin B (**2**) [18], lanceoletin (**3**) [19], 3,2′,4′-trihydroxy-4,3′-dimethoxychalcone (**4**) [19], and 4′-hydroxy-3,4,2′-trimethoxychalcone (**6**) [20].

Compound **5** was obtained as yellow amorphous powder and showed a yellow color on TLC plate after spraying with 10% H_2SO_4 and heating. The molecular formula was determined to be $C_{18}H_{18}O_6$ from the molecular ion peak $[M+Na]^+$ at *m/z* 353.1005 (calcd for $C_{18}H_{18}O_6Na$, 353.1001, Δ = +1.0 ppm) in the positive HRFABMS. The IR spectrum showed absorbance bands of hydroxyl (3421, 3355 cm^{-1}), ketone (1698 cm^{-1}), and aromatic double bond (1630, 1509 cm^{-1}) groups. Analysis of the DEPT and gHSQC spectra of compound **5** unambiguously confirmed the assignment of the ^1H and ^{13}C NMR signals (Tables 1 and 2). In the ^1H NMR spectrum, proton signals of two olefinic methines δ_H 7.83 (1H, d, *J* = 8.0 Hz, H-6′) and δ_H 6.46 (1H, d, *J* = 8.0 Hz, H-5′) due to a 1,2,3,4-tetrasubstituted benzene ring moiety; three olefinic methines δ_H 7.22 (1H, dd, *J* = 8.0, 1.2 Hz, H-6), δ_H 7.13 (1H, d, *J* = 1.2 Hz, H-2), δ_H 6.88 (1H, d, *J* = 8.0 Hz, H-5) due to a 1,2,4-trisubstituted benzene ring moiety; two olefinic methines δ_H 7.82 (1H, d, *J* = 15.6 Hz, H-*β*), δ_H 7.41 (1H, d, *J* = 15.6 Hz, H-*α*), which had trans-conformation based on the large coupling constant (*J* = 15.6 Hz); and three methoxys δ_H 3.93 (3H, s, 3-OCH_3), δ_H 3.91 (3H, s, 4-OCH_3), and δ_H 3.83 (3H, s, 2′-OCH_3) were observed (Table 1). The ^1H NMR data indicated that compound **5** was a chalcone with three methoxy groups. The ^{13}C NMR spectrum showed 18 carbon signals including three methoxy groups [δ_C 56.0 (3-OCH_3), 56.0 (4-OCH_3), 55.3 (2′-OCH_3)] confirming that compound **5** was composed of a chalcone and three methoxy groups. The signals of the chalcone moiety included those of one conjugated ketone [δ_C 191.7 (C-7)], five oxygenated olefinic quaternaries [δ_C 166.6 (C-4′), 166.0 (C-2′), 151.4 (C-4), 150.0 (C-3), 133.9 (C-3′)], two olefinic quaternaries [δ_C 129.1 (C-1), 113.3 (C-1′)], and seven olefinic methines [δ_C 144.5 (C-*β*), 127.7 (C-6′), 123.2 (C-6), 117.9 (C-α), 114.1 (C-2), 111.1 (C-5), 110.2 (C-5′)]. The ^{13}C NMR data indicated that the A- and B-ring structures of compound **5** were a 3,4-dioxyphenyl and a 2,3,4-trioxyphenyl, respectively. In the gHMBC spectrum, three methoxy proton signals [δ_H 3.93 (3-OCH_3), δ_H 3.91 (4-OCH_3), and δ_H 3.83 (2′-OCH_3)] showed cross-peaks with the three oxygenated olefinic methine carbon signals [δ_C 150.0 (C-3), δ_C 151.4 (C-4), and δ_C 166.0 (C-2′)], respectively, suggesting that three methoxy groups were at the C-3, C-4, and C-2′ positions. Based on comparison of spectroscopic data with those of a similar structure, 3,2′,4′-trihydroxy-4,3′-dimethoxychalcone (**4**) [19] the chemical structure of compound **5** was determined as 3′,4′-dihydroxy-3,4,2′-trimethoxychalcone, which was named lanceolein A.

Compound **7** was obtained as a red amorphous powder and showed a yellow color on TLC plate after spraying with 10% H_2SO_4 and heating. The molecular formula was determined to be $C_{22}H_{24}O_{10}$ from the molecular ion peak $[M-H]^-$ at *m/z* 447.1289 (calcd for $C_{22}H_{23}O_{10}$, 447.1291, Δ = –0.5 ppm) in the negative HRFABMS. The IR spectrum showed absorbance bands of hydroxyl (3298 cm^{-1}), ketone (1681 cm^{-1}), and aromatic double bond (1647, 1591, 1501 cm^{-1}) groups. The ^1H NMR and ^{13}C NMR spectra of compound **7** were very similar to those of compound **5** with the exception of signals due to an A-ring structure and an additional hexose. The ^1H NMR spectrum showed four olefinic methine proton signals δ_H 7.61 (2H, d, *J* = 8.8 Hz, H-2/H-6), δ_H 6.84 (2H, d, *J* = 8.8 Hz, H-3/H-5) indicating that the A-ring was a para-disubstituted benzene moiety. The ^{13}C NMR showed one oxygenated olefin quaternary (δ_C 161.9, C-4), one olefinic quaternary (δ_C 132.1, C-1), and four olefinic methines [(δ_C 127.6, C-2/C-6), (δ_C 117.0, C-3/C-5)] indicating the A-ring was a monohydroxy phenyl structure. The signals due to the hexose moiety included a hemiacetal (δ_H 5.07, 1H, d, *J* = 7.6 Hz, H-1″; δ_C 101.8), four oxygenated methines [(δ_H 3.42, 1H, d, overlapped, H-3″), (δ_H 3.48, 1H, d, overlapped, H-5″), (δ_H 3.55, 1H, *J* = 7.6, 7.6 Hz, H-4″), (δ_H 3.43, 1H, *J* = 7.6, 7.6 Hz, H-2‴); δ_C 78.4, 77.9, 74.7, 71.2], and one oxygenated methylene [(δ_H 3.90, 1H, dd, *J* = 11.6, 2.0 Hz, H-6″a), (δ_H 3.71, 1H, dd, *J* = 11.6, 5.6 Hz, H-6″b; δ_C 62.4] indicating that the sugar was a *β*-glucopyranose. In the gHMBC spectrum, one hemiacetal proton signal (δ_H 5.07, H-1″) showed a cross-peak with the oxygenated olefinic quaternary carbon signal (δ_C 157.3), which also showed a cross-peak with the olefinic methine proton signal (δ_H 7.86, H-6′), but did not show a correlation with the olefinic methine proton signal (δ_H 6.81, H-5′) suggesting that the *β*-glucopyranose was at the C-2′ position, and not the C-4′ position. Also, one methoxy proton signal (δ_H 3.87) showed a cross-peak with the oxygenated olefinic quaternary carbon signal (δ_C 138.2, C-3′) suggesting the methoxy group was at the C-3′ position. The absolute configuration of *β*-glucopyranose was determined to be D, based on GC analysis of a chiral derivative of the monosaccharide obtained by hydrolysis of compound **7** followed by derivatization using *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) (see Experimental Section). Based on comparison of spectroscopic data with those of kukulkanin B (**2**) [18], the chemical structure of compound **7** was determined as 4,4′-dihydroxy-3′-methoxychalcone 2′-*O*-*β*-D-glucopyranoside and designated lanceolein B.

Compound **8** was obtained as red amorphous powder and showed a red color on TLC plate after spraying with 10% H_2SO_4 and heating. The molecular formula was determined to be $C_{21}H_{22}O_{11}$ from the molecular ion peak $[M-H]^-$ at *m/z* 449.1082 (calcd for $C_{21}H_{21}O_{11}$, 449.1083, Δ = –0.4 ppm) in the negative HRFABMS. The IR spectrum showed absorbance bands of hydroxyl (3298 cm^{-1}), ketone (1680 cm^{-1}), and aromatic double bond (1654, 1592 cm^{-1}) groups. The ^1H NMR and ^{13}C NMR spectra of compound **8** were very similar to those of compound **7** with the exception of signals due to an A-ring structure and the absence of any methoxy group. The ^1H NMR spectrum showed three olefinic methine proton signals δ_H 6.85 (1H, dd, *J* = 8.4, 1.2 Hz, H-5), δ_H 7.10 (1H, d, *J* = 8.4 Hz, H-6) and δ_H 7.19 (1H, d, *J* = 1.2 Hz, H-2) indicating the A-ring was a 1,2,4-trisubstituted benzene structure. The ^1H NMR data indicated that compound **8** was a chalcone glycoside. The ^{13}C NMR showed two oxygenated olefinic quaternaries [(δ_C 150.1, C-4) and (δ_C 149.8, C-3)], one olefinic quaternary (δ_C 128.2, C-1), and three olefinic methines [(δ_C 122.5, C-6), (δ_C 116.6, C-2), and (δ_C 113.7, C-5)], indicating that the A-ring had a dihydroxy phenyl structure. In the gHMBC spectrum, one hemiacetal proton signal (δ_H 5.10, H-1″) showed a cross-peak with the oxygenated olefinic quaternary carbon signal (δ_C 151.6), which also showed a cross-peak with the olefinic methine

proton signal (δ_H 7.58, H-6′), suggesting the β-D-glucopyranose was at the C-2′ position. The absolute configuration of β-glucopyranose was determined to be D through the previously described method. By comparison of spectroscopic data with okanin [21], the chemical structure of compound **8** was determined as 3,4,3′,4′-tetrahydroxychalcone 2′-*O*-β-D-glucopyranoside and named lanceolein C.

Compound **9** was obtained as a red amorphous powder and showed a red color on TLC plate after spraying with 10% H_2SO_4 and heating. The molecular formula was determined to be $C_{22}H_{24}O_{11}$ from the molecular ion peak $[M+H]^+$ at *m/z* 465.1400 (calcd for $C_{22}H_{25}O_{11}$, 465.1396, Δ = +0.7 ppm) in the positive HRFABMS. The IR spectrum showed absorbance bands of hydroxyl (3356 cm^{-1}), ketone (1737 cm^{-1}), and aromatic double bond (1634, 1605, 1514 cm^{-1}) groups. The ^1H NMR and ^{13}C NMR spectra of compound **9** were very similar to those of compound **8** with the exception of an additional methoxy group (δ_H 3.93, 3H, s; δ_C 56.5, 3-OCH_3). In the gHMBC spectrum, the methoxy proton signal (δ_H 3.93) showed a cross-peak with the oxygenated olefinic quaternary carbon signal (δ_C 149.4, C-3) suggesting that the methoxy group was at the C-3 position. The absolute configuration of β-glucopyranose was determined to be D. Taking these data together, the chemical structure of compound **9** was determined as 4,3′,4′-trihydroxy-3-methoxychalcone 2′-*O*-β-D-glucopyranoside and named lanceolein D.

Compound **10** was obtained as a red amorphous powder and showed a red color on TLC plate after spraying with 10% H_2SO_4 and heating. The molecular formula was determined to be $C_{22}H_{24}O_{11}$ from the molecular ion peak $[M-H]^-$ at *m/z* 463.1242 (calcd for $C_{22}H_{23}O_{11}$, 463.1240, Δ = +0.4 ppm) in the negative HRFABMS. The IR spectrum showed absorbance bands of hydroxyl (3356 cm^{-1}), ketone (1700 cm^{-1}), and aromatic double bond (1631, 1600, 1514 cm^{-1}) groups. The ^1H NMR and ^{13}C NMR spectra of compound **10** were almost the same as those of compound **9** with the exception of the the position of one methoxy group [**9**: (δ_H 3.93, 3H, s; δ_C 56.5, 3-OCH_3), **10**: (δ_H 3.89, 3H, s; δ_C 61.5, 3′-OCH_3)]. In the gHMBC spectrum, the methoxy proton signal (δ_H 3.89) showed a cross-peak with the oxygenated olefinic quaternary carbon signal (δ_C 138.1, C-3′) suggesting that the methoxy group was at the C-3′ position. From the above data the chemical structure of compound **10** was determined as 3,4,4′-trihydroxy-3′-methoxychalcone 2′-*O*-β-D-glucopyranoside, named lanceolein E.

Compound **11** was obtained as red amorphous powder and showed a red color on TLC plate after spraying with 10% H_2SO_4 and heating. The molecular formula was determined to be $C_{23}H_{26}O_{11}$ from the molecular ion peak $[M-H]^-$ at *m/z* 477.1393 (calcd for $C_{23}H_{25}O_{11}$, 477.1396, Δ = –0.9 ppm) in the negative HRFABMS. The IR spectrum showed absorbance bands of hydroxyl (3356 cm^{-1}), ketone (1720 cm^{-1}), and aromatic double bond (1671, 1599, 1509 cm^{-1}) groups. The ^1H NMR and ^{13}C NMR spectra of compound **11** were almost identical to those of compound **10** with the exception of an additional methoxy group (δ_H 3.90, 3H, s; δ_C 56.6, 4-OCH_3). In the gHMBC spectrum, the methoxy proton signal (δ_H 3.90) showed a cross-peak with the oxygenated olefinic quaternary carbon signal (δ_C 152.2, C-4) suggesting the methoxy group was at the C-4 position. Therefore, the structure of compound **11** was determined as 3,4′-dihydroxy-4,3′-dimethoxychalcone 2′-*O*-β-D-glucopyranoside, named lanceolein F.

Compound **12** was obtained as red amorphous powder and showed a red color on TLC plate after spraying with 10% H_2SO_4 and heating. The molecular formula was determined to be $C_{29}H_{36}O_{15}$ from the molecular ion peak $[M+Na]^+$ at *m/z* 647.1954 (calcd for $C_{29}H_{36}O_{15}Na$, 647.1951, Δ = +0.4 ppm) in the positive HRFABMS. The IR spectrum showed absorbance bands of hydroxyl (3355 cm^{-1}), ketone (1728 cm^{-1}), and aromatic double bond (1632, 1591, 1508 cm^{-1}) groups. The ^1H NMR and ^{13}C NMR spectra of compound **12** were very similar to those of compound **11** with the exception of an additional deoxyhexose, which included a hemiacetal signal (δ_H 4.70, 1H, br. s, H-1‴; δ_C 104.2), four oxygenated methine signals [(δ_H 4.03, 1H, br. d, *J* = 9.2 Hz, H-3‴), (δ_H 3.47, 1H, overlapped, H-2‴), (δ_H 3.46, 1H, overlapped, H-4‴), (δ_H 3.45, 1H, overlapped, H-5‴); δ_C 74.0, 72.5, 72.3, 70.9)] and one methyl signal (δ_H 1.20, 3H, d, *J* = 6.0 Hz, H-6‴; δ_C 18.1), indicating the sugar was a α-rhamnopyranose. The gHMBC spectrum showed a correlation between the hemiacetal proton signal (δ_H 5.53, H-1‴) and an oxygenated methylene carbon signal (δ_C 70.0, C-6″) suggesting the α-rhamnopyranose was at the C-6″ position. The oxygenated methylene carbon signal (δ_C 70.0, C-6″), which is usually observed at δ_C 62.3 in β-glucopyranose [22], was shifted downfield due to the glycosidation effect. The absolute configuration of β-glucopyranose and α-rhamnopyranose was determined to be D and L, respectively, based on GC analysis of chiral derivatives of the monosaccharides obtained by hydrolysis of compound **12** followed by derivatization using MSTFA (see Experimental Section). Therefore, the structure of compound **12** was determined as 3,4′-dihydroxy-4,3′-dimethoxychalcone 2′-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside, named lanceolein G.

EtOAc and *n*-BuOH fractions and the isolated chalcones from *C. lanceolata* flowers were evaluated for an inhibitory effect on NO production in LPS-stimulated RAW264.7 cells, which is known to be important in the induction of inflammation [23]. LPS-stimulated macrophages were treated with multiple concentrations of the fractions and isolated compounds (25, 50, 100 μg/mL or μM). *n*-BuOH fraction and compounds **2**, **7**, and **9–12** were not cytotoxic in RAW264.7 cells at concentrations lower than 100 μM and compounds **1**, **4–6**, and **8** were not cytotoxic at concentrations lower than 50 μM (Fig. 2A). As shown in Fig. 2B, the LPS-treated group clearly showed increased NO production. The EtOAc fraction and compounds **1**, **2**, **4–6,** and **10** significantly and

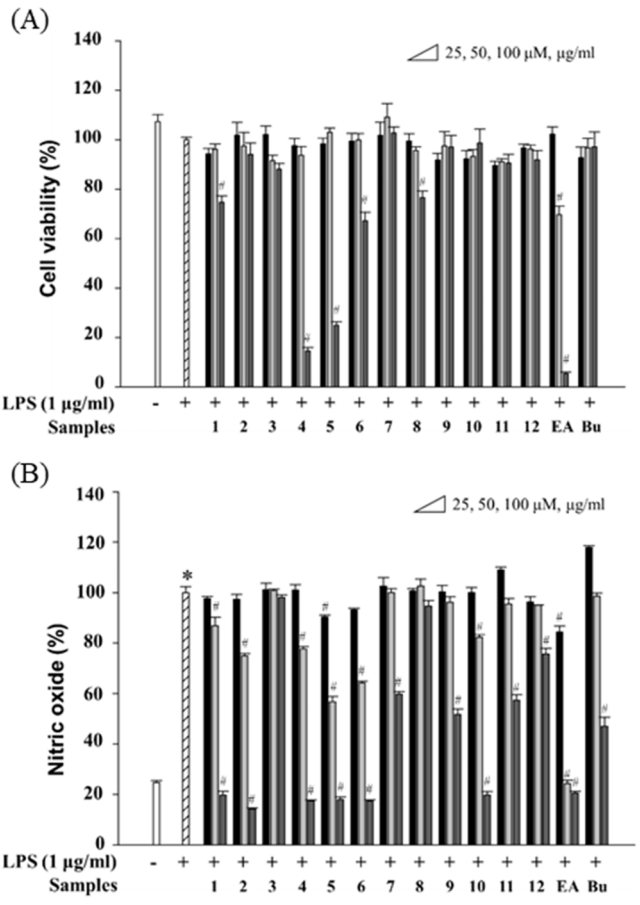

Fig. 2. (A) Cytotoxicity of compound **1–12** on RAW264.7 cells. (B) Inhibition effects of compounds **1–12** on NO production in RAW264.7 cells. EA: *Coreopsis lanceolata* flowers EtOAc fraction, Bu: *Coreopsis lanceolata* flowers *n*-BuOH fraction.

Table 3
Inhibitory effects of compounds **1–12** on the growth of HCT-15 colon cancer cells (IC_{50}[a] ± SD (μM)).

Compounds	HCT-15 (μM)
1	92.4 ± 2.36
2	43.7 ± 2.17
3	97.8 ± 3.10
4	98.5 ± 3.11
5	35.6 ± 0.24
6	47.9 ± 1.18
7	> 100
8	> 100
9	> 100
10	> 100
11	> 100
12	> 100
Cisplatin	29.5 ± 1.25

[a] IC_{50}: drug concentration required to inhibit cell viability by 50%, results are means ± standard errors of at least three independent experiments.

dose-dependently inhibited LPS-induced NO production. Especially, compounds **2** and **10** most efficiently inhibited LPS-induced NO production at all concentrations without any apparent toxicity to RAW264.7 cells. Compounds **7**, **9**, and **11–12** moderately inhibited NO production at a concentration of 100 μM.

In addition, the isolated chalcones from *C. lanceolata* flowers were evaluated for their inhibitory effect on the viability of human colon cancer cells HCT15 using the MTT assay. In general, aglycon compounds **1–6** exhibited a little higher cytotoxicity against HCT15 cells than other glycosides **7–12** (Table 3). The cytotoxic mechanism of the isolated chalcones was investigated by examining the induction of apoptosis in HCT15 cells. HCT15 cells were treated with compounds **1–12** for 24 h and the number of cells in various states was counted using the Tali apoptosis analysis method. The number of apoptotic cells increased after treatment with compounds **2**, **5,** and **9** (Fig. 3). The expression of proteins related to apoptosis such as poly-(ADP-ribose)-polymerase (PARP) and caspase-3 was also investigated to determine whether compounds **1–12** induced apoptosis in the colon cancer cell line HCT15. PARP is the most specific proteolytic substrate of caspases that is cleaved in the phase of apoptosis, thus interfering with the function of the DNA repair enzyme [24]. In addition, PARP degradation can ultimately lead to apoptosis [25]. In agreement with data from the Tali assay, PARP was cleaved after treatment with compounds **2** and **5**. As PARP degradation is induced by the activation of caspase-3, which is the end point of the caspase activation cascade [26], the protein

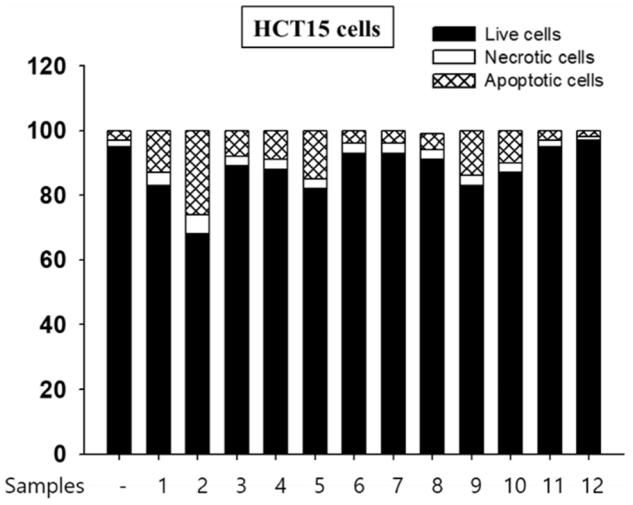

Fig. 3. Tali apoptotic analysis of compounds **1–12** in HCT15 colon cancer cells.

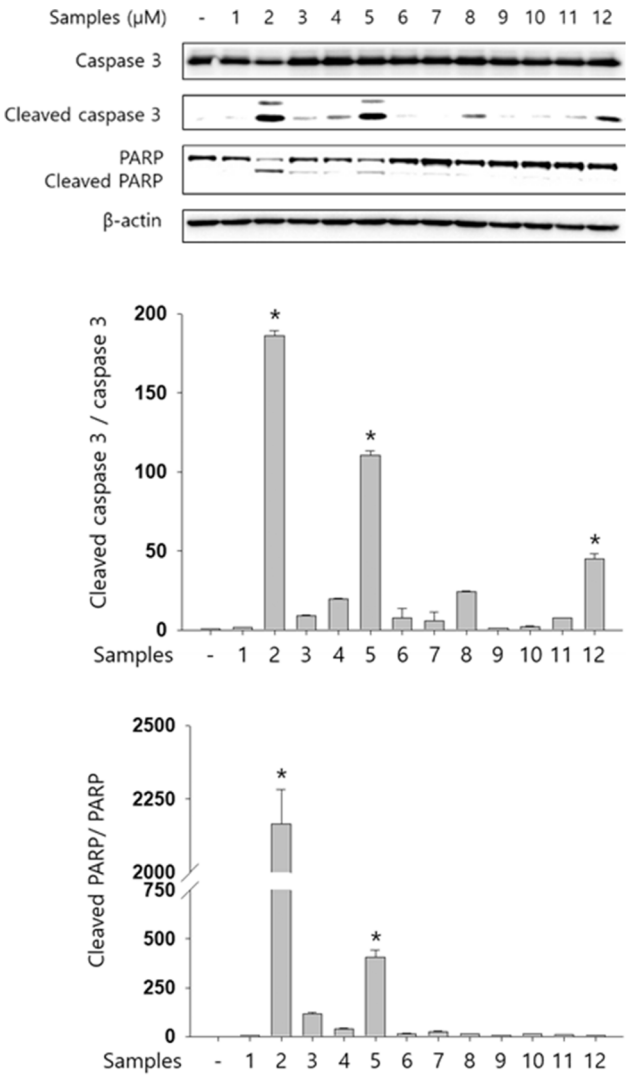

Fig. 4. Promotion Effects of compounds **1–12** on the expression of apoptosis proteins.

expression of caspase-3 and cleaved caspase-3 in HCT15 cancer cells was also examined. Caspase-3 cleavage was observed after treatment with compounds **2**, **5,** and **12** (Fig. 4).

These results suggest that chalcones **2** and **5** from the flowers of *C. lanceloata* can prevent cancer *via* the induction of cellular cytotoxicity and apoptosis. In addition, chalcones **2** and **10** inhibited NO production in LPS-stimulated RAW 264.7 cells.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This work was supported by the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry, and Fisheries (317071-03-1-SB020).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.01.003.

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