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RESEARCH ARTICLE

Chemical constituents on the aerial parts of *Artemisia selengensis* and their IL-6 inhibitory activity

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Abstract Ten compounds, 1′,3′-propanediol,2′-amino-1′-(1,3-benzodioxol-5-yl) (**1**), artanomaloide (**2**), canin (**3**), eupatilin (**4**), quercetin-3-*O*-*β*-D-glucoside-7-*O*-α-L-rhamnoside (**5**), 1,3-di-*O*-caffeoylquinic acid (**6**), isoquercitrin (**7**), pinoresinol-4-*O*-*β*-D-glucoside (**8**), scopolin (**9**), and isofraxidin-7-*O*-*β*-D-glucopyranoside (**10**) were isolated from the aerial parts of *A. selengensis*. The structures of compounds (**1–10**) were identified based on 1D and 2D NMR, including ^{1}H–^{1}H COSY, HSQC, HMBC and NOESY spectroscopic analyses. Among them, compound **1** was isolated from this plant for the first time as a naturally occurring compound. The inhibitory activity of these isolated compounds against interleukin-6 (IL-6) production in TNF-α stimulated MG-63 cells was also examined.

Keywords *Artemisia selengensis* Turcz. · Compositae · 1′,3′-propanediol,2′-amino-1′-(1,3-benzodioxol-5-yl) · IL-6 inhibitory activity

Introduction

Artemisia selengensis Turcz. is a perennial herb belonging to the Compositae that grows mainly in wetlands, and waterside in Korea (Lee 1993). The aerial parts of this plant have been used traditionally as an anti-inflammation, hemostasis, invigorating the blood circulation, and relieving dysmenorrhea (Ahn 1998; Hu and Feng 1999). Only a few phytochemical investigation on this plant resulted in the isolation of a sesquiterpene endoperoxide, and sesquiterpene (Hu and Feng 1999; Jang and Lee 1993). Concerning the biological studies of *A. selengensis*, anti-tumor, antioxidant and immune-modulating activities of its various extracts has been reported so far (Koo et al. 1994; Shi et al. 2010).

In an ongoing investigation into anti-inflammatory compounds from natural products, the methanol extract of *A. selengensis* was found to inhibit IL-6 production in TNF-α stimulated MG-63 cells. By means of repeated column chromatography using silica gel, Sephadex LH-20, and LiChroprep RP-18, ten compounds were isolated from the aerial parts of *A. selengensis*. The structures of these compounds were identified as 1′,3′-propanediol,2′-amino-1′-(1,3-benzodioxol-5-yl) (**1**), artanomaloide (**2**), canin (**3**), eupatilin (**4**), quercetin-3-*O*-*β*-D-glucoside-7-*O*-α-L-rhamnoside (**5**), 1,3-di-*O*-caffeoylquinic acid (**6**), isoquercitrin (**7**), pinoresinol-4-*O*-*β*-D-glucoside (**8**), scopolin (**9**), and isofraxidin-7-*O*-*β*-D-glucopyranoside (**10**), by comparing their spectroscopic data with those reported in the literature. These compounds were isolated from this plant for the first time. Furthermore, compound **1** was isolated for the first time as a new natural product even though it was synthesized previously. For theses isolated compounds, the inhibitory activity of IL-6 production in TNF-α stimulated MG-63 cells was examined. Among these compounds, artanomaloide (**2**), and canin (**3**) showed potent inhibitory activity against IL-6 production in TNF-α stimulated MG-63 cells.

This paper reports the isolation, structure elucidation, and the inhibitory activity of IL-6 production of isolated

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compounds from the aerial parts of *A. selengensis*. In addition, the complete NMR assignments of compound **1** are presented here for the first time.

Materials and methods

General procedure

Optical rotations were measured using an Autopol-IV polarimeter (Rudolph Research Flangers). The UV spectra were obtained on a Shimadzu UV/Visible Spectrophotometer. The IR spectra were recorded on an IMS 85 (Bruker). The HR-TOF -MS spectra were recorded on a Q-TOF (Synapt HDMS system, Waters, USA) mass spectrometer. The NMR spectra were recorded on a Varian Unity Inova 500 and Unity Inova 600 spectrometer (KBSI-Gwangju center). Semi-preparative HPLC was performed using a Waters HPLC system equipped with Waters 600 Q-pumps, a 996 photodiode array detector, and a YMC-Pack ODS-A column (250 × 10 mm i.d., 5 μm), flow rate 4.0 mL/min. TLC and column chromatography were performed on precoated Si Gel F_{254} plates (Merck, art. 5715), RP-18 F_{254} plates (Merck, art. 15389) and silica gel 60 (40–63 and 63–200 μm, Merck), MCI gel CHP20P (75–150 μ, Mitsubishi Chemical Co.), Sephadex LH-20 (25–100 μm, Sigma), as well as LiChroprep RP-18 (40–63 μm, Merck).

Plant material

The aerial parts of *A. selengensis* Turcz. (Compositae) were collected from the Herbarium of College of Pharmacy, Chosun University, Korea, in Aug 2007. A voucher specimen was deposited in the Herbarium of College of Pharmacy, Chosun University (CSU-1041-17).

Extraction and isolation

The air-dried aerial parts of *A. selengensis* (3.5 kg) were extracted three times with MeOH under reflux and 218.9 g of residues were produced. The MeOH extract was suspended in water, which was then partitioned sequentially with equal volumes of dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc) and *n*-butanol (BuOH). Each fraction was evaporated *in vaccuo* to yield residues of CH_2Cl_2 (61.8 g), EtOAc (12.1 g), *n*-BuOH (27.6 g), and water extract (117.4 g). The CH_2Cl_2 fraction (26.0 g) was chromatographed over a silica gel column chromatography (CC), using a gradient solvent system of Hexane:EtOAc (10:1 → 1:5), to give six subfractions (D1–D6). Subfraction D5 (1.70 g) was subjected to a silica gel CC eluting with a gradient solvent system of Hexane:EtOAc (3:1 → 1:2) to yield nine subfractions (D5-1–D5-9). Subfraction D5-5 (210.81 mg) was eluted with Hexane:Acetone (4:1) to yield thirteen subfractions (D5-5-1–D5-5-13). Subfraction D5-5-9 (41.84 mg) was subjected to RP-18 CC eluting with a gradient solvent system of MeOH: H_2O (1:1.5 → 2:1) to give **2** (8.56 mg), **3** (9.12 mg), and **4** (3.53 mg), respectively. Subfraction D3 (3.1 g) was purified by repeated silica gel CC (Hexane:EtOAc, 10:1 → 2:1), followed by MCI gel CC (MeOH:H_2O, 50:1), to give **1** (12.37 mg). The *n*-BuOH fraction (2.5 g) was chromatographed over a HP-20 CC, using a gradient solvent system of MeOH:H_2O (0:100 → 100:0) to give six subfractions (B1–B6). Subfraction B4 (0.55 g) was subjected to a silica gel CC eluting with a gradient solvent system of $CHCl_3$:MeOH:H_2O (7.5:1:0.1 → 1:1:0.1) to yield twenty-four subfractions (B4-1–B4-24). Subfraction B4-18, -19, and -20 (61.09 mg) was eluted with $CHCl_3$:MeOH:H_2O (3:1:0.1) to yield **5** (4.18 mg), and **6** (3.77 mg). Subfraction B4-9-21, and -22 (39.24 mg) was purified by silica gel CC eluting with a gradient solvent system of $CHCl_3$:MeOH:H_2O (4:1:0.1 → 2:1:0.1), followed by Lichroprep RP-18 CC (MeOH:H_2O, 1:3 → 1:2.5) to give **7** (3.07 mg), and **8** (3.10 mg). Subfraction B3 (2.54 g) was purified by MCI gel CC eluting with a gradient solvent system of MeOH:H_2O (1:4 → 1:1), followed by silica gel CC ($CHCl_3$:MeOH:H_2O,10:1:0.1 → 1:1:0.1) to give **9** (6.78 mg), and **10** (1.49 mg).

1′,3′-Propanediol,2′-amino-1′-(1,3-benzodioxol-5-yl) (**1**)—White amorphous powder; $[\alpha]_D^{25}$ 12.7° ($CHCl_3$; *c* 0.1); HR-ESI-MS (positive mode) *m/z*: 212.0962 $[M + H]^+$ (calcd for $C_{10}H_{14}NO_4$, 212.0923); ^1H NMR (500 MHz, $CDCl_3$) δ: 3.05 (1H, dd, *J* = 4.5, 6.5 Hz, H-2′), 3.87(1H, dd, *J* = 3.5, 9.5 Hz, H-3′a), 4.23 (1H, dd, *J* = 6.5, 9.5 Hz, H-3′b), 4.71 (1H, d, *J* = 4.5 Hz, H-1′), 5.95 (2H, s, O-CH_2-O), 6.78 (1H, d, *J* = 8.0 Hz, H-7), 6.80 (1H, dd, *J* = 1.5, 8.0 Hz, H-6), 6.85 (1H, br s, H-4); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 101.0 (O-$\underline{C}H_2$-O), 106.5 (C-4), 134.9 (C-5), 119.3 (C-6), 108.1 (C-7), 147.9 (C-8), 147.0 (C-9), 85.7 (C-1′), 54.3 (C-2′), 71.7 (C-3′).

Artanomaloide (**2**)—Colorless gum; $[\alpha]_D^{25}$ −17° ($CHCl_3$; *c* 0.2); HR-EI-MS *m/z*: 548.2440 $[M]^+$ (calcd for $C_{32}H_{36}O_8$ 548.2447); ^1H NMR (500 MHz, $CDCl_3$) δ: 1.23 (3H, s, H-14′), 1.51 (1H, d, *J* = 12.0 Hz, H-13a), 1.54 (3H, s, H-15′), 1.83 (2H, m, H-9′), 1.84 (1H, m, H-8′a), 1.98 (1H, d, *J* = 9.8 Hz, H-5′), 2.04 (3H, s, -OAc), 2.10 (1H, m, H-8′b), 2.31 (3H, br s, H-14), 2.36 (1H, d, *J* = 2.5 Hz, H-9a), 2.37 (3H, s, H-15), 2.43 (1H, d, *J* = 12.0 Hz, H-13b), 2.90 (1H, dd, *J* = 10.5, 13.0 Hz, H-9b), 3.06 (1H, m, H-7), 3.15 (1H, m, H-7′), 3.78 (1H,br d, H-6), 3.79 (1H, br d, H-5), 4.22 (1H, t, *J* = 9.5 Hz, H-6′), 5.15 (1H, dd. *J* = 2.5, 10.5 Hz, H-8), 5.47 (1H, d, *J* = 3.0 Hz, H-13′a), 5.88 (1H, d, *J* = 5.5 Hz, H-3′), 6.04 (1H, d, *J* = 3.0 Hz, H-13′b), 6.17 (1H, s, H-3), 6.28 (1H, d, *J* = 5.5 Hz, H-2′); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 136.0 (C-1), 197.3 (C-2), 136.6 (C-3), 174.6 (C-4), 51.2 (C-5), 81.0 (C-6), 57.6 (C-

7), 68.1 (C-8), 44.1 (C-9), 145.9 (C-10), 61.5 (C-11), 178.5 (C-12), 38.0 (C-13), 20.5 (C-14), 20.2 (C-15), 65.1 (C-1′), 143.3 (C-2′), 134.2 (C-3′), 58.2 (C-4′), 67.9 (C-5′), 81.9 (C-6′), 44.9 (C-7′), 22.1 (C-8′), 35.7 (C-9′), 73.1 (C-10′), 142.8 (C-11′), 172.7 (C-12′), 119.9 (C-13′), 29.6 (C-14′), 15.2 (C-15′), 25.0, 172.7 (OAc).

Canin (**3**)—Colorless powder; $[\alpha]_D^{25}$ −14.7° ($CHCl_3$; *c* 0.4); HR-ESI-MS (positive mode) *m/z*: 279.1236 $[M + H]^+$ (calcd for $C_{15}H_{19}O_5$ 279.1230); ^1H NMR (500 MHz, $CDCl_3$) δ: 1.27 (3H, s, H-14), 1.57 (3H, s, H-15), 1.84 (1H, m, H-9a), 2.05 (1H, m, H-9b), 2.10 (1H, m, H-8a), 2.33 (1H, m, H-8b), 2.63 (1H, d, *J* = 11.5 Hz, H-5), 3.45 (1H, m, H-7), 3.70 (1H, br s, H-2), 4.07 (1H, br s, H-3), 4.34 (1H, dd, *J* = 9.5, 11.5 Hz, H-6), 5.49 (1H, d, *J* = 3.0 Hz, H-13a), 6.21 (1H, d, *J* = 3.0 Hz, H-13b); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 73.3 (C-1), 64.6 (C-2), 64.3 (C-3), 83.3 (C-4), 57.8 (C-5), 79.8 (C-6), 45.0 (C-7), 23.5 (C-8), 35.0 (C-9), 72.2 (C-10), 139.3 (C-11), 169.4 (C-12), 120.0 (C-13), 26.5 (C-14), 22.1 (C-15).

Eupatilin (**4**)—Yellow amorphous powder; EI-MS *m/z*: 344 $[M]^+$; ^1H NMR (500 MHz, $CDCl_3$) δ: 3.94 (3H, s, OCH_3), 3.98 (3H, s, OCH_3), 3.99 (3H, s, OCH_3), 6.61 (1H, s, H-3), 6.56 (1H, s, H-8), 6.98 (1H, d, *J* = 9.0 Hz, H-5′), 7.34 (1H, d, *J* = 2.0 Hz, H-2′), 7.53 (1H, dd, *J* = 2.0, 8.5 Hz, H-6′); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 163.9 (C-2), 104.5 (C-3), 182.6 (C-4), 153.2 (C-5), 132.6 (C-6), 158.7 (C-7), 90.6 (C-8), 153.1 (C-9), 106.1 (C-10), 123.8 (C-1′), 108.7 (C-2′), 149.3 (C-3′), 152.2 (C-4′), 111.1 (C-5′), 120.1 (C-6′), 60.86 (OCH_3), 56.33 (OCH_3), 56.11 (OCH_3).

Quercetin-3-*O*-β-D-glucoside-7-*O*-α-L-rhamnoside (**5**)—Yellow amorphous powder; ^1H NMR (500 MHz, CD_3OD) δ: 1.12 (3H, d, *J* = 6.0 Hz, H-6‴), 3.08 (1H, m, H-4″), 3.24 (1H, d, *J* = 7.5 Hz, H-3″), 3.24 (1H, d, *J* = 7.5 Hz, H-2″), 3.29 (1H, d, *J* = 9.0 Hz, H-4‴), 3.31 (2H, m, H-6″), 3.40 (1H, m, H-5‴), 3.60 (1H, m, H-5″), 3.63 (1H, dd, *J* = 2.9, 9.5 Hz, H-3‴), 3.83 (1H, dd, *J* = 2.0, 2.9 Hz, H-2‴), 4.52 (d, *J* = 1.2 Hz, H-1‴), 5.11 (1H, d, *J* = 7.5 Hz, H-1″), 6.21 (1H, d, *J* = 2.0 Hz, H-6), 6.40 (1H, d, *J* = 2.0 Hz, H-8), 6.87 (1H, d, *J* = 8.5 Hz, H-5′), 7.63 (1H, dd, *J* = 2.0, 8.5 Hz, H-6′), 7.67 (1H, d, *J* = 2.0 Hz, H-2′); ^{13}C NMR (125 MHz, CD_3OD) δ: 159.4 (C-2), 135.7 (C-3), 179.6 (C-4), 163.1 (C-5), 100.2 (C-6), 166.5 (C-7), 95.1 (C-8), 158.7 (C-9), 104.9 (C-10), 123.7 (C-1′), 117.8 (C-2′), 146.0 (C-3′), 150.0 (C-4′), 116.2 (C-5′), 123.2 (C-6′), 105.7 (C-1″), 75.9 (C-2″), 76.4 (C-3″), 71.5 (C-4″), 78.3 (C-5″), 68.7 (C-6″), 102.6 (C-1‴), 74.1 (C-4‴), 71.4 (C-5‴), 72.3 (C-3‴), 69.9 (C-2‴), 18.0 (C-6‴).

Isoquercitrin (**6**)—Yellow amorphous powder; $[\alpha]_D^{25}$ −85° (MeOH; *c* 0.06); EI-MS *m/z*: 464 $[M]^+$; ^1H NMR (500 MHz, CD_3OD) δ: 3.71–3.35 (6H, m, H-2″-6″), 5.25 (1H, d, *J* = 8.0 Hz, H-1″), 6.20 (1H, d, *J* = 2.0 Hz, H-6), 6.39 (1H, d, *J* = 2.0 Hz, H-8), 6.87 (1H, d, *J* = 8.5 Hz, H-5′), 7.59 (1H, dd, *J* = 2.0, 8.5 Hz, H-6′), 7.78 (1H, d, *J* = 2.0 Hz, H-2′); ^{13}C NMR (125 MHz, CD_3OD) δ: 159.1 (C-2), 135.7 (C-3), 179.6 (C-4), 163.2 (C-5), 100.1 (C-6), 166.5 (C-7), 94.9 (C-8), 158.6 (C-9), 104.4 (C-10), 123.2 (C-1′), 117.7 (C-2′), 146.1 (C-3′), 150.0 (C-4′), 116.1 (C-5′), 123.3 (C-6′), 105.7 (C-1″), 75.9 (C-2″), 78.5 (C-3″), 71.3 (C-4″), 78.3 (C-5″), 62.7 (C-6″).

1,3-Di-*O*-caffeoylquinic acid (**7**) - Yellowish gum; $[\alpha]_D^{25}$ −24.7° (MeOH; *c* 0.21); ^1H NMR (500 MHz, CD_3OD) δ: 1.98 (1H, m, H-6a), 2.25 (1H, dd, *J* = 3.5, 13.0 Hz, H-2b), 2.69 (2H, m, H-2a,-6b), 3.72 (1H, dd, *J* = 3.5, 9.5 Hz, H-4), 4.22 (1H, dd, *J* = 3.5, 6.5 Hz, H-5), 5.45 (1H, m, H-3) (quinic acid moiety); 6.32 (2H, d, *J* = 16.0 Hz, H-2), 6.77 (2H, dd, *J* = 2.5, 8.0 Hz, H-8), 6.94 (2H, dd, *J* = 2.0, 8.5 Hz, H-9), 7.05 (2H, t, *J* = 2.0 Hz, H-5), 7.57 (2H, dd, *J* = 4.5, 16.0 Hz, H-3) (caffeoyl groups); ^{13}C NMR (125 MHz, CD_3OD) δ: 82.7 (C-1), 35.5 (C-2), 70.5 (C-3), 73.2 (C-4), 69.6 (C-5), 37.1 (C-6), 176.8 (C-7) (quinic acid moiety); 167.8, 166.9 (C-1), 115.5, 114.2 (C-2), 145.5, 145.4 (C-3), 126.8, 126.5 (C-4), 113.7, 113.6 (C-5), 145.3, 144.8 (C-6), 148.2, 147.8 (C-7), 115.1, 115.0 (C-8), 121.6, 121.4 (C-9) (caffeoyl groups).

Pinoresinol-4-*O*-β-D-glucoside (**8**)—Yellow amorphous powder; $[\alpha]_D^{25}$ −84.0° (MeOH; *c* 0.16); ^1H NMR (500 MHz, CD_3OD) δ: 3.13 (2H, m, H-8, 8′), 3.39–3.86 (sugar H), 3.84 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 3.86 (2H, m, H-9a, 9a′),4.24 (2H, m, H-9b, 9b′), 4.71 (1H, d, *J* = 4.0 Hz, H-7′), 4.76 (1H, d, *J* = 4.5 Hz, H-7), 4.87 (1H, H-1″), 6.77 (1H, d, *J* = 8.5 Hz, H-5′), 6.81 (1H, dd, *J* = 1.5, 8.5 Hz, H-6′), 6.92 (1H, dd, *J* = 1.5, 8.5 Hz, H-6), 6.94 (1H, d, *J* = 1.5 Hz, H-2′), 7.03 (1H, d, *J* = 2.0 Hz, H-2), 7.15 (1H, d, *J* = 8.0 Hz, H-5); ^{13}C NMR (125 MHz, CD_3OD) δ: 137.6 (C-1), 133.9 (C-1′), 111.7 (C-2), 111.1 (C-2′), 147.6 (C-3), 147.4 (C-3′), 151.1 (C-4), 149.3 (C-4′), 118.1 (C-5), 116.2 (C-5′), 119.9 (C-6), 120.2 (C-6′), 87.2 (C-7), 87.6 (C-7′), 55.5 (C-8), 55.7 (C-8′), 72.8 (C-9), 72.8 (C-9′), 56.9 (C-OCH_3), 56.5 (C-OCH_3), 102.9 (C-1″), 75.0 (C-2″), 78.0 (C-3″), 71.5 (C-4″), 78.3 (C-5″), 62.6 (C-6″).

Scopolin (**9**)—White powder; ^1H NMR (500 MHz, DMSO-d_6): δ 3.15 (1H, m), 3.28 (2H, m), 3.45 (2H, m), 3.69 (1H, m), 3.82 (3H, s, 6-OCH_3), 5.08 (1H, d, *J* = 7.5 Hz, H-1′), 6.33 (1H, d, *J* = 9.0 Hz, H-3), 7.15 (1H, s, H-8), 7.30 (1H, s, H-5), 7.96 (1H, d, *J* = 9.5 Hz, H-4); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 160.5 (C-2), 113.3 (C-3), 144.2 (C-4), 109.6 (C-5), 146.0 (C-6), 149.9 (C-7), 103.0 (C-8), 148.9 (C-9), 112.2 (C-10), 56.0 (6-OCH_3), 99.6 (C-1′), 73.0 (C-2′), 76.7 (C-3′), 69.6 (C-4′), 77.1 (C-5′), 60.6 (C-6′).

Isofraxidin-7-*O*-β-D-glucopyranoside **(10)**—Colorless amorphous powder; $[\alpha]_D^{25}$ +44.1° (MeOH; *c* 0.25); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 3.09 (2H, m), 3.24 (2H, m), 3.38 (1H, m), 3.59 (1H, m), 3.82 (3H, s, 6-OCH_3), 3.91 (3H, s, 8-OCH_3), 5.15 (1H, d, *J* = 5.0 Hz, H-1′), 6.40 (1H, d, *J* = 9.5 Hz, H-3), 7.13 (1H, s, H-5), 7.96 (1H, d, *J* = 9.5 Hz, H-4); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 159.8 (C-2), 114.7 (C-3), 144.4 (C-4), 105.4 (C-5), 149.4 (C-6), 141.6 (C-7), 140.2 (C-8), 142.4 (C-9), 114.5 (C-10), 56.5 (6-OCH_3), 61.3 (8-OCH_3), 102.2 (C-1′), 74.1 (C-2′), 77.5 (C-3′), 70.0 (C-4′), 76.5 (C-5′), 60.7 (C-6′).

Bioassay of IL-6

IL-6 bioassay was carried out using a slight modification of an established method (Kim et al. 2003; Liu et al. 2006). Briefly, 500 μL of the MG-63 cells (3 × 10^4 cells/mL) in DMEM containing 10 % FBS were dispensed into a 24-well plate; the culture was incubated for 24 h at 37 °C. Then, 5 μL of TNF-α (10 ng/mL), 5 μL of BAY 11-7085 (10 ng/mL), and 5 μL of the DMSO with or without the compounds (100 μg/mL) were added. After incubation at 37 °C with 5 % CO_2 for 24 h, the medium was stored at −20 °C until measurement. The IL-6 content of the medium was measured in an ELISA procedure. 96-well plates were coated with 100 μL of purified rat anti-human IL-6 monoclonal antibody in 0.1 M $NaHCO_3$ (pH 9.6) by overnight incubation at 4 °C. The wells were blocked with 200 μL of 3 % BSA in PBS for 2 h at room temperature (RT) and then incubated with 100 μL of specific antibody for 2 h at RT. 100 μL of HRP conjugated rabbit anti-goat IgG (1:1,000 dilution) was added to each well and incubated for 2 h at RT. 100 μL of TMB (3,3′,5,5′-tetramethylbenzidine) substrate solution was added and incubated for 10 min at RT. The color reaction was stopped with 50 μL of 0.4 N HCl and the optical density was read at 450 nm using a Microplate Reader (Molecular Devices Co., Ltd., U.S.A.).

Results and discussion

The MeOH extract of the aerial parts of *A. selengensis* was partitioned into CH_2Cl_2, EtOAc, *n*-BuOH-soluble fractions. Separation of the CH_2Cl_2 and *n*-BuOH soluble fraction with silica gel, MCI gel filtration, and repeated RP-18 CC led to the isolations of compounds **1–10** (Fig. 1).

Compound **1** was obtained as white amorphous powder, $[\alpha]_D^{25}$ 12.7° (MeOH). Its molecular formula was determined to be $C_{10}H_{14}NO_4$ by HR-ESI-MS data at *m/z* 212.0962 $[M+H]^+$ (calcd for $C_{10}H_{14}NO_4$ 212.0923). In the IR spectrum, absorption bands for hydroxyl (3,400 cm^{-1}) and aromatic ring (1600, 1518 cm^{-1}) groups were observed. The ^{1}H NMR spectrum of **1** showed ABX-trisubstituted aromatic protons at δ_H 6.78 (1H, d, *J* = 8.0 Hz, H-7), 6.80 (1H, dd, *J* = 1.0, 8.0 Hz, H-6), 6.85 (1H, br s, H-4), one hydroxyl propyl proton at δ_H 3.87(1H, dd, *J* = 3.5, 9.5 Hz, H-3′a), 4.23 (1H, dd, *J* = 6.5, 9.5 Hz, H-3′b), one oxymethine proton at δ_H 4.71 (1H, d, *J* = 4.5 Hz, H-1′), one aminomethine proton at δ_H 3.05 (1H, dd, *J* = 4.5, 6.5 Hz, H-2′) and one methylene dioxy proton at δ_H 5.95 (2H, s, H-2). In the ^{13}C NMR spectrum, 10 carbon signals appeared, which included two oxygenated quaternary carbons at δ_C 147.9 (C-8), and 147.0 (C-9), three aromatic carbons at δ_C 106.5 (C-4), 119.3 (C-6), and 108.1 (C-7), one quaternary carbon at δ_C 134.9 (C-5), one oxygenated carbon at δ_C 85.7 (C-1′), one aminomethine carbon at δ_C 54.3 (C-2′), one methylene dioxycarbon at δ_C 101.0 (C-2) and one hydroxyl propyl carbon δ_C at 71.7 (C-3′). From these results, compound **1** was indicated to be a phenylpropanoid derivative with one amino and two hydroxyl groups. In the HMBC spectrum, correlations between H-2 and C-9/C-8, H-1′ and C-5/C-3′ were observed. Furthermore, in the ^{1}H-^{1}H COSY spectrum, the hydroxyl proton at δ_H 3.87 (H-3′a) and 4.23 (H-3′b) showed couplings with H-2′, and H-1′ (Fig. 2). Accordingly, compound **1** was determined as 1′,3′-propanediol,2′-amino-1′-(1,3-benzodioxol-5-yl), as shown in Fig. 1. Compound **1** was isolated for the first time as a new natural product even though it was synthesized previously (Cellitti et al. 2008). Furthermore, its spectral data are presented here for the first time.

Artanomaloide (**2**) was obtained as colorless gum, $[\alpha]_D^{25}$ −17°. The ^{1}H, ^{13}C NMR, and HSQC spectroscopic data of **2** showed the presence of 32 carbons, which were assignable to four tertiary methyl groups [δ_H 1.23 (3H, s, H-14′), 1.54 (3H, s, H-15′), 2.31 (3H, br s, H-14), 2.37 (3H, s, H-15); δ_C 29.6 (C-14′), 15.2 (C-15′), 20.5 (C-14), 20.2 (C-15), respectively], three carbonyl groups [δ_C 197.3 (C-2), 178.5 (C-12), 172.7 (C-12′)], three acetal methine groups [δ_H 3.78 (1H, br s, H-6); δ_C 81.0 (C-6), δ_H 4.22 (1H, t, *J* = 9.5 Hz, H-6′); δ_C 81.9 (C-6′), δ_H 5.15 (1H, dd, *J* = 2.5, 10.5 Hz, H-8); δ_C 68.1 (C-8)], an *exo* methylene group [δ_H 5.47 (1H, d, *J* = 3.0 Hz, H-13′a) and δ_H 6.04 (1H, d, *J* = 3.0 Hz, H-13′b); δ_C 119.9 (C-13′)], one oxygenated quaternary carbon δ_C 73.1(C-10′), one acetyl group [δ_H 2.04 (3H, s); δ_C 172.7], two olefinic groups [δ_H 6.17 (1H, s, H-3); δ_C 136.6 (C-3), δ_H 5.88 (1H, d, *J* = 5.5 Hz, H-3′); δ_C 134.2 (C-3′), δ_H 6.28 (1H, d, *J* = 5.5 Hz, H-2′); δ_C 143.3 (C-2′), respectively], four methylene groups δ_C 44.1(C-9), 38.0 (C-13), 35.7 (C-9′), and 22.1 (C-8′), four methine groups [δ_H 1.98 (d, *J* = 9.8 Hz, H-5′); δ_C 67.9 (C-5′), δ_H 3.06 (1H, m, H-7); δ_C 57.6 (C-7), δ_H 3.15 (1H, m, H-7′); δ_C 44.9 (C-7′), δ_H 3.79 (1H, br s, H-5); δ_C 51.2 (C-5), respectively], and two

Fig. 1 Structures of compounds **1–10**

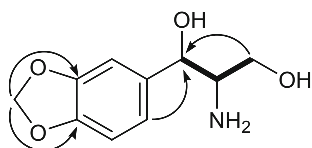

Fig. 2 Selected ^{1}H-^{1}H COSY (–), HMBC (H → C) correlations of compound **1**

quaternary carbons δ_C 65.1(C-1′) and 61.5 (C-11). From these results, compound **2** was indicated to be a dimeric sesquiterpene lactone (Jakupovic et al. 1987). Furthermore, in the HMBC spectrum, long range correlations between H-3/H-6 and C-1, H-7 and C-11, H-3 and C-2 were observed. In addition, correlations between H-5′ and C-11, H-7′ and C-12′, H-6′ and C-11′ were supported the proposed structure of **2**. Accordingly, compound **2** was determined as artanomaloide on the basis of the above evidences, together with a comparison with the literature (Jakupovic et al. 1987).

Compound **3** was obtained colorless powder, $[\alpha]_D^{25}$ −14.7°. The ^{1}H-, ^{13}C NMR, and HSQC spectroscopic data of **3** showed the presence of 15 carbons, which were assignable to two tertiary methyl groups [δ_H 1.27 (3H, s, H-14); δ_C 26.5 (C-14) and δ_H 1.57(3H, s, H-15); δ_C 22.1 (C-15), respectively], an *exo* methylene group [δ_H 6.21 (1H, d, $J = 3.0$ Hz, H-13b) and δ_H 5.49 (1H, d, $J = 3.0$ Hz, H-13a); δ_C 120.0], three acetal methine groups [δ_H 4.34 (1H, dd, $J = 9.5$, 11.5 Hz, H-6); δ_C 79.8 (C-6), δ_H 4.07 (1H, br s, H-3); δ_C 64.3 (C-3) and δ_H 3.70 (1H, br s, H-2); δ_C 64.6 (C-2), respectively], three oxygenated quaternary carbons δ_C 72.2 (C-10), 73.3 (C-1), and 83.3 (C-4), one carbonyl carbon δ_C 169.4 (C-12), two methine groups [δ_H 2.63 (1H, d, $J = 11.5$ Hz, H-5); δ_C 57.8 (C-5) and δ_H 3.45 (1H, m, H-7); δ_C 45.0 (C-7), respectively], two methylene groups [δ_H 1.84 (1H, m, H-9a), δ_H 2.05 (1H, m, H-9b); δ_C 35.0 (C-9) and δ_H 2.10 (1H, m, H-8a), δ_H 2.33 (1H, m, H-8b); δ_C 23.5 (C-8), respectively] and one quaternary carbon δ_C 139.3 (C-11). These observations suggested that compound **3** was a 1,2;3,4-diepoxyguaianolide sesquiterpene lactone with two tertiary methyls and one hydroxyl group. Furthermore, HMBC and NOESY spectral data were good agreement with the reported data (Li et al. 2010). Accordingly, compound **3** was determined as canin

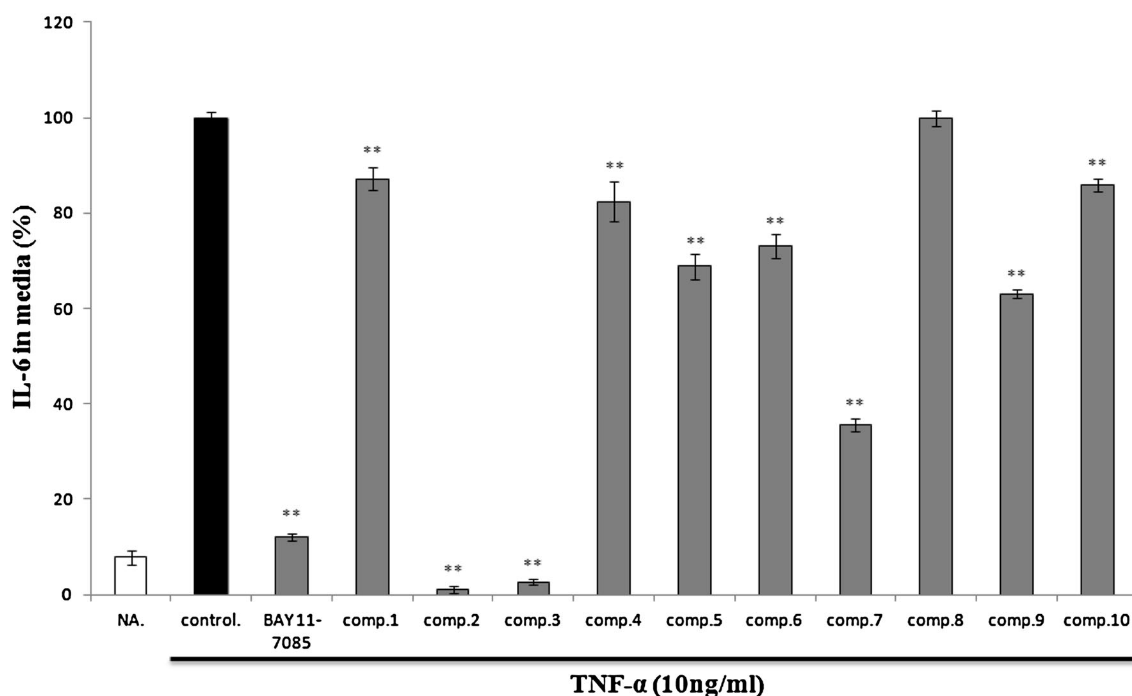

Fig. 3 Inhibitory effect of compounds **1–10** against IL-6 production in TNF-α stimulated MG-63cells. MG-63 cells (3×10^4) were incubated for 24 h. Cultures were incubated with or without compounds (100 μg/mL) for 30 min and then stimulated with TNF-α (10 ng/mL) for 24 h. IL-6 in the supernatant was measured by ELISA as described in "Materials and methods" section. Results are expressed as the mean ± S.E. from three different experiments. BAY 11-7085 was used as a positive control. *$P < 0.05$ or **$P < 0.01$ compared with TNF-α treated value

Table 1 Inhibitory effect of compounds **1–10** against IL-6 production in TNF-α stimulated MG 63 cells

Treatment	IL-6 (pg/mL)	Inhibition (%)
None	19.7 ± 3.7	–
TNF-α	250.6 ± 3.4	–
BAY 11-7085	30.2 ± 2.1**	87.9**
Compound **1**	218.0 ± 5.95**	12.8**
Compound **2**	5.0 ± 1.71**	97.7**
Compound **3**	12.5 ± 1.52**	94.7**
Compound **4**	205.5 ± 10.54**	17.6**
Compound **5**	390.9 ± 6.9**	31.2**
Compound **6**	175.4 ± 6.4**	26.9**
Compound **7**	182.9 ± 3.4**	64.5**
Compound **8**	228.0 ± 4.1**	0.0
Compound **9**	213.0 ± 2.1**	36.9**
Compound **10**	218.0 ± 3.4**	14.0**

MG-63 cells (3×10^4) were incubated for 24 h. Cultures were incubated with or without compounds (100 μg/mL) for 30 min and then stimulated with TNF-α (10 ng/mL) for 24 h. IL-6 in the supernatant was measured by ELISA as described in "Materials and methods" section. Results are expressed as the mean ± S.E. from three different experiments. BAY 11-7085 was used as a positive control

* $P < 0.05$ or ** $P < 0.01$ compared with TNF-α treated value

on the basis of the above evidences, together with a comparison with the literature (Li et al. 2010).

The known compounds **4–10** were also identified as eupatilin **(4)** (Li et al. 2010), quercetin-3-*O*-β-D-glucoside-7-*O*-α-L-rhamnoside **(5)** (Kim et al. 2013), isoquercitrin **(6)** (Duan et al. 2009), 1,3-di-*O*-caffeoylquinic acid **(7)** (An et al. 2008; Jiang et al. 2010; Lee et al. 2013), pinoresinol-4-*O*-β-D-glucoside **(8)** (Wang et al. 2012; Kim et al. 2005), scopolin **(9)** (Chung et al. 1999; Lee et al. 2005), and isofraxidin-7-*O*-β-D-glucopyranoside **(10)** (Heo et al. 2005; Hu et al. 2011), respectively, by comparing the NMR spectral data with those reported in the literature. All compounds have not been previously isolated from this plant.

IL-6 is a cytokine, originally identified as a T cell derived factor that regulates B-cell growth and differentiation (Hirano et al. 1986). Human IL-6 is an important component of the inflammatory cascade. Dysregulation of IL-6 production has been implicated in a variety of inflammatory/autoimmune disease states, including rheumatoid arthritis, cardiac myxoma, Castleman's disease, and mesangial proliferative glomerulonephritis (Hirano et al. 1990). The proinflammatory cytokines IL-1 and TNF-α markedly stimulate the production IL-6 (Van Damme et al. 1987).

The inhibitory activity of the isolated compounds (**1–10**) against IL-6 production in TNF-α stimulated MG-63 cells was examined. None of these isolates exhibited cellular cytoxicity in MG 63 cells at the tested concentration (data not shown). Among these compounds, compounds **2**, **3** and **7** showed potent inhibitory activity against IL-6 production in TNF-α stimulated MG-63 cells, while compounds **5, 6,** and **9** showed moderate inhibitory activity (Fig. 3; Table 1).

In conclusion, this paper reports the isolation, characterization, and inhibitory activity of 10 isolates, including one new compound and nine known compounds, from the aerial parts of *A. selengensis*.

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References

Ahn, D.K. 1998. *Illustrated book of Korean medicinal herbs*, 550. Seoul: Kyo-Hak Publishing.

An, R.-B., D.-H. Sohn, G.-S. Jeong, and Y.-C. Kim. 2008. In vitro hepatoprotective compounds from *Suaeda glauca*. *Archives of Pharmacal Research* 31: 594–597.

Cellitti, S.E., D.H. Jones, L. Lagpacan, X. Hao, Q. Zhang, H. Hu, S.M. Brittain, A. Brinker, J. Caldwell, B. Bursulaya, G. Spraggon, A. Brock, Y. Ryu, T. Uno, P.G. Schultz, and B.H. Geierstanger. 2008. In vivo incorporation of unnatural amino acids to probe structure, dynamics, and ligand binding in a large protein by nuclear magnetic resonance spectroscopy. *Journal of American Chemical Society* 130: 9268–9281.

Chung, S.-C., B.Y. Hwang, G.-J. Oh, S.-J. Kang, M.-J. Kim, W.-H. Choi, K.S. Lee, and J.S. Ro. 1999. Chemical components from the stem bark of *Rhus javanica* L. *Korean Journal of Pharmacognosy* 30: 295–300.

Duan, C.H., J.-N. Lee, C.-M. Lee, G.-T. Lee, and K.-K. Lee. 2009. Phytochemical constituents from *Metasequoia glyptostroboids* leaves. *Natural Product Sciences* 15: 12–16.

Hirano, T., K. Yasukawa, H. Harada, T. Taga, Y. Watanabe, T. Matsuda, S. Kashiwamura, K. Nakajima, K. Koyama, A. Iwamatsu, S. Tsunsawa, F. Sakiyama, H. Matsui, Y. Takahara, T. Taniguchi, and T. Kishimoto. 1986. Complementary DNA for a novel human interleukin (BSF-2) that induced lymphocytes to produce immunoglobulin. *Nature* 324: 73–76.

Heo, J.E., J.L. Jin, Y.Y. Lee, and H.S. Yun-Choi. 2005. Chemical constituents of the aerial parts of *Chloranthus japonicas* Sieb. *Natural Product Sciences* 11: 41–44.

Hirano, T., S. Akira, T. Taga, and T. Kishimoto. 1990. Biological and clinical aspects of interleukin 6. *Immunology Today* 11: 443–449.

Hu, J.-F., and X.-Z. Feng. 1999. New guaianolides from *Artemisia selengensis*. *Journal of Asian Natural Product Report* 1: 169–176.

Hu, H.-B., X.-D. Zheng, Y.-F. Jian, J.-X. Liu, and J.-H. Zhu. 2011. Constituents of the root of *Anemone tomentosa*. *Archives of Pharmacal Research* 34: 1097–1105.

Jang, W.Y., and K.R. Lee. 1993. A new endoperoxide from *Artemisia selengensis*. *Korean Journal of Pharmacognosy* 24: 107–110.

Jakupovic, J., Z.-L. Chen, and F. Bohlmann. 1987. Artanomaloide, a dimeric guaianolide and phenylalanine derivatives from *Artemisia anola*. *Phytochemistry* 26: 2777–2779.

Jiang, J., B.B. Zhang, and Z.X. Liao. 2010. A new dicaffeoyl quinic acid from *Nannoglottis ravida*. *Chinese Chemical Letters* 21: 203–205.

Kim, B.H., E.Y. Chung, J.-C. Ryu, S.-H. Jung, K.R. Min, and Y. Kim. 2003. Anti-inflammatory mode of isoflavone glycosides sophoricoside by inhibition of interleukin-6 and cyclooxygenase-2 in inflammatory response. *Archives of Pharmacal Research* 26: 306–311.

Kim, D.K., J.P. Lim, J.W. Kim, H.W. Park, and J.S. Eun. 2005. Antitumor and anti-inflammatory constituents from *Celtis sinensis*. *Archives of Pharmacal Research* 28: 39–43.

Kim, H.J., B.-G. Kim, and J.-H. Ahn. 2013. Regioselective synthesis of flavonoid bisglycosides using *Escherichia coli* harboring two glycosyltransferases. *Applied Microbiology Biotechnology* 97: 5275–5282.

Koo, K.A., J.H. Kwak, K.R. Lee, O.P. Zee, E.-R. Woo, H.K. Park, and H.J. Youn. 1994. Antitumor and immunomodulating activities of the polysaccharide fraction from *Artemisia selengensis* and *Artemisia iwayomogi*. *Archives of Pharmacal Research* 17: 371–374.

Lee, T.B. 1993. *Illustrated flora of Korea*, 759. Seoul: Hyang-Moon Publishing.

Lee, J.H., W.J. Jeon, E.S. Yoo, C.M. Kim, and Y.S. Kwon. 2005. The chemical constituents and their antioxidant activity of the stem of *Rhododendron mucronulatum*. *Natural Product Sciences* 11: 97–102.

Lee, Y.G., J.-Y. Cho, C.-M. Kim, S.-H. Lee, W.-S. Kim, T.-I. Jeon, K.-H. Park, and J.-H. Moon. 2013. Coumaroyl quinic acid derivatives and flavonoids from Immature Pear (*Pyrus pyrifolia* Nakai) fruit. *Food Science Biotechnology* 22: 803–810.

Li, D., X.H. Han, S.S. Hong, C. Lee, M.S. Lee, D. Lee, M.K. Lee, and B.Y. Hwang. 2010. Inhibitors of nitric oxide production from *Artemisia princeps*. *Natural Product Sciences* 16: 143–147.

Liu, Q.H., J.-E. Jeong, E.J. Choi, Y.H. Moon, and E.-R. Woo. 2006. A new furofuran lignan from *Geranium thunbergii*. *Archives of Pharmacal Research* 29: 1109–1113.

Shi, F., X. Jia, C. Zhao, and Y. Chen. 2010. Antioxidant activities of various extracts from *Artemisia selengensis* Turcz (LuHao). *Molecules* 15: 4934–4946.

Van Damme, J., G. Opdenakker, R.J. Simpson, M.R. Rubira, S. Cayphas, A. Vink, A. Billiau, and J. Van Snick. 1987. Identification of the human 26-kD protein, interferon beta 2 (IFN-beta 2), as a B cell hybridoma/plasmacytoma growth factor induced in interleukin 1 and tumor necrosis factor. *Journal of Experimental Medicine* 165: 914–919.

Wang, D.-M., W.-J. Pu, Y.-H. Wang, Y.-J. Zhang, and S.-S. Wang. 2012. A New isorhamnetin glycoside and other phenolic compounds from *Callianthemum taipaicum*. *Molecules* 17: 4595–4603.