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A new iridoid and effect on the rat aortic vascular smooth muscle cell proliferation of isolated compounds from *Buddleja officinalis*

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ABSTRACT

A new iridoid, named methylscutelloside (**1**) together with 19 known compounds belonging to the iridoids (**2**–**4**), monoterpenoids (**5**), flavonoids (**6**–**8**), triterpenoids (**9**–**14**), and phenylethanoids (**15**–**20**) were isolated from the flowers of *Buddleja officinalis*. Their chemical structures were elucidated on the basis of physicochemical properties, and by spectroscopic methods including 1D, 2D NMR, and MS. All isolated compounds were tested in vitro for their effects on the proliferation of rat aortic vascular smooth muscle cells (VSMCs). Among them, iridoids were the main active components and showed significant inhibitory effects on PDGF-BB-induced proliferation in rat aortic VSMCs.

Buddleja officinalis Maxim. (Buddlejaceae), a shrub tree, is cultivated widely throughout the temperate regions of the Americas, Africa and Asia. Phytochemical studies on *Buddleja* species have led to the isolation of flavonoids,[1] triterpenoids,[2] and phenylethanoids[3] which are the main active components for the treatment of stroke, headache, neurological disorders, conjunctival congestion, clustered nebulae, removal of heat, replenishment of the liver, and clearing of corneal opacity in several folk medicine remedies.[4,5] Recent studies showed that, the water extract of *B. officinalis* inhibited high glucose-induced matrix metalloproteinase activity,[6] vascular inflammation[7,8] in human umbilical vein endothelial cells (HUVECs), and suppressed high glucose-induced atherosclerotic processes through inhibition of p38 mitogen-activated protein kinases (MAPK), JNK phosphorylation, nuclear factor-κB (NF-κB) and matrix metalloproteinase (MMP) signal pathways in human aortic smooth muscle cells.[9] Previously, flavonoids and phenylethanoids from *B. officinalis* were reported to act as anti-oxidative stress inhibitors against peroxynitrite.[3] For continuous investigation on the chemical components and bioactivities of *B. officininalis*, we report the isolation and structure elucidation of a new iridoid and 19 known compounds. In addition, their inhibitory effects on rat aortic vascular smooth muscle cell (VSMC) proliferation through the homodimer platelet-derived growth factor beta polypeptide (PDGF-BB) signal pathway are also discussed.

Dried flowers of *B. officinalis* were ultrasonically extracted with methanol. The crude extract was then successively partitioned with *n*-hexane, dichloromethane, ethyl acetate, and *n*-butanol. Using various types of column chromatography, a new iridoid named methylscutelloside (**1**) together with 19 known compounds 6-*O*-vanilloyl ajugol (**2**),[10] methylcatalpol (**3**),[11,12] buddlejoside A_4 (**4**),[11] betulalbusides A (**5**),[13,14] apigenin (**6**),[3] isorhoifolin (**7**),[3] linarin (**8**),[3] β-phenylethoxy-β-D-glucopyranoside (**9**),[3] salidroside (**10**),[3] bioside (**11**),[3] acteoside (**12**),[3] poliumoside (**13**),[3] echinacoside (**14**),[3] buddlejasaponin III (**15**),[15,16] buddlejasaponin IV (**16**),[15] buddlejasaponin I (**17**),[17,18] buddlejasaponin Ia (**18**),[19,20] buddlejasaponin IVa (**19**),[15] and mimengoside B (**20**)[21,22] were isolated.[3,23] Their chemical structures (see Fig. 1) were elucidated base on the results of 1D, 2D NMR, MS data, as well as by comparison with compounds reported in the literatures. Compound **1** was obtained from *n*-butanol extract as a pale yellow amorphous powder, $[\alpha]_D^{22} = -7.6$ (*c* 0.31, MeOH). Its molecular formula was determined to be $C_{16}H_{26}O_{11}$ based on the quasi-molecular ion peak observed at *m/z* 395 $[M+H]^+$ in positive mode, electrospray ionization mass spectrometry (ESI-MS) and *m/z* 395.1561 $[M+H]^+$ (calcd $C_{16}H_{27}O_{11}$ for 395.1553) in high resolution ESI-MS (HR-ESI-MS) analysis. The ^{13}C NMR and Distortionless Enhancement by Polarization Transfer (DEPT) spectral data of **1** showed 16 carbons including one methoxy carbon, three methylene carbons, 11 tertiary carbons, and one quaternary carbon. Among them, one

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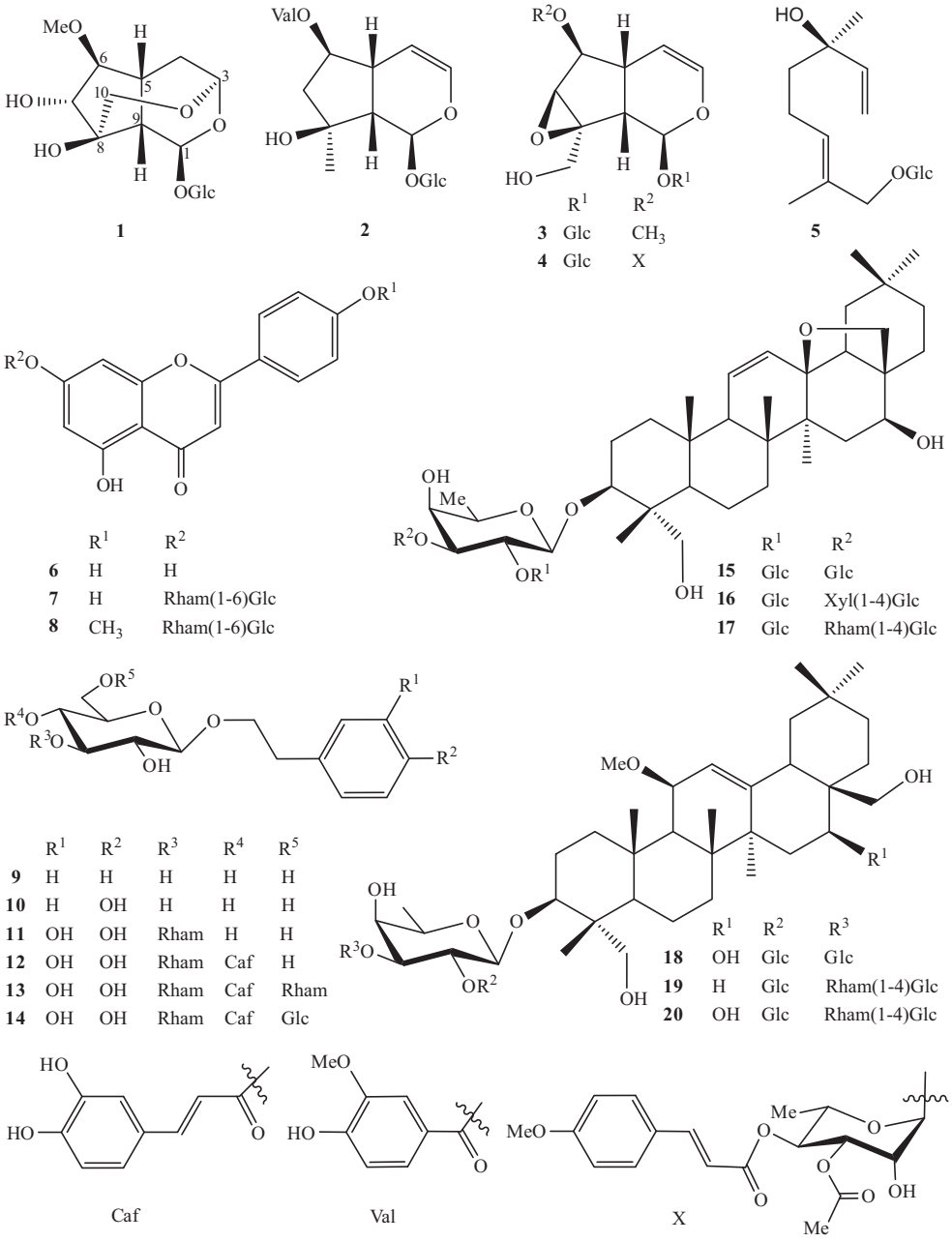

Figure 1. Chemical structures of isolated compounds.

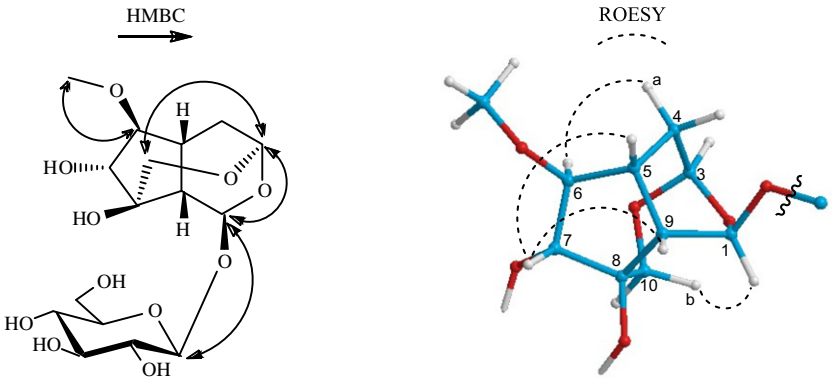

Figure 2. Selected HMBC and ROESY correlations for **1**.

Table 1
The ^{1}H and ^{13}C NMR data for **1** in CD_3OD

Position	δ_H[a] (mult, *J* in Hz)	δ_C[b]
1	5.60 (br s)	93.3
3	5.23 (br s)	95.7
4	1.63 (dd, 13.8, 2.4)	35.3
	2.43 (m)	
5	2.37 (t, 8.4)	33.3
6	3.68 (dd, 1.8, 7.8)	95.0
7	4.06 (d, 7.8)	72.4
8	—	80.2
9	2.47 (br d, 8.4)	48.1
10	3.57 (d, 11.4)	62.1
	3.95 (d, 11.4)	
1-*O*-Glc		
1′	4.66 (d, 7.8)	98.9
2′	3.15 (dd, 8.1, 7.8)	74.7
3′	3.26 (m)	78.1
4′	3.26 (m)	71.6
5′	3.34 (m)	78.1
6′	3.62 (dd, 13.8, 3.0)	62.7
	3.83 (d, 13.8)	
6-OMe	3.38 (s)	57.9

Assignments were done by HMQC, HMBC, ^{1}H–^{1}H COSY, and ROESY experiments.
[a] Measure at 600 MHz.
[b] Measure at 150 MHz.

anomeric carbon observed at δ_C 98.9, four oxygenated methine carbons at δ_C 71.6–78.1, and one oxygenated methylene carbon at δ_C 62.7 were assigned to the sugar moiety. One methyl carbon at δ_C 57.9 ppm was assigned to the methoxy group and the nine remaining carbons belonged to the aglycone moiety. These data suggested that **1** had nine carbons in a catalpol-like iridoid structure linked to a β-glucopyranosyl unit at C-1, which was also in agreement with the correlations observed between anomeric proton H-1′ (δ_H 4.66) and C-1 (δ_C 93.3), and between proton H-1 (δ_H 5.60) and anomeric carbon C-1′ (δ_C 98.9) in the HMBC spectra (see Fig. 2). The coupling constant of the anomeric proton was 7.8 Hz in doublet multiplicity in the ^{1}H NMR spectra which confirmed the β-configuration of glucose. The presence of a D-glucose unit was further confirmed by acid hydrolysis and gas chromatography (GC) analysis, followed by comparisons of retention times with authentic samples.[24] Next, the stereostructure of **1** was elucidated by careful examination of its 1D and 2D NMR spectra (Table 1). The broad singlet appearance of H-1 and the cross-peak between H-1(δ_H 5.60) and H-9 (δ_H 2.47) on COSY spectra confirmed the β-orientation of the H-9, since H-1 is known to be α-oriented in naturally occurring iridoid glycosides.[25,26] The stereochemistry of the ring fusion was *cis*, β-oriented which was also confirmed by the large coupling constant (*J* = 8.4 Hz) between H-9 (δ_H 2.47) and H-5 (δ_H 2.37). The ROESY (Fig. 2) spectra showed a correlation signal between H-7 and H-9 indicating the β-configuration of H-7, thus confirming the α-configuration of the hydroxyl group attached to C-7. Moreover, the ROESY correlation between H-10β (δ_H 3.57) and H-1 (δ_H 5.60) also confirmed the α-orientation of C-10 attached to C-8. The absence of olefinic proton signals in the ^{1}H NMR spectra indicated that no double bond existed between C-3 and C-4 as described for common iridoids.[25] In addition, the long range correlations between proton H-3 (δ_H 5.23) and carbon C-10 (δ_C 62.1), and between proton H-10 (δ_H 3.95, 3.57) and C-3 (δ_C 95.7) indicated an ether linkage between C-3 and C-10 and the α-configuration of C-3. Finally, the strong movement of the chemical shift ($\Delta\delta_{C\text{-}6}$ 9.0) at C-6 to the downfield of **1** ($\delta_{C\text{-}6}$ 95.0) in comparison with scutelloside[27] ($\delta_{C\text{-}6}$ 86.0) indicated a methoxy group attached to C-6. These differences were similar to those of methylcatalpol (**3**, $\delta_{C\text{-}6}$ 88.6) and catalpol[28] ($\delta_{C\text{-}6}$ 79.6; $\Delta\delta_{C\text{-}6}$ 9.0). Furthermore, the long range correlation signals between methoxy proton (δ_H 3.38, 6-OMe) and C-6 (δ_C 95.0), H-6 (δ_H 3.68) and methoxy carbon (δ_C 57.9, 6-OMe) in HMBC spectra also confirmed the methoxy group attached to C-6. The strereochemistry of the methoxy group at C-6, however, was determined to be the β-configuration due to the *trans*-orientation between protons H-6 and H-7 observed in the ^{1}H NMR spectra with a coupling constant of 7.8 Hz, and the ROESY correlation between H-6 (δ_H 3.68) and H-4α (δ_H 1.63). Based on the above data, compound **1** was determined to be a new compound named methylscutelloside (Fig. 1). In a preliminary assay of atherosclerosis treatment using *B. officinalis*, the isolated compounds were screened for their effects on rat aortic VSMCs proliferation.[29–31] The results showed that

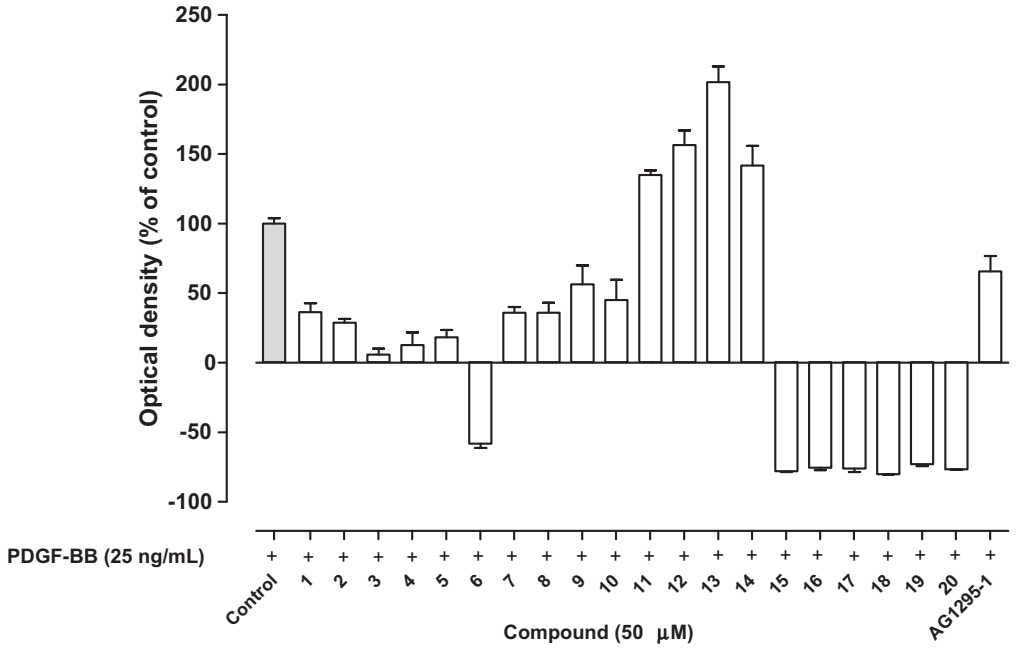

Figure 3. Effects of isolated compounds on PDGF-BB-induced proliferation in rat aortic VSMCs. AG1295 (1 μM), a PDGF-BB receptor inhibitor, was used as a positive control of anti-proliferative agents. Data are expressed as Mean ± SD (n = 4, P <0.05 vs stimulus control).

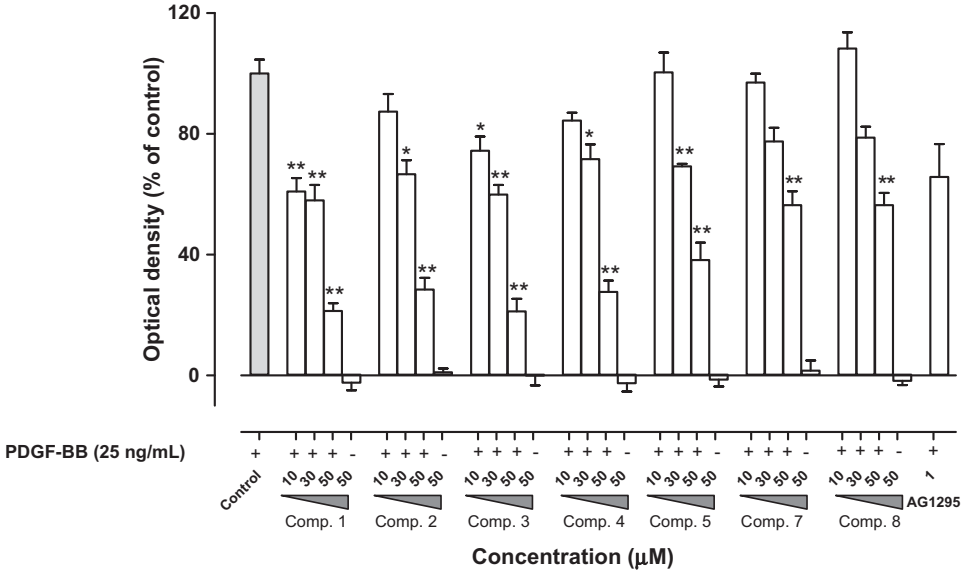

Figure 4. Effects of selected compounds on PDGF-BB-induced proliferation in rat aortic VSMCs. AG1295 (1 μM), a PDGF-BB receptor inhibitor, was used as a positive control of anti-proliferative agents. Data are expressed as Mean ± SD ($n = 4$, $^{*}P < 0.05$, $^{**}P < 0.01$ vs stimulus control).

most of compounds significantly inhibited VSMCs proliferation at a concentration of 50 μM (Fig. 3). Of these, compounds **1**–**5**, **7** and **8** showed the potent inhibitory effects, while compounds **9**–**14** did not show inhibitory effects on VSMCs proliferation after treatment with 25 ng/mL homodimer platelet-derived growth factor beta polypeptide (PDGF-BB). Compounds **6**, **15**–**20** showed the pattern of cytotoxic effects. Based on the above results, compounds **1**–**5**, **7** and **8** were then further tested for concentration-dependent effects on VSMCs (0–50 μM) as they showed no cytotoxicity at concentration of 50 μM (Fig. 4). Compound **1** significantly decreased cells proliferation at 10, 30 and 50 μM, with inhibition of 39.1%, 42.0% and 79.6%, respectively. Compounds **7** and **8** showed moderate inhibition by 22.6% and 21.2%, respectively, at a concentration of 30 μM. With the exception of compounds **1** and **3**, none of these compounds showed significantly inhibition effects on the proliferation of VSMCs at a concentration of 10 μM. These results clearly indicated that the iridoids from *B. officinalis* have potent inhibitory effects on rat aortic VSMC proliferation and may be useful for the treatment of atherosclerosis.

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23. Dried flowers of *B. officinalis* Maxim. (2.0 kg) were extracted with methanol at room temperature three times. After removal of the solvent under reduced pressure, the crude extract (94.37 g) was dissolved in 1.0 L of H_2O to form a suspension that was successively partitioned with dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc), and *n*-butanol (BuOH) to give CH_2Cl_2 (10.77 g), EtOAc (12.70 g), and BuOH (27.29 g) extracts, respectively. The BuOH extract was then subjected to column chromatography using SiO_2 (70–230 mesh), eluting with $Me_2CO/CHCl_3/H_2O$ (3:1:0.2, v/v/v) to give five fractions (**1a**–**e**). Repeated silica gel column chromatography of fraction **1a** with $CH_2Cl_2/MeOH/H_2O$ (4.5:1:0.1, v/v/v) and $Me_2CO/CHCl_3/H_2O$ (3.5:1:0.1) to give compounds **3** (13 mg) and **4** (18 mg). Fraction **1b** was then subjected to a silica gel column using an isocratic eluent of $CHCl_3/Me_2CO/MeOH/H_2O$ (3:2:1:0.1, v/v/v/v) and further by YMC column with $MeOH/H_2O$ (1:2, v/v) afford compounds **1**(9 mg), **2** (15 mg) and **5** (12 mg). Compounds **15** (14 mg), **16** (12 mg), and **19** (15 mg) were isolated from fraction **1d** using a silica gel column with $CH_2Cl_2/MeOH/H_2O$ (4:1:0.1, v/v/v), further by YMC column and eluting with Me_2CO/H_2O (2:1, v/v). Finally, fraction **1e** was chromatographed using a YMC column with $MeOH/H_2O$ (2:3, v/v) to afford five fractions (**2a**–**e**). Compounds **17** (90 mg), **18** (30 mg), and **20** (18 mg) were obtained from YMC gel column chromatography of sub-fractions **2d** and **2e**. The purity of isolated compounds was more than 97% by high-performance liquid chromatography (HPLC) analysis.
24. Compound **1** (2.0 mg) was dissolved in 1.0 N HCl (dioxane/H_2O, 1:1, v/v, 1.0 mL) and then heated to 80 °C in a water bath for 3 h. The acidic solution was neutralized with silver carbonate and the solvent thoroughly driven out under N_2 gas overnight. After extraction with ethyl acetate, the aqueous layer was concentrated to dryness using N_2 gas. The residue was dissolved in 0.1 mL of dry pyridine, and then L-cysteine methyl ester hydrochloride in pyridine

(0.06 M, 0.1 mL) was added to the solution. The reaction mixture was heated at 60 °C for 2 h, and 0.1 mL of trimethylsilylimidazole solution was added, followed by heating at 60 °C for 1.5 h. The dried product was partitioned with *n*-hexane and H_2O (0.1 mL, each), and the organic layer was analyzed by gas liquid chromatography (GC): Column: column SPB-1 (0.25 mm × 30 m); detector FID, column temp 210 °C, injector temperature 270 °C, detector temperature 300 °C, carrier gas He. The absolute configuration of the monosaccharide was confirmed to be D-glucose by comparison of the retention time of the monosaccharide derivative (t_R 14.11 min) with that of authentic sugar derivative samples prepared in the same manner (D-glucose derivative t_R 14.11 min, L-glucose derivative t_R 14.26 min).

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29. VSMC proliferation was assessed using the WST-1 (CCK-8) assay kit according to the manufacturer's instructions. In brief, cells were seeded at a concentration of 4×10^4 cells/well in 96-well plates and grown in Dulbecco's Modified Eagle Medium (DMEM) containing 10% FBS for 48 h. The cells were then cultured with serum-free medium containing various isolated compounds for 24 h. Cells were then stimulated with 5% FBS for another 24 h. WST-1 reagent was added to each well at 22 h, and the plate was further incubated the plate at 37 °C for 2 h. The optical density was read at 450 nm in an ELISA reader (Molecular, USA). Cell proliferation was measured by direct counting. Initially, the cells were seeded into 12-well culture plates at 4×10^4 cells/well and cultured in DMEM containing 10% FBS at 37 °C for 24 h. When cells reached up to 70% confluence, the medium was replaced with serum-free DMEM containing various concentrations (0–50 μM) of isolated compounds. After 24 h incubation, they were treated with 25 ng/ml PDGF-BB (Upstate Biotechnology, NY, USA) and further incubated for another 24 h. The cells per well were collected after treatment with trypsin-EDTA and then counted using a hemocytometer.
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