DOI 10.5012/bkcs.2010.31.04.929

# Chemical Constituents from *Acer mandshuricum* and Their Effects on the Function of Osteoblastic MC3T3-E1 Cells

**Yan Ding, Chun Liang, Huu Tung Nguyen, Eun Mi Choi, Jeong Ah Kim, and Young Ho Kim***

*College of Pharmacy, Chungnam National University, Daejeon 305-764, Korea. *E-mail: yhk@cnu.ac.kr*
*Received October 26, 2009, Accepted February 16, 2010*

A new compound, 4-methoxyl 5-hydroxymethyl benzoic 3-*O*-β-D-glucopyranoside (**1**), has been isolated from the leaves and stems of *Acer mandshuricum*, along with nine known compounds (**2**-**10**). Their structures were determined by a variety of spectroscopic analyses. The effect of compounds **1**-**10** on the function of osteoblastic MC3T3-E1 cells was examined by determining alkaline phosphatase (ALP) activity, collagen synthesis, and mineralization. Compound **1** significantly increased the function of osteoblastic MC3T3-E1 cells; 5.0 μM of **1** increased ALP activity, collagen synthesis, and mineralization of MC3T3-E1 cells to 114.7, 119.5, and 108.2% ($P < 0.05$) of the basal value, respectively. In addition, compounds **2**-**10** also potently increased the function of osteoblastic MC3T3-E1 cells.

**Key Words**: *Acer mandshuricum*, Aceraceae, 4-Methoxyl 5-hydroxymethyl benzoic 3-*O*-β-D-glucopyranoside, Osteoblast differentiation, MC3T3-E1 cell

## Introduction

Osteoporosis is a major health concern for aging communities. The associated progressive decrease in bone mass leads to an increased susceptibility to fractures, resulting in substantial morbidity and mortality.[1] Normal bone remodeling is achieved by a balance of bone formation and bone resorption. These processes are closely regulated and under the control of both systemic hormones and locally derived cytokines. Osteoblasts, typically located on bone-lining surfaces, are physically positioned to influence bone resorption. Since many osteoporotic patients have already lost a substantial amount of bone, a method of increasing bone mass by stimulating new bone formation is required.[2]

*Acer mandshuricum* MAXIM, belonging to Aceraceae, is a type of deciduous tree that grows in Korea, Russia, and the northern areas of China. *A. mandshuricum* is used as an ornamental tree and its breeding technique has been extensively studied in China. However, to date, there appears to be no study focusing on the phytochemistry and bioactivity of this plant.

In our ongoing efforts to find active natural compounds to differentiate MC3T3-E1 osteoblastic cells, potent activity was found in the MeOH extract of the stems and leaves of *A. mandshuricum*. Based on a bioactivity-guided fractionation and isolation method, one new compound (**1**) has been isolated, along with nine known compounds. This paper describes the isolation method and structure elucidation of 4-methoxyl 5-hydroxymethyl benzoic 3-*O*-β-D-glucopyranoside (**1**) along with the evaluation of the effects of compounds **1**-**10** on osteoblast differentiation in MC3T3-E1 cells.

## Experimental

**General methods.** Optical rotations: JASCO DIP-360 digital polarimeter; NMR spectra: Bruker DRX 400 and 500 NMR spectrometers using TMS as an internal standard, δ in ppm, *J* in Hz. HR-FAB-MS: JMS-T100TD spectrometer (Tokyo, Japan). GC spectra: Shidmazu-2010 spectrometer. Column chromatography (CC): silica gel 60 (40 - 63 and 63 - 200 μm particle size, Merck) and RP-18 (40 - 63 μm particle size, Merck).

**Plant material.** Stems and leaves of *Acer mandshuricum*, were collected in Kangwon Province in August 2005, and identified by one of us (Y. H. Kim). A voucher specimen (CNU05012) is deposited at the herbarium of the College of Pharmacy, Chungnam National University, Korea.

**Extraction and isolation.** Air-dried stems and leaves of *A. mandshuricum* (2.0 kg) were extracted three times with MeOH (50 °C). The MeOH extract (170 g) was concentrated under vacuum to yield a gummy residue that was suspended in $H_2O$ (2.0 L). This solution was partitioned with $CH_2Cl_2$ and EtOAc (each 2 L × 3), successively, yielding the $CH_2Cl_2$ fraction (74 g), EtOAc fraction (39 g), and the $H_2O$ fraction (35 g). The $H_2O$ soluble fraction was subjected to a Dianion HP 20 CC and eluted with $H_2O$ and 25, 50, 75, and 100% MeOH gradiently to give four fractions (Fr. 1A - 1D). The EtOAc soluble fraction was subjected to CC ($SiO_2$; $CH_2Cl_2$-MeOH 30:1 → 0:1) to afford five fractions (Fr. 2A - 2E). Fraction 1A was further separated by passage over a RP column (YMC-18; MeOH-$H_2O$ 2:3) to afford 2 subfractions (Fr. 3A and 3B). Compound **1** (400 mg) was isolated from fraction 3A by CC ($SiO_2$; $CH_2Cl_2$-MeOH 12:1). The combined fractions 3B and 1B were separated by CC ($SiO_2$; $CH_2Cl_2$-MeOH 4:1) to give five fractions (Fr. 4A - 4E). Fraction 4B was subjected to RP CC (YMC-18; MeOH-$H_2O$ 2:3) to give three subfractions (Fr. 5A - 5C). Compounds **2** (5.0 mg) and **4** (75 mg) were obtained from fraction 5B by CC ($SiO_2$; $CH_2Cl_2$-MeOH-$H_2O$ 4:1:0.5). Fraction 4D was subjected to RP CC (YMC-18; MeOH-$H_2O$ 2:3) to give compound **8** (36 mg). Next, the combined fractions of 1C and 1D were separated by CC ($SiO_2$; $CH_2Cl_2$-MeOH-$H_2O$ 10:1:0.1 → 2:1:0:1) to afford four fractions (Fr. 6A - 6D). Fraction 6A was subjected to RP CC (YMC-18; MeOH-$H_2O$ 1:1) to give three fractions (Fr. 7A-7C). Compound **3** (1500 mg) was purified from fraction 7B by crystallization in MeOH-$H_2O$ (2:3). Fractions 6B and 6C were subjected to RP CC (YMC-18; MeOH-$H_2O$ 1:1) to yield com-

pounds **5** (400 mg) and **9** (200 mg), respectively. In addition, compound **6** (1.5 mg) was obtained from fraction 2B using a RP CC (YMC-18; MeOH-$H_2O$ 2:1). Fractions 1C and 1D were separated using CC ($SiO_2$; $CH_2Cl_2$-MeOH-$H_2O$ 10:1:0.1 → 2:1:0:1) to afford four fractions (Fr. 8A - 8D). Fraction 8C was subjected to RP CC (YMC-18; MeOH-$H_2O$ 1:1) to yield compounds **7** (40 mg) and **10** (30 mg).

**4-Methoxyl 5-hydroxymethyl benzoic 3-*O*-β-D-glucopyranoside (1):** White amorphous powder; mp 193 - 194°; $[\alpha]_D^{20}$ −9.14 (*c* 0.20, MeOH); IR (KBr) 3375 (OH), 1703 (C=O), 1578, 1502, 1403 (aromatic C=C), 1052 (C-O-C); UV (MeOH) 210.6 (1.33), 255.4 (0.39); $^1$H-NMR ($CD_3OD$, 500 MHz) δ 7.23 (1H, d, *J* = 2.0 Hz, H-2), 7.15 (1H, d, *J* = 2.0 Hz, H-6), 4.87 (1H, d, *J* = 7.0 Hz, H-1'), 3.79 (6H, *s*, H-8, 9), 3.67 (1H, dd, *J* = 12.0, 2.0 Hz, H-6'a), 3.48 (1H, dd, *J* = 12.0, 6.0 Hz, H-6'b), 3.10 - 3.40 (4H, m, H-2', 3', 4', 5'); $^{13}$C-NMR ($CD_3OD$, 125 MHz) δ 165.8 (s, C-7), 151.1 (s, C-3), 150.7 (s, C-5), 141.6 (s, C-4), 124.5 (s, C-1), 111.3 (d, C-6), 108.7 (d, C-2), 101.3 (d, C-1'), 77.2 (d, C-5'), 76.7 (d, C-3'), 73.4 (d, C-2'), 69.6 (d, C-4'), 60.6 (t, C-6'), 60.3 (q, C-9), 52.1 (q, C-8); ESI-MS 383 $[M + Na]^+$; HR-FAB-MS 359.0975 $[M - H]^-$, (calcd. for $C_{19}H_{35}O_8$: 359.0978).

**(2*R*)-4-(4-Hydroxyphenyl)-2-butanol 2-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (2)[3]:** A colorless amorphous powder; $[\alpha]_D^{20}$ −49.23 (*c* 0.03, MeOH); $^1$H-NMR ($CD_3OD$, 500 MHz) δ 7.04 (2H, d, *J* = 8.5 Hz, H-2', 6'), 6.68 (2H, d, *J* = 8.5 Hz, H-3', 5'), 5.02 (1H, d, *J* = 2.0 Hz, H-1'''), 4.30 (1H, d, *J* = 8.0 Hz, H-1''), 3.86 (1H, m, H-2), 2.62 (2H, m, H-4), 1.84 (1H, m, H-3a), 1.68 (1H, m, H-3b), 1.19 (3H, d, *J* = 6.0 Hz, H-1); ESI-MS 459 $[M - H]^-$.

**Aceroside I (3)[4]:** Amorphous solid; $[\alpha]_D^{20}$ +13.51 (*c* 0.05, MeOH); $^1$H-NMR ($CD_3OD$, 500 MHz) δ 7.35 (1H, dd, *J* = 8.5, 2.0 Hz, H-15), 7.26 (1H, dd, *J* = 8.5, 2.0 Hz, H-19), 7.15 (1H, dd, *J* = 8.5, 2.5 Hz, H-16), 7.08 (1H, d, *J* = 8.2 Hz, H-3), 6.95 (1H, dd, *J* = 8.5, 2.5 Hz, H-18), 6.62 (1H, dd, *J* = 8.5, 2.0 Hz, H-4), 5.71 (1H, d, *J* = 2.0 Hz, H-6), 5.01 (1H, d, *J* = 8.0 Hz, H-1'), 3.20 (1H, dt, *J* = 10.0, 5.0 Hz, H-11), 2.96 (1H, dt, *J* = 13.0, 4.0 Hz, H-13a), 2.68 (1H, dt, *J* = 13.0, 4.0 Hz, H-13b), 2.44 (2H, m, H-7), 1.85 (1H, dq, *J* = 14.0, 4.0 Hz, H-12a), 1.60 (1H, ddt, *J* = 13.5, 13.5, 4.5 Hz, H-12b), 1.45 (1H, m, H-8a), 1.24 (1H, m, H-8b), 1.10 (1H, m, H-10a), 1.01 (1H, m, H-9a), 0.95 (1H, m, H-10b), 0.84 (1H, m, H-9b); ESI-MS 483 $[M + Na]^+$.

**Kaempferol 3-*O*-neohesperidoside (4)[5]:** Yellow powder; $[\alpha]_D^{20}$ −39.6 (*c* 0.10, MeOH); $^1$H-NMR ($CD_3OD$, 600 MHz) δ 8.05 (2H, d, *J* = 9.0 Hz, H-2', 6'), 6.89 (2H, d, *J* = 9.0 Hz, H-3', 5'), 6.38 (1H, d, *J* = 2.4 Hz, H-8), 6.18 (1H, d, *J* = 2.4 Hz, H-6), 5.74 (1H, d, *J* = 7.8 Hz, H-1''), 5.22 (1H, s, H-1'''); ESI-MS 595 $[M + H]^+$.

**Astragalin (5)[6]:** yellow power; $[\alpha]_D^{20}$ +16.0° (*c* 0.20, MeOH); $^1$H-NMR (acetone-$d_6$, 400 MHz) δ 8.11 (2H, d, *J* = 8.6 Hz, H-2', 6'), 6.96 (2H, d, *J* = 8.6 Hz, H-3', 5'), 6.49 (1H, d, *J* = 2.2 Hz, H-8), 6.26 (1H, d, *J* = 2.2 Hz, H-6), 5.25 (1H, d, *J* = 7.6 Hz, H-1''); ESI-MS 471 $[M + Na]^+$.

**Kaempferol (6)[7]:** Yellow power; $[\alpha]_D^{20}$ +11.8° (c 0.20, MeOH); $^1$H-NMR (acetone-$d_6$, 400 MHz) δ 8.09 (2H, d, *J* = 9.0 Hz, H-2', 6'), 6.91 (2H, d, *J* = 9.0 Hz, H-3', 5'), 6.40 (1H, d, *J* = 2.1 Hz, H-8), 6.19 (1H, d, *J* = 2.1 Hz, H-6); ESI-MS 287 $[M + H]^+$.

**Kaempferol-3-*O*-α-L-arabinofuranoside (7)[8]:** Pale yellow powder; $[\alpha]_D^{20}$ −40.8° (*c* 0.20, MeOH); $^1$H-NMR ($CD_3OD$, 500 MHz) δ 7.96 (2H, d, *J* = 9.0 Hz, H-2', 6'), 6.92 (2H, d, *J* = 9.0 Hz, H-3', 5'), 6.41 (1H, d, *J* = 2.0 Hz, H-8), 6.21 (1H, d, *J* = 2.0 Hz, H-6), 5.49 (1H, s, H-1''); ESI-MS 419 $[M + H]^+$.

**Quercetin 3-*O*-neohesperidoside (8)[5]:** Yellow powder; $[\alpha]_D^{20}$ +19.3 (*c* 0.10, MeOH); $^1$H-NMR ($CH_3OD$, 500 MHz) δ 7.60 (1H, dd, *J* = 8.0, 2.2 Hz, H-6'), 6.87 (1H, d, *J* = 8.0 Hz, H-5'), 6.62 (1H, d, *J* = 2.2 Hz, H-2'), 6.37 (1H, d, *J* = 2.0 Hz, H-8), 6.18 (1H, d, *J* = 2.0 Hz, H-6), 5.75 (1H, d, *J* = 8.0 Hz, H-1''), 5.23 (1H, d, *J* = 1.2 Hz, H-1'''); ESI-MS 611 $[M + H]^+$.

**Isoquercitrin (9)[6]:** Yellow power. $[\alpha]_D^{20}$ −22.5° (*c* 0.10, MeOH); $^1$H-NMR ($CD_3OD$, 500 MHz) δ 7.71 (1H, d, *J* = 2.5 Hz, H-2'), 7.58 (1H, dd, *J* = 8.5, 2.5 Hz, H-C(6')), 6.87 (1H, d, *J* = 8.5 Hz, H-5'), 6.39 (1H, d, *J* = 2.0 Hz, H-8), 6.20 (1H, d, *J* = 2.0 Hz, H-6), 5.25 (1H, d, *J* = 7.5 Hz, H-1''); ESI-MS 465 $[M + H]^+$.

**(-)-Catechin (10)[9]:** Pale yellow powder; $[\alpha]_D^{20}$ −11.4° (*c* 0.20, MeOH); $^1$H-NMR ($CD_3OD$, 500 MHz) δ 6.84 (1H, d, *J* = 1.5 Hz, H-2'), 6.77 (1H, d, *J* = 8.0 Hz, H-6'), 6.72 (1H, dd, *J* = 8.0, 1.5 Hz, H-5'), 5.94 (1H, d, *J* = 2.0 Hz, H-6), 5.87 (1H, d, *J* = 2.0 Hz, H-8), 4.57 (1H, d, *J* = 8.0 Hz, H-2), 3.98 (1H, dt, *J* = 8.0, 5.5 Hz, H-3), 2.85 (1H, dd, *J* = 16.0, 5.5 Hz, H-4a), 2.51 (1H, dd, *J* = 16.0, 8.0 Hz, H-4b); ESI-MS 291 $[M + H]^+$.

**Cell culture.** MC3T3-E1 cells (RCB1126, an osteoblast-like cell line from C57BL/6 mouse calvaria) were obtained from RIKEN Cell Bank (Tsukuba, Japan). MC3T3-E1 cells were cultured at 37° in a 5% $CO_2$ atmosphere in an α-modified minimum essential medium (α-MEM; GIBCO). The medium contained 10% heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin.

**Alkaline phosphatase (ALP) activity.** The cells were treated, at 90% confluence, with a culture medium containing 10 mM β-glycerophosphate and 50 μg/mL ascorbic acid, to initiate differentiation. The medium was changed every 2 - 3 d. After 8 d, the cells were cultured with a medium containing 0.3% BSA and samples individually for 2 d. On harvesting, the medium was removed and the cell monolayer was gently washed twice with PBS. The cells were lysed with 0.2% Triton X-100, with the lysate centrifuged at 14,000 × *g* for 5 min. The clear supernatant was used to measure the ALP activity, which was determined using an ALP activity assay kit (Asan Co. Korea).

**Collagen contents.** The cells were treated, at 90% confluence, with a culture medium containing 10 mM β-glycerophosphate and 50 μg/mL ascorbic acid. The medium was changed every 2 - 3 d. After 8 d, the cells were cultured with a medium containing 0.3% BSA and samples individually for 2 d. On harvesting, the medium was removed and the cell monolayer was gently washed twice with PBS. The collagen content was quantified by a Sirius Red-based colorimetric assay. The cultured osteoblasts were washed with PBS, followed by fixation with Bouin's fluid for 1 h. After fixation, the fixation fluid was removed and the culture dishes were washed by immersion in running tap water for 15 min. The culture dishes were air dried and stained by Sirius Red dye reagent for 1 h under mild shaking on a shaker. Thereafter, the solution was removed and the cultures were washed with 0.01 N HCl to remove any non-bound dye. The stained material was dissolved in 0.1 N NaOH. And the absorbance was measured at 550 nm.

**Calcium deposition assay.** The cells were treated, at 90% confluence, with a culture medium containing 10 mM β-glycero-

phosphate and 50 μg/mL ascorbic acid. After 12 d, the cells were cultured with a medium containing 0.3% BSA and samples individually for 2 d. Staining with Alizarin Red S is a standard method for the visualization of nodular patterns and calcium depositions of osteoblast cultures *in vitro*. At harvest, the cells were fixed with 70% ethanol for 1 h and stained with 40 mM Alizarin Red S for 10 min with gentle shaking. To quantify the bound dye, the stain was solubilized with 1 mL 10% (w/v) acetylpyridinum chloride for 15 min while shaking shielded from light. The absorbance of the solubilized stain was measured at 561 nm.

**Statistics.** Statistical analysis was performed using one-way ANOVA with SAS statistical software. Data are presented as mean ± S.E.M. (n = 3), and significance was accepted at $P < 0.05$.

## Results and Discussion

Compound **1** was obtained as a white amorphous powder with its molecular formula determined to be $C_{15}H_{20}O_{10}$ by HR-FAB-MS. The $^1$H-NMR spectrum revealed the presence of one β-glucopyranose unit from the anomeric proton at δ 4.87 (d, $J$ = 7.0 Hz), two methyl groups at δ 3.79 (s, 6H), and two aromatic protons at δ 7.23 (d, $J$ = 2.0 Hz) and 7.15 (d, $J$ = 2.0 Hz) of the aglycone moiety. The $^{13}$C-NMR spectrum of **1** indicated the presence of one sugar unit along with nine carbon signals for the aglycone moiety. These signals were assigned to two methyl signals, two methine signals and five quaternary carbons by HMQC spectra. In addition, the partial structures of **1** were confirmed from the HMBC spectrum; key correlations for this experiment are illustrated in Figure 2. The HMBC spectrum revealed correlations of H-8 at δ 3.79 to C-7 at δ 165.8 and H-9 at δ 3.79 to C-4 at δ 141.6. Moreover, the glycosidic linkages were determined from the following HMBC correlations` H-1' at δ 4.87 to C-3 at δ 151.1, indicating the linkage position with the glucose unit at C-3. The peak of the acid hydrolysate of **1** was detected at $t_R$ (min) 14.21 by GC analysis and identified as D-glucose through comparison with the retention times of the authentic samples after treatment with trimethylsilylimidazole in pyridine. Thus, the structure of **1** was determined to be 4-methoxyl 5-hydroxymethyl benzoic 3-*O*-β-D-glucopyranoside. A previous study[10] already reported this compound as a synthetic by-product, but no NMR data or any information of this structure was reported. Thus, this compound was here first isolated from nature.

The structures of other nine known compounds were determined by comparison of their spectroscopic data with those reported in the literature. All of them were isolated from *A. mandshuricum* for the first time, and compounds **2**, **6**, **8**, and **9** were found to the first time in the genus Acer.

To investigate the effect of the isolated compounds on bone metabolism, a cell culture system was employed in our study. MC3T3-E1 cells, derived from newborn mouse calvarias, display osteoblast-like characteristics.[11] ALP, collagen and mineralization are the most widely recognized biochemical markers for osteoblastic activity. Therefore, the effects of the isolated compounds on the ALP activity, collagen synthesis, and mineralization of the osteoblastic MC3T3-E1 cells were examined. Osteoblastic MC3T3-E1 cells, at confluence, were cultured with a differentiation-inducing medium and incubated in a medium containing the individual isolated compounds. Among the ten compounds tested, compounds **3**, **7**, and **8** significantly increased the ALP activity as shown in Figure 3. After treating with compounds **3** and **8** (5.0 - 20 μM), ALP activity was increased dose dependently to 122.7 and 119.8%, respectively, at a concentration of 20 μM ($P < 0.05$), compare to that of control (100%) and stronger than the positive control, 17β-estradiol (106.3%). However, compound **7** increased ALP activity up to 118.8% at a concentration of 5 μM, stronger than the positive control. Furthermore, their effects on collagen synthesis were evaluated using a Sirius Red-based colorimetric assay. As the results shown in Figure 4, compounds **1**, **2**, **3**, **4**, **7**, **8**, **9,** and **10** increased collagen synthesis. At a concentration of 5.0 μM, compounds **1**, **3**, **4**, and **10** significantly increased collagen synthesis up to 119.5, 117.5, 118.8, and 117.5%, respectively, compare to that of the control (100%) and stronger than the positive control, 17β-estradial (113.3%). Even if higher concentrations of the samples did not shown cytotoxic effects, they did not shown better effect to increase ALP activity than lower con-

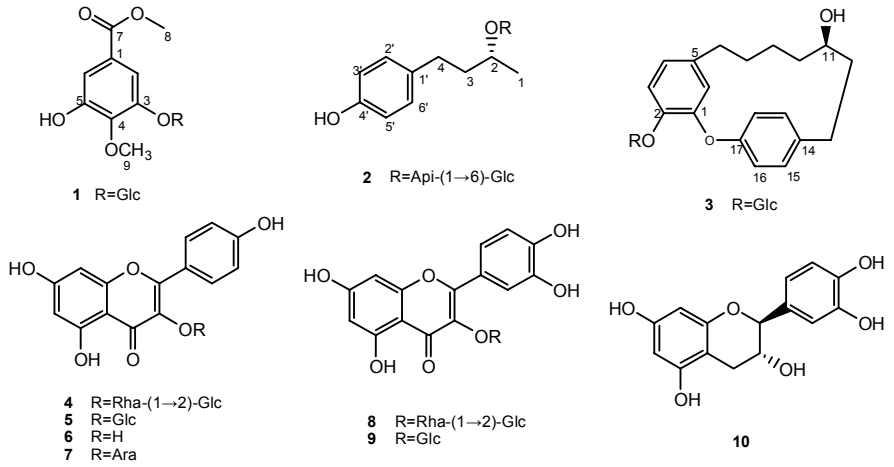


**Figure 1.** Structures of compounds **1**-**10**.

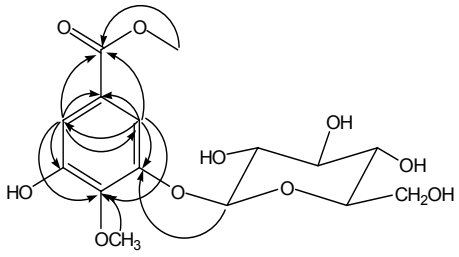


**Figure 2.** Selected HMBC correlations of compound **1.**

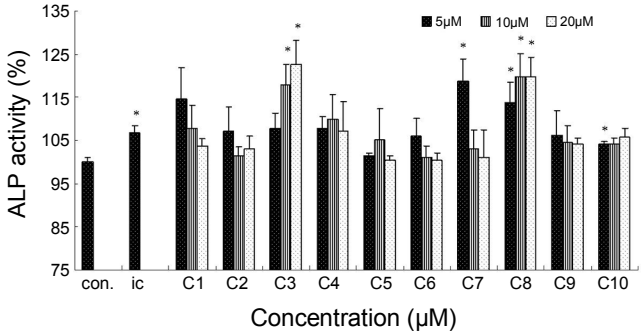


**Figure 3.** Effect of compounds **1-10** on the ALP activity of MC3T3-E1 Cells. Data were expressed as a percentage of control (2.9937 ± 0.3194 Unit/$10^6$cells), ic = inhibitor control (17β-estradiol). *$P < 0.05$ *vs*. control.

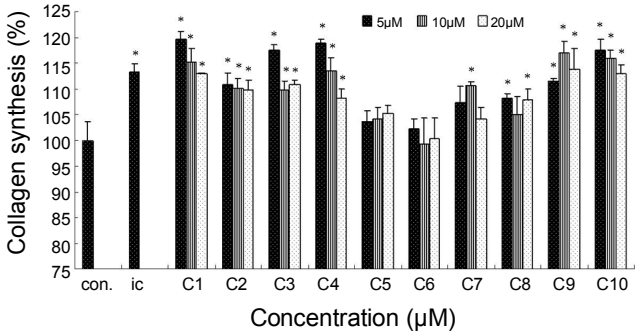


**Figure 4.** Effect of compounds **1-10** on the collagen synthesis of MC3T3-E1 Cells. Data were expressed as a percentage of control (0.183 ± 0.006 mg/$10^6$cells), ic = inhibitor control (17β-estradiol). *$P < 0.05$ *vs*. control.

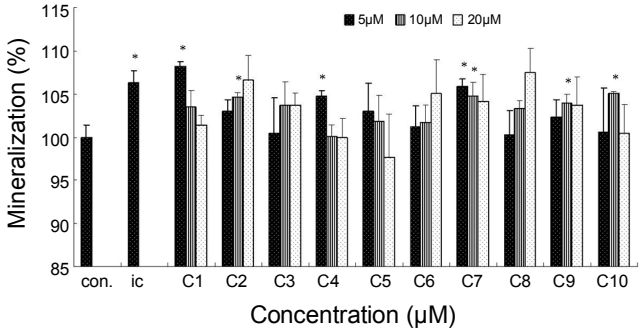


**Figure 5.** Effect of compounds **1-10** on the mineralization of MC3T3-E1 Cells. Data were expressed as a percentage of control (0.692 ± 0.040 O.D), ic = inhibitor control (17β-estradiol). *$P < 0.05$ *vs*. control.

centrations of the samples. The declined effect of some sample with higher concentration might be due to the characteristics of the samples and tested cells. In addition, compounds **2**, **7**, **8**, and **9** showed comparatively weaker effects. The effect of mineralization, another important process in differentiation, was then examined by measuring the calcium deposition by Alizarin Red staining. Compounds **2** and **8** dose-dependently increased mineralization to 106.6 and 107.5 at the concentration of 20 μM, respectively, compare to that of control (100%) and stronger than the positive control, 17β-estradiol (106.4%), as shown in Figure 5. However, compounds **1** and **7** showed increased mineralization to 108.2 and 105.9% at concentration of 5 μM, respectively, compare to that of control (100%). When cell viability was measured using the MTT assay, no toxic effects were observed after incubation of the cells with 5.0 to 20 μM of the ten compounds (data not shown). This result indicated that the tested compounds were not toxic at the concentrations used in this study. Each compound could increase cell viability of MC3T3-E1 cells.

Osteoblasts are the bone-forming cells of the skeleton synthesizing and regulating the deposition and mineralization of the extra-cellular matrix of the bone. MC3T3-E1 cells, an osteoblast-like cell line, have been reported to retain their capacity to differentiate into osteoblasts, and may provide very useful information about the effects of phytochemicals on the differentiation of osteoblasts.[12] These results indicated that compounds **1-4** and **7-10**, which could stimulate osteoblastic bone formation, may be useful for the prevention and treatment of osteoporosis. In addition, of the ten isolated compounds, compounds **4-10** belong to the flavonoid group. Recently, many researchers have reported that flavonoids showed good activity towards osteoporosis and inflammatory bone diseases.[13,14] In our work, the effects of isolated flavonoids compounds were carried out using a different cell line MC3T3-E1. Furthermore, the cell viability, ALP activity, collagen synthesis, and mineralization of MC3T3-E1 cell were studied systemically to check the effect of each sample. As a result, our data further indicated that these kinds of compounds could play an important role in the therapy of bone diseases.

**Acknowledgments.** This work was supported by the Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0093815). We are deeply grateful to the Korean Basic Science Institute (KBSI) for performing the NMR experiments.

## References

1. Riggs, B. L.; Melton, L. J., III. *N. Engl. J. Med.* **1992**, *327*, 620.
2. Hatano, T.; Yasuhara, T.; Yoshihara, R.; Ikegami, Y.; Matsuda, M.; Yazaki, K. *Planta Med.* **1991**, *57*, 83.
3. Hiroyuki, F.; Tetsuya, S.; Nobutoshi, T. *Chem. Pharm. Bull.* **1995**, *43*, 1937.
4. Inoue, T.; Ishidate, Y.; Fujita, M.; Kubo, M.; Fukushima, M.; Na-

gai, M. *Yakugaku Zasshi* **1978**, *98*, 41.
5. Kazuma, K.; Noda, N.; Suzuki, M. *Phytochemistry* **2003**, *62*, 229.
6. Han, J. T.; Bang, M. H.; Chun, O. K.; Kim, D. O.; Lee, C. Y.; Baek, N. I. *Arch. Pharm. Res.* **2004**, *27*, 390.
7. Chen, C. Y.; Hsieh, S. L.; Hsieh, M. M.; Hsieh, S. F.; Hsieh, T. J. *Chinese Pharm. J.* **2004**, *56*, 141.
8. Cai, Y. Z.; Xing, J.; Sun, M.; Zhan, Z. Q.; Corke, H. *J. Agric. Food Chem.* **2005**, *53*, 9940.
9. Park, K. M.; Yang, M. C.; Lee, K. H.; Kim, K. R.; Choi, S. U.; Lee, K. R. *Arch Pharm. Res.* **2006**, *29*, 1086.
10. Xie, J. X.; Wang, L.; Liu, C. X.; Zhang, D. Y. *Yaoxue Xuebao* **1981**, *16*, 425.
11. Stein, G. S.; Lian, J. B. *Endocr. Rev.* **1993**, *14*, 424.
12. Kodama, H. A.; Suda, Y.; Kasai, H.; Yamamoto, S. *Jpn. J. Oral. Biol.* **1981**, *23*, 899.
13. Wang, X. L.; Wang, N. L.; Zhang, Y.; Gao, H.; Pang, W. Y.; Wong, M. S.; Zhang, G.; Qin, L.; Yao, X. S. *Chem. Pharm. Bull.* **2008**, *56*, 46.
14. Yamaguchi, M.; Hamamoto, R.; Uchiyama, S.; Ishiyama, K. *Mol. Cell Bio.* **2007**, *303*, 83.