Bioorganic & Medicinal Chemistry Letters 20 (2010) 1528–1531

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Triterpene compounds isolated from *Acer mandshuricum* and their anti-inflammatory activity

Yan Ding [a], Chun Liang [a], Jun Ho Kim [b], Young-Mi Lee [b], Jae-Hee Hyun [c], Hee-Kyoung Kang [c], Jeong-Ah Kim [a], Byung Sun Min [d], Young Ho Kim [a,*]

[a] College of Pharmacy, Chungnam National University, Daejeon 305-764, Republic of Korea
[b] Department of Oriental Pharmacy, College of Pharmacy, Wonkwang University, Iksan, Jeonbuk 570-749, Republic of Korea
[c] Department of Pharmacology, School of Medicine, Cheju National University, Jeju 690-756, Republic of Korea
[d] College of Pharmacy, Catholic University of Daegu, Gyeongbuk 712-702, Republic of Korea

ARTICLE INFO

Article history:
Received 25 September 2009
Revised 23 December 2009
Accepted 16 January 2010
Available online 25 January 2010

Keywords:
Acer mandshuricum
Aceraceae
Aceranol acetate
Triterpene
Sterol
Cytotoxic
Anti-inflammatory
TNF-α

ABSTRACT

In our preliminary screening study on the anti-inflammatory activity, a new triterpene compound, aceranol acetate (**1**), was isolated along with five known compounds: β-amyrin acetate (**2**); glutinol acetate (**3**); friedelin (**4**); glutinol (**5**); (3β)-D-glucopyranoside-stigmast-5-en-3-yl (**6**), from the stems and leaves of *Acer mandshuricum*. The structure of the new triterpene was determined to be 5α,6α-epidioxy-5β,6β-epoxy-9,13-dimethyl-25,26-dinoroleanan-3β-ol acetate by spectroscopic studies. Compounds **2**–**6** were isolated from this plant for the first. Five triterpene compounds (**1**–**5**) showed significant cytotoxic activity with GI_{50} in the range of 11.1–17.9 μM, whereas steroid compound (**6**) exhibited moderate activity against four human cancer cell lines (HL-60, SK-OV-3, A549, and HT-29). Furthermore, the anti-inflammatory effects of compounds **1**–**6** in the non-cytotoxic concentrations (1–100 nM) were evaluated for the inhibitory activity of TNF-α secretion in the lipopolysaccharide (LPS)-stimulated murine RAW264.7 macrophage cell line. Among the compounds tested, compound **2** showed the strongest anti-inflammatory activity with the inhibition rate up to 38.40% at the concentration of 100 nM, whereas other five compounds (**2**–**6**) exhibited moderate activity.

Acer mandshuricum Maxim, which belongs to Aceraceae family, is a type of deciduous tree that grows in Korea, Russia, and the northern areas of China. It is typically used as an ornament and its breed technique has been studied in China.[1] In our investigation into biologically active compounds from medicinal plants, five triterpenes (**1**–**5**) and one sterol (**6**) (Fig. 1), were isolated from the CH_2Cl_2 layer of a MeOH extract of *A. mandshuricum*. Among them, compound **1** was determined as a new compound, named aceranol acetate, and five known compounds **2**–**6** were isolated for the first time from this plant.

Inflammatory cytokines are produced by innate immune cells, such as macrophage and dendritic cells, during infection. Lipopolysaccharide (LPS) is an endotoxin, which induces septic shock syndrome and stimulates the production of inflammatory mediators such as NO, tumor necrosis factor-α (TNF-α), interleukins, prostanoids and leukotrienes.[2] Among these, TNF-α activates various cells and induces cell death/survival, differentiation, proliferation, and migration.[3] TNF-α is like a double-edged sword in that it has both beneficial and harmful physiological functions. The beneficial functions of TNF-α are the killing of tumor cells, haematopoiesis, and protection from infection. On the other hand, its harmful functions are tumorigenesis, induction of autoimmunity, and linking with diseases. Increased TNF-α concentrations are co-related with many diseases, such as transplant rejection, rheumatoid arthritis, heart failure, type II diabetes, and atherosclerosis.[4] Therefore, the reduction of the secretion of TNF-α will greatly help in the treatment or mitigation of chronic inflammatory diseases like infection, rheumatic arthritis, rhinitis, and allergy.[5]

Many of the triterpenoids[6,7] and sterol analogs[8,9] were previously reported to have anti-inflammatory activities by suppressing the secretion of inflammatory cytokines, such as TNF-α. Therefore, the effects of the compounds isolated from *A. mandshuricum* on the inhibitory activity of TNF-α secretion in the presence of LPS in a murine RAW264.7 macrophage cell line within non-cytotoxic concentrations (1–100 nM) were reported herein.

Air-dried stems and leaves of *A. mandshuricum* (2 kg)[10] were extracted three times with MeOH at 50 °C. The MeOH extract (170 g) was suspended in H_2O (3 L) and partitioned with CH_2Cl_2 (2 L × 3) and EtOAc (2 L × 3) to give 74 g of a CH_2Cl_2-soluble fraction, 39 g of a EtOAc-soluble fraction and 35 g of an H_2O-soluble, respectively. This CH_2Cl_2 fraction was fractionated over a silica gel column using a stepwise gradient of hexane–EtOAc (100:0–0:1; v/v), followed by silica gel and YMC reversed-phase column

* Corresponding author. Tel.: +82 42 821 5933; fax: +82 42 823 6566.
E-mail address: yhk@cnu.ac.kr (Y.H. Kim).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.01.096

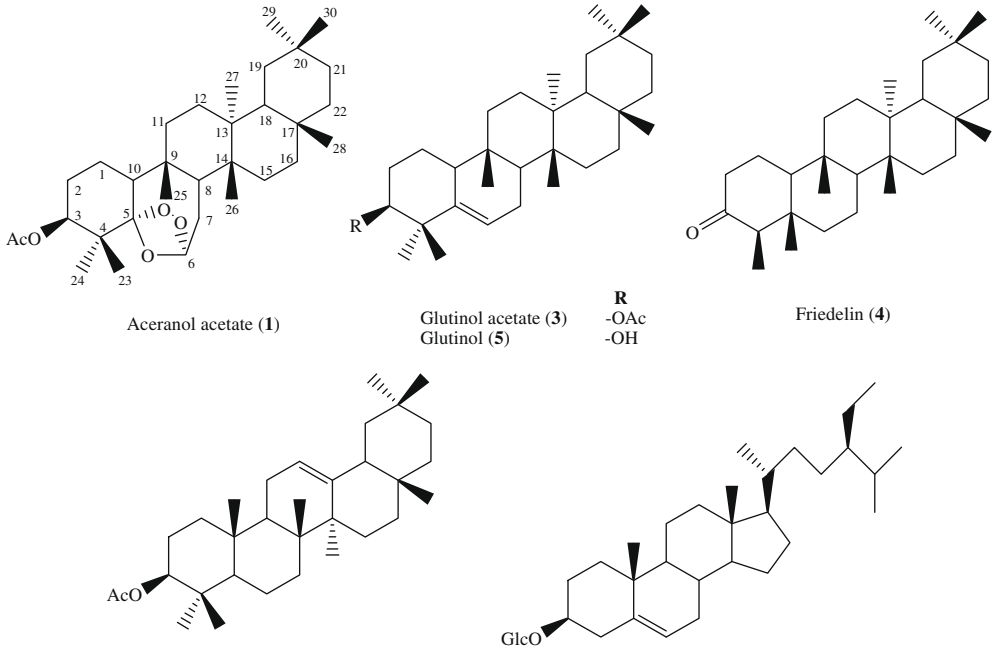

Figure 1. Structures of compounds **1**–**6**.

chromatography[11] to yield one new triterpene compound, named aceranol acetate (**1**), along with five known ones including β-amyrin acetate (**2**),[12] glutinol acetate (**3**),[13] friedelin (**4**),[14] glutinol (**5**),[13] and (3β)-D-glucopyranoside-stigmast-5-en-3-yl (**6**).[15]

The structure of compound **1**[16] were completely determined through spectroscopic analysis, using ^{1}H and ^{13}C NMR, COSY, HMQC, HMBC, and MS spectral data, and were compared with the data in the literature. Compound **1** was obtained as a white amorphous powder. The HRFABMS displayed the $[M-H]^-$ at *m/z* 515.3728 ($C_{32}H_{52}O_5$, calcd 515.3737). The ^{1}H NMR spectrum showed nine methyl signals at δ_H 2.09 (s), 1.15 (s), 1.13 (s), 1.09 (s), 1.06 (s), 1.02 (s), 0.99 (s), 0.97 (s), and 0.96 (s), an oxygenated methine proton at δ_H 4.75 (t, J = 4.0 Hz), and an hemiacetal proton as a broad singlet at δ_H 5.69 (Table 1). In addition, the ^{13}C NMR data indicated the presence of an acetate group along with 30 carbon signals, giving the evidence of a triterpene skeleton. These signals were assigned to eight methyl signals, ten methylene signals, five methane signals, and seven quaternary carbons based on the DEPT-135 spectrum. These signals were similar to those of glutinol acetate (**3**),[13] except for two signals at δ_C 113.3 and 104.2, which were assigned to C-5 and C-6 by analysis of HMBC spectral data (Fig. 2). Higher field moving of these two signals indicated that the two carbon atoms were doubly substituted by oxygen which is supported by those of 5,7α-epidioxy-5α-B-homo-6-oxacholestan-3β-ol.[17] Thus, analysis of the two-dimensional NMR data of compound **1** revealed its structure to be 5,6-epidioxy-5,6-epoxy-9,13-dimethyl-25,26-dinoroleanan-3β-ol acetate. From the coupling patterns of the adjacent well-resolved proton signals in the ^{1}H NMR spectrum, the H-6 at δ_H 5.69 appeared a broad singlet indicating that its orientation is equatorial. The H-8α at δ_H 2.76 was remarkably shifted downfield by the anisotropic effect of the oxygen function at C-6 in diaxial correlation and its splitting pattern (double doublet, J = 1.5 and 12.0 Hz) suggested the stereochemical correlation between H-8α, H-7α, and H-7β; their coupling constants were $J_{8\alpha,7\alpha}$ = 1.5 Hz and $J_{8\alpha,7\beta}$ = 12.0 Hz, respectively. These characteristic features can only be satisfied by a 5α,6α-epidioxy-5β,6β-epoxy structure which was consistent with those of adian-5-ene ozonide.[18] Furthermore, the strong NOE correlation between H-6/H-7β and H-3/H-7α also supported this structure. On the basis of the evidences, the structure of compound **1** was determined to

Table 1
^{1}H and ^{13}C NMR spectral data of compound **1**

Pos.	δ_C[a,b]	DEPT	δ_H[a,c] (J in Hz)	HMBC
1	25.2	CH_2	1.87 m, 1.64 m	10
2	19.7	CH_2	1.86 m, 1.60 m	
3	77.4	CH	4.75 t (4.0)	1, 2, 4, 5, –CO
4	43.8	C		
5	113.3	C		
6	104.2	CH	5.69 br s	5, 7, 8
7	32.6	CH_2	1.89 m, 1.76 m	6, 8, 9, 14
8	44.2	CH	2.76 dd (12.0, 1.5)	6, 7, 9, 14, 15, 25, 26
9	39.3	C		
10	52.1	CH	1.73 m	1, 2, 5, 9, 11
11	37.7	CH_2	1.62 m, 1.52 m	
12	30.3	CH_2	1.32 m, 1.27 m	
13	39.2	C		
14	39.3	C		
15	34.3	CH_2	1.57 m, 1.39 m	
16	36.3	CH_2	1.64 m, 1.31 m	
17	30.3	C		
18	43.0	CH	1.54 m	
19	35.5	CH_2	1.37 m, 1.19 m	20
20	28.5	C		
21	33.0	CH_2	1.47 m, 1.29 m	
22	39.4	CH_2	1.52 m, 0.93 m	
23	24.3	CH_3	1.06 s	3, 4, 5, 24
24	19.6	CH_3	0.97 s	3, 4, 5, 23
25	20.3	CH_3	1.13 s	8, 9, 10, 11
26	20.6	CH_3	1.09 s	8, 14, 15
27	19.0	CH_3	1.02 s	12, 14, 18
28	31.7	CH_3	1.15 s	16, 17, 18
29	32.0	CH_3	0.99 s	20, 21, 30
30	35.0	CH_3	0.96 s	19, 20, 21, 29
CO	171.0	C		
CH_3CO	21.5	CH_3	2.09 s	–CO

[a] Measured in $CDCl_3$.
[b] at 125 MHz.
[c] at 500 MHz.

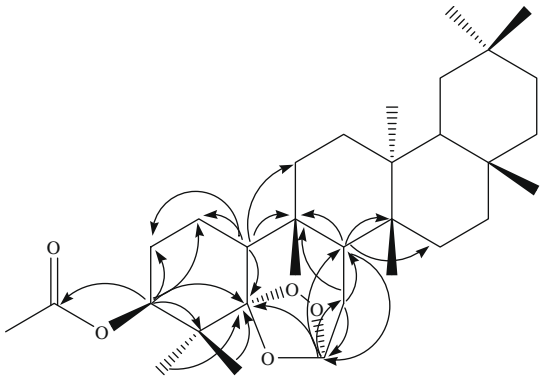

Figure 2. Selected HMBC correlations of compound **1**.

Table 2
The effects of compounds **1**–**6** on the growth of human cancer cells

Compounds	GI_{50}[a] (μM)			
	HL-60 (Leukemia)	SK-OV-3 (Ovary)	A549 (Lung)	HT-29 (Colon)
1	13.7 ± 1.2	12.8 ± 0.9	11.4 ± 0.9	14.6 ± 1.3
2	14.6 ± 1.3	14.6 ± 1.3	11.4 ± 0.9	12.5 ± 1.1
3	16.2 ± 1.6	12.1 ± 0.9	17.9 ± 1.6	16.3 ± 1.3
4	12.2 ± 1.1	12.7 ± 1.0	11.1 ± 0.9	13.5 ± 1.1
5	16.0 ± 1.6	12.6 ± 0.9	11.6 ± 0.9	14.1 ± 1.3
6	50.8 ± 3.0	29.8 ± 2.1	35.5 ± 2.7	46.8 ± 2.6
MX[b]	7.8 ± 0.8	11.0 ± 1.0	8.3 ± 0.8	8.9 ± 0.8

[a] Results were obtained from triplicate experiments. Data are represented as a mean ± SEM.
[b] Mitoxantrone was used as positive control.

be 5α,6α-epidioxy-5β,6β-epoxy-9,13-dimethyl-25,26-dinorolean-3β-ol acetate, which was named aceranol acetate (**1**).

Cytotoxic activity of compounds **1**–**6** were evaluated against human acute promyeloid leukemia (HL-60), human ovarian cancer (SK-OV-3), human lung adenocarcinoma epithelial (A549), and human colon cancer (HT-29) cell line using 3-(dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, as described by Mosmann.[19] Data were shown in Table 2. Among of six compounds tested in the present experiment, five triterpene compounds (**1**–**5**) showed significant cytotoxic activity against all cell lines with GI_{50} values in the range of 11.1–17.9 μM. β-Amyrin acetate (**2**) was reported to have cytotoxicity against A2780 ovarian cancer cell line with IC_{50} of 12.1 μg/ml which is very similar value of our MCF-7 breast cancer cell line.[20] Furthermore, steroid compound (**6**) was first reported to show moderate growth inhibitory effects with GI_{50} values of 50.8, 29.8, 35.5, and 46.8 μM against HL-60, SK-OV-3, A549, and HT-29 cell lines, respectively.

Many of the inflammation can be modulated by the cytokines secreted from immune defense cells. Innate immune cells, such as macrophage or dendritic cell, secrete diverse cytokines for defense modulation. During the inflammation stage, some of the cytokines, such as IL-1, IL-6, and TNF-α, are increased, while others, such as IL-10, are decreased by the macrophage cells. To study the anti-inflammatory activity of each isolated compound, the regulation of inflammatory cytokine secretion was assayed in non-cytotoxic concentrations within the range of 1–100 nM. A murine macrophage RAW264.7 cell line was used for the measurement of secreted inflammatory cytokines of TNF-α in the absence or presence of those compounds isolated from *A. mandshuricum* using inflammatory cytokine assay.[21]

As shown in Figure 3, when the RAW264.7 cells were cultured with each of the compounds isolated from *A. mandshuricum*, the secretion of TNF-α was obviously decreased to 38.4% of that in the LPS-treated control group by the treatment with compound **2** at 100 nM concentrations. Compounds **3**, **4**, and **6**, however, inhibited the TNF-α to 25.4%, 23.5%, and 29.3%, respectively, in the LPS-treated control group, at concentrations of 100 nM. However, compounds **1** and **5** showed relatively weak activity, inhibiting the secretion of TNF-α to 21.0%, and 16.9%, respectively, at concentrations of 100 nM. In terms of structure–activity relationships in this assay, two types of compounds, both triterpene and sterol, showed inhibitory effects on TNF-α secretion in the range of non-cytotoxic concentrations. Although further investigation are required to clarify the detailed relationship with the active compounds and their inhibitory effects on TNF-α secretion, the observation on previous reports[22] prompts us to propose that of the active compounds, 3-acetyl 12-en oleanane (**2**), 3-acetyl 5-en glutinane (**3**), and 3-one friedelane (**4**) can be regarded as sources of potential candidates for anti-inflammatory agents.

Acknowledgments

This work was supported by Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded

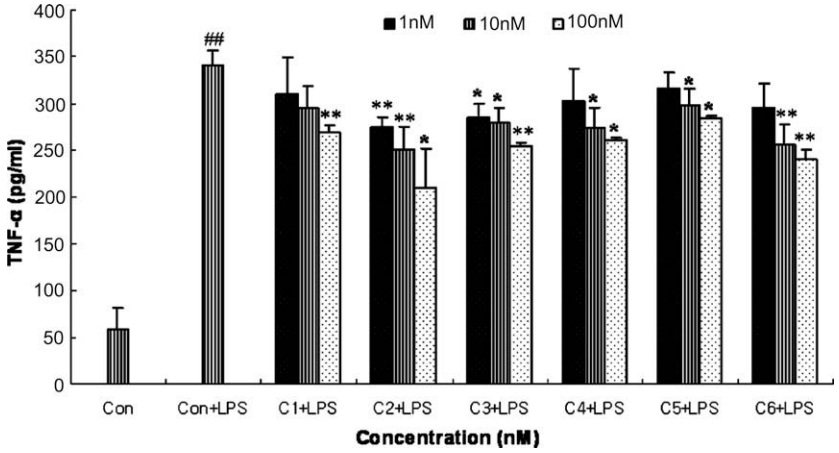

Figure 3. Effects of the compounds isolated from *A. mandshuricum* on TNF-α secretion in the murine RAW264.7 macrophage cell line. The cells in triplicate were treated with each of the compounds at the concentrations of 1, 10, and 100 nM in the 24-well culture plates for 24 h, in the presence of 500 ng/ml LPS. The control group cells (Con) were cultured without LPS and the compounds. Each value is mean ± S.D. ($n = 3$). * Significantly different from the control group, $^{\#\#}p$ <0.01 compared to the control. $^{*}p$ <0.05, $^{**}p$ <0.01 compared to LPS only stimulation.

by the Ministry of Education, Science and Technology (2009-0093815). The authors also wish to thank Korea Basic Science Institute (KBSI) for the NMR analysis.

References and notes

1. Cao, L.; Wang, Q. C.; Cui, D. H. *Yingyong Shengtai Xuebao* **2006**, *17*, 769.
2. Watson, W. H.; Zhao, Y.; Chawla, R. K. *Biochem. J.* **1999**, *342*, 21.
3. Janeway, C. A.; Travers, P.; Walport, M.; Shlomchik, M. J. *Immunobiology: the Immune System in Health and Disease*; Garland Science: New York, 2005.
4. Aggarwal, B. B. *Nat. Rev. Immunol.* **2003**, *3*, 745.
5. Bradley, J. R. *J. Pathol.* **2008**, *214*, 149.
6. Cho, J. Y.; Yoo, E. S.; Cha, B. C.; Park, H. J.; Rhee, M. H.; Han, Y. N. *Planta Med.* **2006**, *72*, 1279.
7. Thimmulappa, R. K.; Fuchs, R. J.; Malhotra, D.; Scollick, C.; Traore, K.; Bream, J. H.; Trush, M. A.; Liby, K. T.; Sporn, M. B.; Kensler, T. W.; Biswal, S. *Antioxid. Redox Signal.* **2007**, *9*, 1963.
8. Akihisa, T.; Nakamura, Y.; Tagata, M.; Tokuda, H.; Yasukawa, K.; Uchiyama, E.; Suzuki, T.; Kimura, Y. *Chem. Biodivers.* **2007**, *4*, 224.
9. Abdel-Ghani, A. E.; Dora, G. A. *Mansoura J. Pharm. Sci.* **2004**, *20*, 104.
10. *Sample preparation*: stems and leaves of *A. mandshuricum* were collected in Kangwon Province in August 2005, and identified by Prof. KiHwan Bae, College of Pharmacy, Chungnan National University. A voucher specimen (CNU05012) is deposited at the herbarium of the College of Pharmacy, Chungnam National University, Korea.
11. *Isolation*: this CH_2Cl_2 fraction was subjected to silica gel column chromatography (CC) using a stepwise gradient of hexane/EtOAc (100:0–0:1; 500 mL each), to yield five fractions (2A–2E). Fraction 2B was subjected to silica gel CC with a stepwise gradient of hexane/EtOAc (40:1–25:1–20:1–5:1) elution solvent to give 11 fractions (3A–3K). Compound **2** (15 mg) was purified from fraction 3B by crystallization in acetone/MeOH (2:3). Fraction 3C was separated by reverse phase CC with a MeOH/acetone (3:1) elution solvent and gave compound **3** (45 mg). Compound **4** (6 mg) was purified from fraction 3E by crystallization in MeOH. Fraction 3G was separated by reverse phase CC with a MeOH/acetone (10:1) elution solvent and gave compound **1** (2 mg). Combined fractions 3I and 3J were separated by reverse phase CC with a MeOH/acetone (10:1) elution solvent and gave compound **5** (8 mg). Fraction 2B was subjected to silica gel CC with a stepwise gradient of CH_2Cl_2/MeOH (30:1–20:1 vs 10:1) elution solvent to give three fractions (4A–4C). Fraction 4B was separated by reversed phase CC with a MeOH/acetone (5:1) elution solvent and gave compound **6** (9 mg).
12. Choi, S. Z.; Choi, S. U.; Lee, K. R. *Arch. Pharm. Res.* **2004**, *27*, 164.
13. El-Seedi, H. R. *Nat. Prod. Res.* **2005**, *19*, 197.
14. David, J. M.; Santos, F. A.; Guedes, M. L. D. S.; David, J. P. *Quim. Nova* **2003**, *26*, 484.
15. Rahman, A.; Ahmed, B.; Ali, M.; Khan, N. Z. *Indian J. Chem.* **2003**, *42B*, 1183.
16. Aceranol acetate (**1**): White powder; $[\alpha]_D^{20}$ + 31.2 (*c* 0.04, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; FABMS *m*/*z*: 517.4 $[M+H]^+$; HRFABMS: $m/z = 515.3728$ $[M-H]^-$, (calcd for $C_{32}H_{52}O_5$: 515.3737).
17. Jaworski, K.; Smith, L. L. *J. Org. Chem.* **1988**, *53*, 545.
18. Ageta, H.; Shiojima, K.; Kamaya, R.; Masuda, K. *Tetrahedron Lett.* **1978**, *10*, 899.
19. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55.
20. Chaturvedula, V. S.; Schilling, J. K.; Miller, J. S.; Andriantsiferana, R.; Rasamison, V. E.; Kingston, D. G. *J. Nat. Prod.* **2002**, *65*, 1222.
21. *Inflammatory cytokine assay*: RAW264.7 cells were cultured at 2×10^5 cells/mL in DMEM containing 10% fetal bovine serum in 24-well tissue culture plates (500 L/well). The cells were pretreated with 1, 10, and 100 nM concentrations of compound **1h** before LPS stimulation. Twenty four hours after LPS stimulation, TNF-α levels in the supernatant were measured by ELISA according to the commercial instruction (R&D System, Minneapolis, MN). Each of the 1-mL culture supernatants was transferred to an Eppendorf tube and was spun down for 3 min at 1000 rpm and used for cytokine assay. For the cytokine assay using the sandwich method, a capture antibody (1:250 dilution) solution was incubated overnight at 4 °C in 96-well plates. The plates were washed and blocked with assay diluent (10% FBS in PBS) for 1 h at room temperature, and then 100 μL of the culture supernatants was added to the wells and was incubated at room temperature. After 2 h, the plates were washed and incubated for 1 h with a detection antibody solution (1:250 dilution), biotinylated anti-mouse monoclonal antibody against TNF-α, together with a Strepavidin–horseradish peroxidase conjugate solution (1:250 dilution). The plates were washed and incubated for 30 min with a 1:1 mixture of tetramethylbenzidine (TMB) and hydrogen peroxide. A stop solution was then added to the plates, and the optical density of 450 nm was read. The concentrations of the cytokines were calculated using the cytokines' standard calibration curve.
22. Khanna, D.; Sethi, G.; Ahn, K. S.; Pandey, M. K.; Kunnumakkara, A. B.; Sung, B.; Aggarwal, A.; Aggarwal, B. B. *Curr. Opin. Pharmacol.* **2007**, *7*, 344.