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Letter

Diacylglycerol Compounds from Barks of *Betula platyphylla* with Inhibitory Activity against Acyltransferase

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ABSTRACT

Objective To identify the active compounds from the barks of *Betula platyphylla* for inhibitory on diacylglycerol acyltransferase (DGAT1). **Methods** Bioassay-guided fractionation resulted in the isolation of DGAT1 inhibitory activity of lupane triterpenes. **Results** Ten compounds were identified as lupenone (**1**), lupeol (**2**), betulinic acid (**3**), betulinaldehyde (**4**), betulin (**5**), 3-deoxybetulonic acid (**6**), glochidonol (**7**), lup-20/29-ene-1β/3β-diol (**8**), 3α-hydroxy-lup-20(29)-en-23,28-dioic acid (**9**), and 3α,11α-dihydroxy-23-oxo-lup-20(29)-en-28-oic acid (**10**). Compounds **3**-**6**, **9**, and **10** inhibited DGAT1 with IC_{50} values ranging from (11.2 ± 0.3) to (38.6 ± 1.2) μmol/L. **Conclusion** Compounds **6**, **9**, and **10** are first isolated from the barks of *B. platyphylla*., and compounds **3**-**6**, **9**, and **10** from the barks of *B. platyphylla* are responsible for the inhibition on DGAT1.

Key words

Betucaseae; *Betula platyphylla*; diacylglycerol acyltransferase1; lupane triterpenes

1. Introduction

Triglyceride (TG) is the most representative storage form of energy in human body. However, excessive accumulation of TG in certain tissues can induce some serious diseases such as obesity, type II diabetes mellitus (DM), and metabolic syndrome (Lehner and Kuksis, 1996). TG synthesis has been assumed to occur primarily through diacylglycerol acyltransferase (DGAT), which catalyses the final and only committed step in the glycerol phosphate pathway (Cases et al, 1998). Two enzymes that display DGAT activity have been characterized as DGAT1 and DGAT2 (Cases et al, 2001). Mice that lacked DGAT1 ($Dgat1^{-/-}$ mice) were viable and had reduced TG levels in their tissues, including white adipose tissue. These findings suggested that specific components with the inhibitory activity against DGAT1 might be a more feasible therapeutic strategy in combating human obesity and type II DM.

In the course of searching for DGAT1 inhibitors from medicines, *Betula platyphylla* Suk. was selected as an active natural resource. *B. platyphylla*, belonging to the Betucaseae family, is a monoecious deciduous tree that widely spreads in the northeast of China, the eastern of Siberia, North Korea, and Japan. The barks of *B. platyphylla* have been used in folk medicine for the treatment of arthritis, cancer, nephritis, dermatitis, poisoning, and chronic bronchitis. It has been reported that an extract of *B. platyphylla* has anti-oxidative and anticancer activities (Cho et al, 2006). Triterpenoid, extracted from the barks of *B. platyphylla*, is an excellent drug with antiviral, antibacterial, antitumor, and anti-AIDS activities (Fan et al, 2011).

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2. Materials and methods

2.1 Materials and instruments

The whole sample of *Betula platyphylla* Suk. was purchased from The Herbal Medicine Association of Taejon, Korea and was identified by Prof. Ki-hwan Bae, College of Pharmacy, Chungnam National University, and the voucher specimen (No. 06103) was deposited at the College of Pharmacy, Beihua University, Jilin, China.

NMR spectra were recorded at Bruker-Avance II 500 Instrument (^{1}H-NMR 500 MHz, ^{13}C-NMR 125 MHz), with tetramethysilane as internal standard. All accurate mass experiments were performed on a Micromass QTOF2 Mass Spectrometer (Micromass, Wythenshawe, UK). Spots were visualized using UV light or 10% sulfuric acid. Positive phase silica gel was made by National Medicine Group Chemical Reagent Co.

2.2 Extraction and isolation

The barks of *B. platyphylla* (9.0 kg) were finely cut and extracted with MeOH at 50 ^{o}C. After evaporation of the solvents under reduced pressure, the residue (1.8 kg) was suspended in water, and then successively partitioned with CH_2Cl_2 and *n*-BuOH. The CH_2Cl_2 fraction (120.0 g, IC_{50} = 22.5 μg/mL) was subjected to silica gel column chromatography (100−200 mesh, 800.0 g) using a system of *n*-hexane-CH_2Cl_2 (1:5) and CH_2Cl_2-MeOH (200:1) to provide nine fractions (Frs. 1−9). Fr. 2 (2.4 g) was purified by silica gel column chromatography and eluted with CH_2Cl_2-MeOH (100:1) to give nine fractions (Frs. 2.1−2.9). Fr. 2.2 (1.1 g) was further purified by silica gel column with CH_2Cl_2-MeOH (200:1) to gain compounds **1** (29.0 mg), **6** (7.0 mg), and **3** (10.0 mg). Fr. 2.4 (0.4 g) was subjected to silica gel column using CH_2Cl_2-MeOH (100:1) to get compounds **4** (4.5 mg) and **5** (1.5 mg). Fr. 2.6 (0.6 g) was applied to silica gel column using the elution CH_2Cl_2-MeOH (20:1), then compounds **2** (26.0 mg), **7** (39.0 mg), and **8** (10.0 mg) were obtained. Frs. 2.7 (0.2 g) was subjected to silica gel column using CH_2Cl_2-MeOH (15:1) to yield compounds **9** (10.0 mg) and **10** (15.0 mg).

2.3 DGAT1 inhibition assay

Microsomal fractions from rat liver were prepared, as described earlier (Chung et al, 2004). DGAT1 activity was measured as reported previously (Coleman et al, 1992). In brief, the reaction mixture was initiated by the addition of rat liver microsomal fraction, followed by gentle and brief vortexing. After incubation for 10 min at 37 ^{o}C, the reaction was stopped by the addition of 1.5 mL of 2-propanol-heptane-water (80:20:2), 1 mL of heptane, and 0.5 mL of water to extract the lipid. After vortexing, 1.2 mL of the organic phase was transferred into a glass tube and washed once with 2.0 mL of alkaline ethanol solution. The amount of radioactivity was determined in a liquid scintillation counter (1450 Micro Beta Triux). The samples were tested for the inhibitory activity against DGAT1 in three independent experiments. The presence of DMSO in the assay medium at 2.5% concentration had no effect on the enzyme activity (Chung et al, 2004). Kuraridine was the positive control in this assay (Chung et al, 2004).

3. Results and discussion

3.1 Structure identification

Compound **2**: white crystal, ESI-MS *m/z* 426 [M^+], ^{1}H-NMR (500 MHz, $CDCl_3$) *δ*: *exo*-methylene protons at [4.69 (1H, m, H-29β)] and [4.57 (1H, m, H-29α)], a secondary alcohol group at [3.18 (1H, dd, H-3)], a vinyl methyl at [1.68 (3H, s, H-30)], six methyl signals at [1.03 (3H, s, H-27), 0.97 (3H, s, H-26), 0.94 (3H, s, H-23), 0.83 (3H, s, H-24), 0.79 (3H, s, H-25), 0.76 (3H, s, H-28)]. ^{13}C-NMR (125 MHz, $CDCl_3$) *δ*: 38.6 (C-1), 27.3 (C-2), 78.9 (C-3), 38.8 (C-4), 55.2 (C-5), 18.2 (C-6), 34.2 (C-7), 40.7 (C-8), 50.3 (C-9), 37.1 (C-10), 20.9 (C-11), 25.0 (C-12), 38.0 (C-13), 42.7 (C-14), 27.4 (C-15), 35.5 (C-16), 42.9 (C-17), 48.2 (C-18), 47.9 (C-19), 150.8 (C-20), 29.8 (C-21), 39.9 (C-22), 27.9 (C-23), 15.3 (C-24), 16.1 (C-25), 15.9 (C-26), 14.5 (C-27), 17.9 (C-28), 109.3 (C-29), 19.2 (C-30). Therefore, according to the literature (Kong et al, 2013), compound **2** could unambiguously be elucidated as lupeol.

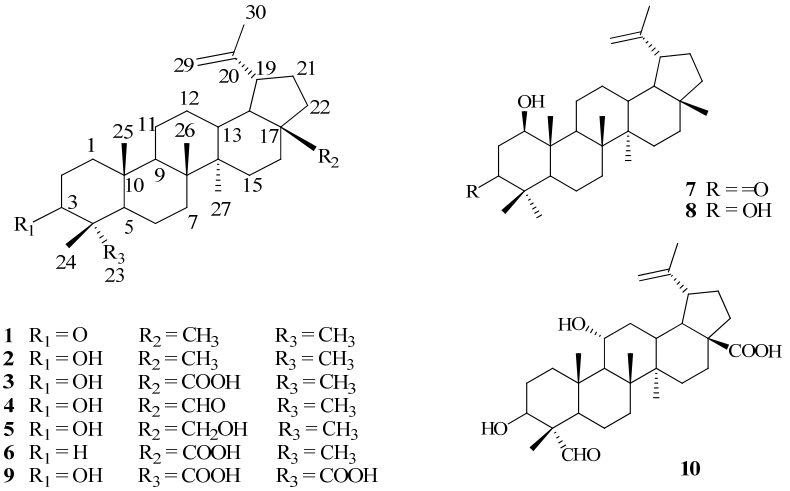
Compound **3**: white crystal, ESI-MS *m/z* 456 [M^+], ^{1}H-NMR (500 MHz, $CDCl_3$) *δ*: *exo*-methylene group at [4.94 (1H, s, H-29α)] and [4.76 (1H, s, H-29β)], 3.54 (1H, td, H-19), a secondary alcohol group at [3.46 (1H, t, H-3)], 2.72 (1H, t, H-15α), 2.63 (1H, d, H-13), 2.23 (1H, m, H-18), 1.93 (1H, d, H-16α), 1.88 (3H, m, H-1α, 12α, 22α), 1.79 (3H, s, H-29), 1.75 (1H, t, H-12β), a vinyl methyl at [1.68 (3H, s, H-30)], 1.59 (1H, d, H-12β), 1.54 (4H, m, H-2α, 6α, 15β, 21α), 1.40 (5H, m, H-6β, 7β, 11α, 16β, 22β), methyl signal at [1.21 (3H, s, H-27)], 1.20 (3H, m, H-9, 11β, 21β), 1.07 (1H, m, H-2β), signals for four methyl at [1.06 (3H, s, H-26), 1.05 (3H, s, H-23), 1.00 (3H, s, H-24)], 0.82 (3H, s, H-25)], 0.67 (1H, m, H-5). ^{13}C-NMR (125 MHz, $CDCl_3$) *δ*: 40.4 (C-1), 29.2 (C-2), 79.0 (C-3), 40.2 (C-4), 56.8 (C-5), 19.7 (C-6), 35.8 (C-7), 42.0 (C-8), 51.9 (C-9), 38.5 (C-10), 22.1 C-11), 27.0 (C-12), 39.5 (C-13), 43.8 (C-14), 32.1 (C-15), 33.8 (C-16), 57.5 (C-17), 48.7 (C-18), 50.9 (C-19), 152.2 (C-20), 31.2 (C-21), 38.4 (C-22), 29.6 (C-23), 29.8 (C-24), 17.2 (C-25), 17.3 (C-26), 15.8 (C-27), 179.7 (C-28), 110.8 (C-29), 20.4 (C-30). Therefore, according to the literature (Salimuzzaman et al, 1988), compound **3** could unambiguously be elucidated as betulinic acid.

Compound **9**: white crystal, ESI-MS *m/z* 487.27 [M + H]$^+$. ^{1}H-NMR (500 MHz, pyridine-d_5) *δ*: *exo*-methylene group at [4.90 (1H, brs, H-29β) and 4.74 (1H, brs, H-29α)], a secondary alcohol group at [4.26 (1H, m, H-3)], 3.54 (1H, m, H-19), 2.70 (1H, m, H-13), a vinyl methyl at [1.78 (3H, s, H-30)], 1.77 (1H, d, H-9), 1.59-1.86 (2H, m, H-11), four methyl singlets at [1.46 (3H, s, H-24), 1.12 (3H, s, H-26), 0.94 (3H, s, H-25), 0.93 (3H, s, H-27)]. ^{13}C-NMR (125 MHz,

pyridine-d_5) δ: 33.0 (C-1), 26.3 (C-2), 73.1 (C-3), 52.1 (C-4), 45.1 (C-5), 21.9 (C-6), 34.9 (C-7), 41.9 (C-8), 51.2 (C-9), 37.6 (C-10), 21.2 (C-11), 26.2 (C-12), 38.2 (C-13), 43.1 (C-14), 30.4 (C-15), 32.9 (C-16), 56.7 (C-17), 49.9 (C-18), 47.9 (C-19), 151.4 (C-20), 31.3 (C-21), 37.6 (C-22), 179.6 (C-23), 18.1 (C-24),16.9 (C-25), 16.8 (C-26), 14.9 (C-27), 179.6 (C-28), 110.0 (C-29), 19.6 (C-30). Therefore, according to the literature (Kiem et al, 2003), compound **9** could unambiguously be elucidated as 3α-hydroxy-lup-20(29)-en-23,28-dioic acid.

Compound **10**: white crystal, ESI-MS *m/z* 509.25 [M + Na]$^+$. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: aldenhyde proton at 10.00 (1H, s, H-23), *exo*-methylene group at [4.85 (1H, brs, H-29β) and 4.63 (1H, brs, H-29α)], a secondary alcohol group at [4.26 (1H, m, H-3)], 3.92 (1H, m, H-11), 3.54 (1H, m, H-19), 2.70 (1H, m, H-13), a vinyl methyl at [1.78 (3H, s, H-30)], 1.77 (1H, d, H-9), four methyl singlets at [1.46 (3H, s, H-24), 1.12 (3H, s, H-26), 0.94 (3H, s, H-25), 0.93 (3H, s, H-27)]. ^{13}C-NMR (125 MHz, pyridine-d_5) δ: 35.5 (C-1), 27.3 (C-2), 73.2 (C-3), 53.1 (C-4), 44.4 (C-5), 21.5 (C-6), 35.7 (C-7), 43.1 (C-8), 56.1 (C-9), 39.2 (C-10), 69.9 (C-11), 38.4 (C-12), 37.8 (C-13), 43.4 (C-14), 30.2 (C-15), 33.0 (C-16), 56.7 (C-17), 49.5 (C-18), 47.9 (C-19), 151.4 (C-20), 31.4 (C-21), 37.7 (C-22), 210.1 (C-23), 17.9 (C-24), 15.1 (C-25), 16.8 (C-26), 14.9 (C-27), 178.9 (C-28), 110.2 (C-29), 19.7 (C-30). Therefore, according to the literature (Kiem et al, 2003), compound **10** could unambiguously be elucidated as 3α,11α-dihydroxy-23-oxo-lup-20(29)-en-28-oic acid.

Bioactivity-guided fractionation of the CH_2Cl_2-soluble extract (IC_{50} = 22.5 μg/mL) from the braks of *B. platyphylla* using for an *in vitro* DGAT1 inhibitory assay, yielded 10 lupane triterpenes: lupenone (**1**), lupeol (**2**), betulinic acid (**3**), betulinaldehyde (**4**), betulin (**5**), 3-deoxybetulonic acid (**6**), glochidonol (**7**), lup-20/29-ene-1β/3β-diol (**8**), 3α-hydroxy-lup-20(29)-en-23,28-dioic acid (**9**), and 3α,11α-dihydroxy-23-oxo-lup-20(29)-en-28-oic acid (**10**). The structures of the compounds (Figure 1) were determined by 1D and 2D NMR analysis .



1 R_1 = O R_2 = CH_3 R_3 = CH_3
2 R_1 = OH R_2 = CH_3 R_3 = CH_3
3 R_1 = OH R_2 = COOH R_3 = CH_3
4 R_1 = OH R_2 = CHO R_3 = CH_3
5 R_1 = OH R_2 = CH_2OH R_3 = CH_3
6 R_1 = H R_2 = COOH R_3 = CH_3
9 R_1 = OH R_3 = COOH R_3 = COOH

Figure 1 Chemical structures of compounds 1−10 isolated from barks of *B. platyphylla*

3.2 Bioactivity results

All the isolates were assayed for their inhibitory activity against DGAT1, and the results were presented in Table 1. The known DGAT1 inhibitor kuraridine [IC_{50} = (9.8 ± 0.2) μmol/L] (Chung et al, 2004) was used as positive control in this assay. Among the isolates, compounds **3**−**6**, **9** and **10** inhibited DGAT1 activity in a dose dependent manner with IC_{50} values ranging from (11.2 ± 0.3) to (38.6 ± 1.2) μmol/L, while compounds **1**, **2**, **7**, and **8** showed very weak inhibitory effects. Compound **2** (IC_{50} > 100 μmol/L), with methyl substituent at C-28, exhibited a significantly lower inhibitory activity against DGAT1 than that in compounds **3**−**6**, **9**, and **10** on which the same position was attached as carboxyl, aldehyde, or methylol group. Furthermore, compounds **3** and **6** showed a higher activity, indicating that as hydroxyl group substituent at C-3 may non-significantly increase the inhibitory activity against DGAT1. Moreover, compounds **3** and **9** exhibited higher activities, indicating only a positional

Table 1 Inhibitory activity of compounds 1−10 against DGAT1 ($\bar{x} \pm s$, n = 3)

Compounds	IC_{50} / (μmol·L^{-1})
lupenone (**1**)	>100
lupeol (**2**)	>100
betulinic acid (**3**)	11.2 ± 0.3
betulinaldehyde (**4**)	18.6 ± 0.9
betulini (**5**)	38.6 ± 1.2
3-deoxybetulonic acid (**6**)	13.2 ± 1.1
glochidonol (**7**)	>100
lup-20/29-ene-1β/3β-diol (**8**)	>100
3α-hydroxy-lup-20(29)-en-23,28-dioic acid (**9**)	15.2 ± 0.2
3α,11α-dihydroxy-23-oxo-lup-20(29)-en-28-oic acid (**10**)	20.2 ± 1.5
kuraridine (positive control)	9.8 ± 0.2

change of groups at C-17 may not affect the inhibitory activity against DGAT1. Although structure-activity relationships of lupine triterpenes bearing other groups at C-28 were not thoroughly investigated, our results indicated that the substitution of electron- withdrawing group on lupine triterpenes may be important for *in vitro* inhibitory activity against DGAT1. Compounds **1**−**10** were known, but compounds **6**, **9,** and **10** were first extracted from the barks of *B. platyphylla*. Therefore, further investigation and optimization of these derivatives might enable the finding of new DGAT1 inhibitors that are potentially useful in the treatment of type II DM as well as obesity.

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