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# Palbinone and triterpenes from Moutan Cortex (*Paeonia suffruticosa*, Paeoniaceae) stimulate glucose uptake and glycogen synthesis via activation of AMPK in insulin-resistant human HepG2 Cells

Do Thi Ha [a,b], Dao Trong Tuan [b], Nguyen Bich Thu [b], Nguyen Xuan Nhiem [a], Tran Minh Ngoc [a], NamHui Yim [a], KiHwan Bae [a,*]

[a] College of Pharmacy, Chungnam National University, Daejeon 305-764, Republic of Korea
[b] National Institute of Medicinal Materials, 3B Quangtrung, Hoankiem, Hanoi, Viet Nam

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ABSTRACT

Moutan Cortex is a well-known herb in traditional Korean, Chinese, and Japanese anti-diabetic formulae. In the current study, we investigated the metabolic effects of isolated triterpenes (**1**–**7**) in HepG2 cells under high glucose conditions. These compounds remakably stimulated AMP-activated protein kinase (AMPK), GSK-3β, and ACC phosphorylation. The compounds also increased glucose uptake and enhanced glycogen synthesis. Among these, compound **1** displayed the greatest potential anti-diabetic activity though the AMPK activation pathway. Compound **1** significantly increased the levels of phospho-AMPK, phospho-ACC, and phospho-GSK-3β and stimulated glucose uptake and glycogen synthesis in a dose-dependent manner. In conclusion, our results suggest that these compounds, especially compound **1**, may have beneficial roles in glucose metabolism via the AMPK pathway.



Diabetes mellitus (DM) refers to disorders that share the common feature of elevated blood glucose levels. The classification accepted by the World Health Organization (WHO)[1,2] and the American Diabetes Association (ADA)[3] combines both clinical stages of hyperglycemia and the etiological types, that is, type 1 and type 2 diabetes. Over the last 30 years, an enormous number of studies have been dedicated to unraveling the pathophysiology of type 2 diabetes mellitus.[4] High levels of circulating glucose, or hyperglycemia, is a serious problem in type 2 diabetes. Among various pathological effects, the failure of hepatic control of glucose homeostasis is a key factor that causes hypeglycemia.[5] The weakened ability of insulin to initiate downstream liver metabolic action, defined as hepatic insulin resistance, also leads to dysregulated lipid synthesis and further causes hepatic steatosis, as well as systemic insulin resistance.[6]

5′ AMP-activated protein kinase (AMPK), an energy sensor that regulates cellular metabolism, was initially identified as an activating enzyme of acetyl-coA carboxylase (ACC).[7] In addition, AMPK has been reported to be involved in the glucose metabolism of various tissues such as liver, skeletal muscle, adipose tissues, and pancreatic β cells, which are key tissues in the pathogenesis of type 2 diabetes.[8] Recent findings showed that AMPK plays a major role in the control of hepatic metabolism.[9] Elevated fasting plasma glucose is associated with type 2 diabetes and is regulated by gluconeogenesis, a process that makes glucose from non-carbohydrate sources in the liver.[10] Thus, by inhibiting hepatic glucose output and increasing the liver stores by conversion of glucose to glycogen, AMPK could control blood glucose levels in the body.[10] Therefore, a defect in AMPK signaling could account for many of the abnormalities observed in insulin resistance related to type-2 diabetes.[11]

Human HepG2 hepatoma cells are a suitable cell model for investigating insulin signaling.[12] Thus, they were used to investigate the effects of isolated compounds from Moutan Cortex on insulin signaling under high glucose conditions.

Moutan Cortex ('Mok-Dan-Pi' in Korean), the root bark of *Paeonia suffruticosa* Andrew (Paeoniaceae), is found in traditional Chinese anti-diabetic formulae.[13,14] However, the mechanisms of action of Moutan Cortex extract, as well as of isolated compounds, in type 2 diabetic diseases remain unclear.[15,16] Previous phytochemical investigations of this plant resulted in the isolation of various compounds such as triterpenes,[17] monoterpene glycosides,[18–21] acetophenones,[18–21] flavonoids,[22] and tannins.[23]

Glucose uptake and glycogen synthesis assays were conducted in HepG2 cells under high glucose conditions using the MeOH

* Corresponding author. Tel.: +82 42 821 5925; fax: +82 42 823 6566.
*E-mail address:* baekh@cnu.ac.kr (KiHwan Bae).

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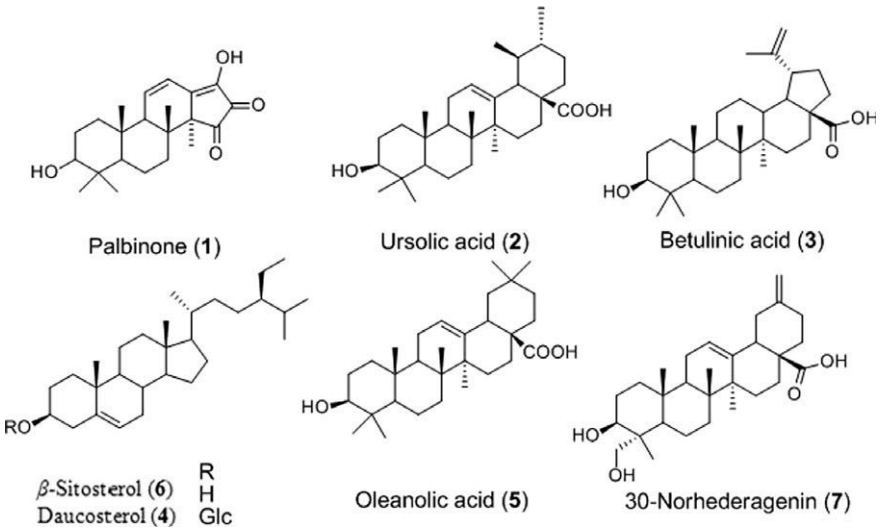


**Figure 1.** Structure of isolated triterpenes (**1**–**7**) from Moutan Cortex.

extract and subsequent partitions with *n*-hexane, EtOAc, and *n*-BuOH of Moutan Cortex. The MeOH extract, as well as the *n*-hexane and EtOAc fractions, considerably enhanced glucose uptake and glycogen synthesis in HepG2 cells (data not shown). Furthermore, repeated chromatography of these fractions on silica gel and YMC gel (ODS) columns resulted in isolation of seven triterpenes (**1**–**7**) (Fig. 1),[24] which were identified as palbinone (**1**), ursolic acid (**2**), betulinic acid (**3**), daucosterol (**4**), oleanolic acid (**5**), β-sitosterol

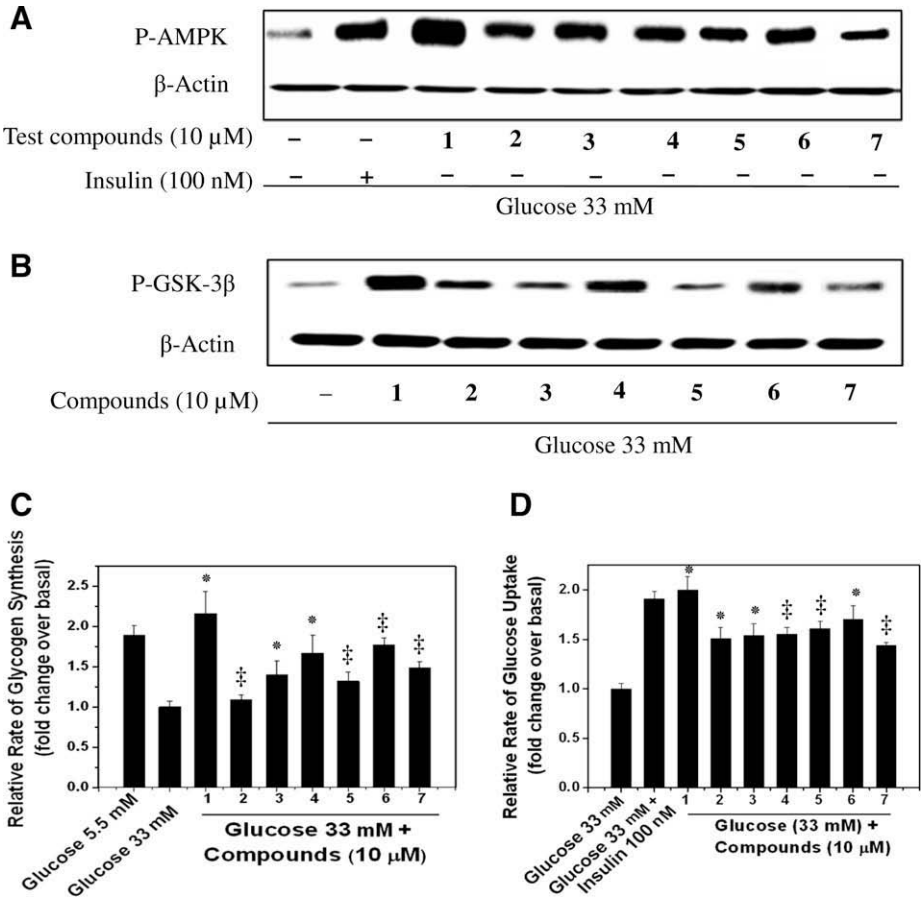


**Figure 2.** The triterpenes (**1**–**7**) stimulate glucose uptake and enhance glycogen synthesis in HepG2 cells under high glucose treatment in HepG2 cells via the AMPK-GSK-3β pathway. Effect of the triterpenes (**1**–**7**) on: (A) phosphorylation of AMPK; (B) phosphorylation of the GSK-3β; (C) glycogen synthesis. The incorporation of [$^{14}$C]glucose into the cellular glycogen pools was allowed for 60 min and the radioactivity incorporated in the glycogen was measured in the glycogen precipitate; (D) glucose uptake. HepG2 cells were treated with 2-[$^{14}$C]DOG with the presence or absence of various concentrations (1, 2, 5, and 10 μM) of **1** for measuring the glucose uptake rate by a scintillation counter; Data compiled from three independent experiments and values are expressed as mean ± SD. $^{*}p$ <0.05 and $^{‡}p$ <0.01, as compared with the control values.

(**6**), and 30-norhederagenin (**7**) by comparing their physicochemical properties and spectroscopic data with those reported in the literature.[25–27] We subsequently evaluated their ability to prevent type 2 diabetic diseases via stimulation of glucose uptake and glycogen synthesis related to activation of AMPK in human HepG2 cells under high glucose conditions. The most bioactive compound (**1**) was studied further as a novel anti-diabetic agent from Moutan Cortex.

Experiments were conducted as previously described with minor modifications: cell culture,[28] cell viability assays,[29] Western Blot analyses,[30] glycogen synthesis assays,[31] and glucose uptake assays.[32] Data are presented as means ± SD. Statistical significance was set at $p$ <0.05 and $p$ <0.01. Statistically significant differences were determined by analysis of variance in SPSS statistical software (SPSS, IL, USA).

Cytotoxicity of the isolated compounds (**1**–**7**) to HepG2 cells was examined under the conditions described by Mosmann.[30] The HepG2 cells were treated with various concentrations of these compounds (5–100 μM) for 24 h (Table 1, Supplementary data). These compounds at up to 10 μM did not influence the viability of HepG2 cells. Thus we employed the test compounds at less than 10 μM in subsequent experiments.

A previous study reported that AMPK activation in liver leads to the inhibition of glucose production and stimulation of fatty acid oxidation by enhancing the phosphorylation of ACC and GSK-3β.[9] Thus, we first determined the phosphorylation state of AMPK by using specific anti-phospho-AMPK antibody. As demonstrated in Figure 2A, compounds **1**–**7** stimulated phosphorylation of AMPK remarkably compared to insulin (100 nM). Among these, **1** was the strongest AMPK activator. The enzyme GSK-3β plays a dominant role in promoting hepatic glycogen synthesis. We therefore examined whether the elevation of glycogen was regulated via GSK-3β activities by treatment with compounds (**1**–**7**). Interestingly, administration of 10 μM of each compound **1**–**7** considerably increased the phosphorylation of GSK-3β (Fig. 2B). We next performed a glycogen synthesis assay in HepG2 cells to test whether the isolated compounds (**1**–**7**) from Moutan Cortex reversed the high glucose-induced inhibition of glycogen synthesis. Interestingly, the presence of 10 μM (**1**–**7**) in HepG2 cells under high glucose conditions remarkably increased glycogen synthesis over the basal level (Fig. 2C). Of these, compound **1** displayed the strongest capacity to elevate glycogen synthesis (more than twice the basal level) (Fig. 3B). Previous studies of hepatic insulin sensitivity indicated that short-term insulin treatment could directly promote glucose uptake in HepG2 cells.[4,32,33] In response to insulin, glucose uptake increased almost twofold over basal levels under high glucose treatment in HepG2 cells (Fig. 2D). As expected, compounds (**1**–**7**) displayed a remarkable ability to stimulate glucose uptake in HepG2 cells after administration of 10 μM of each compound (Fig. 2D). Of these, compound **1** most stimulated the glucose uptake activity in HepG2 cells (more than twice basal levels). These results indicated that AMPK activity is involved in the compound-induced glucose uptake and glycogen synthesis in HepG2 cells.

Palbinone (**1**) is an unusual terpenoid that was first isolated from *Paeonia albiflora* in 1993 and then from *Paeonia delavayi* in 2005.[25,34] However, few studies have reported its bioactivities, except for its potent inhibitory activities on 3α-hydroxysteroid dehydrogenase, 3α-hydroxy dehydrogenase, and human monocyte interleukin-1β reported in 1993.[25,35,36] In the primary screening of compounds (**1**–**7**) in type 2 diabetes treatment, palbinone was found to be a potent anti-diabetic candidate. As shown in Figure 3A, treatment with various concentrations of palbinone dose-dependently induced the phosphorylation of ACC, a well-characterized substrate of AMPK that is commonly used as a sensor of AMPK activation, whereas there was no change in the total level

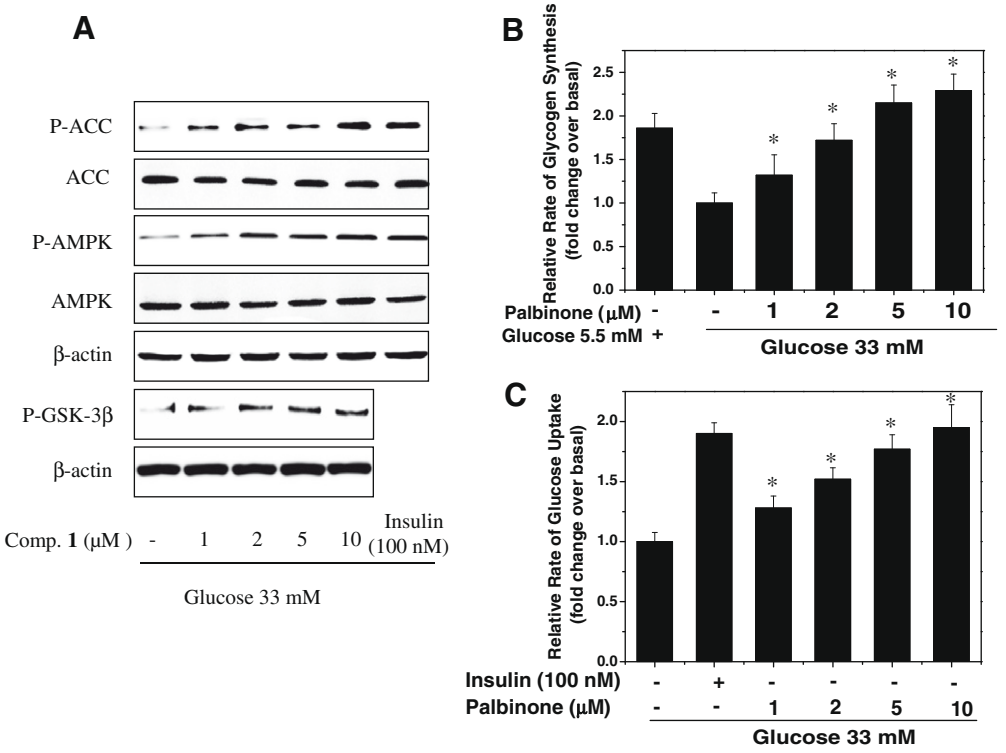


**Figure 3.** As the same manner, palbinone (**1**) stimulates glucose uptake and glycogen synthesis via AMPK downstream pathway in a dose-dependent fashion under high glucose treatment in HepG2 cells. Effect of palbinone (**1**) on: (A) phosphorylation of ACC and GSK-3β via AMPK activation; (B) glycogen synthesis; (C) glucose uptake; Data compiled from three independent experiments and values are expressed as mean ± SD. $^{*}p$ <0.05, as compared with the control values.

of ACC.[37,38] Palbinone treatment also increased the level of phospho-AMPK in a dose-dependent manner, verifying the activation of AMPK without no alteration in the total level of AMPK protein (Fig. 3A). In addition, palbinone administration also caused a remarkable increase in the level of P-GSK-3β in a dose-dependent manner (Fig. 3A). Palbinone began to elevate glycogen synthesis in human HepG2 cells under high glucose conditions at 1 μM, with a maximum increase occurring at 10 μM compared to the basal level (Fig. 3B). Also, palbinone dose-dependently enhanced glucose uptake in human HepG2 cells under high glucose conditions compared with the basal level. Furthermore, upon 10 μM palbinone treatment, the glucose uptake in HepG2 cells was higher than that with 100 nM insulin treatment (Fig. 3C).

To date, only ursolic acid and β-sitosterol, which belong to triterpene skeleton, have been investigated for active mechanisms in type 2 diabetic diseases.[39,40] Ursolic acid potently inhibited protein tyrosine phosphatase 1B (PTP 1B), enhanced insulin receptor phosphorylation, and stimulated glucose uptake in L6 myotube cells.[39] In addition, the beneficial effects of β-sitosterol on glucose and lipid metabolism in L6 myotube cells mediated by AMP-activated protein kinase were also studied.[40] To our knowledge, this is the first report of the stimulation of glucose uptake and glycogen synthesis via activation of AMPK in insulin-resistant human HepG2 cells by triterpenes from Moutan Cortex. Moreover, this study identified a promising candidate, palbinone, for treatment of type 2 diabetes. Its metabolic function was studied and palbinone was shown to increase glucose uptake and glycogen synthesis via the AMPK pathway. Therefore, it may play a crucial role in increasing insulin sensitivity, which is very meaningful in the treatment of type 2 diabetes. Further study directed at the determining the mechanism of palbinone action may lead to the identification of a novel molecular target for the generation of therapeutic agents useful in the prevention of insulin resistance in type 2 diabetes.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.08.048.

## References and notes

1. *WHO Consultation. Report No. 99.2*. Geneva: World Health Organization, 1999, 8.
2. World Health organization Study Group on Diabetes Mellitus. *Technical Report Series 727*. Geneva: World Health Organization, 1985, 25.
3. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus *Diabetes Care* **1997**, *20*, 1183.
4. Stumvoll, M.; Goldstein, B. J.; Van Haeften, T. W. *Lancet* **2005**, *365*, 1333.
5. Kim, S. P.; Ellmerer, M.; Van Citters, G. W.; Bergman, R. N. *Diabetes* **2003**, *52*, 2453.
6. Belfiore, F.; Iannello, S. In *Chapter 3: New Concepts in Diabetes and its Treatment*; Belfiore, F., Mogensen, C. E., Eds.; Basel: Karger, 2000; p 38.
7. Hadie, D. G.; Carling, D.; Sim, A. T. R. *Trends Biochem. Sci.* **1998**, *14*, 20.
8. Long, Y. C.; Zierath, J. R. *J. Clin. Invest.* **2006**, *116*, 1776.
9. Viollet, B.; Foretx, M.; Guigas, B.; Horman, S.; Dentin, R.; Bertrand, L.; Hue, L.; Andreelli, F. *J. Physiol.* **2006**, *574*, 41.
10. Misra, P.; Chakrabarti, R. *Indian J. Med. Res.* **2007**, *125*, 389.
11. Towler, M. C.; Grahame Hardie, D. *Circ. Res.* **2007**, *16*, 328.
12. Gupta, D.; Varma, S.; Khandelwal, R. L. *J. Cell. Biochem.* **2007**, *100*, 593.
13. Ding, H. Y.; Wu, Y. C.; Lin, H. C.; Chan, Y. Y.; Wu, P. L.; Wu, T. S. *Chem. Pharm. Bull.* **1999**, *47*, 652.
14. Shimizu, M.; Zenko, Y.; Tanaka, R.; Matsuzawa, T.; Morita, N. *Chem. Pharm. Bull.* **1993**, *48*, 1469.
15. Hong, H.; Wang, Q.; Zhao, Z.; Liu, G.; Shen, Y.; Chen, G. *Yao Xue Xue Bao* **2003**, *38*, 255.
16. Lau, C. H.; Chan, C. M.; Chan, Y. W.; Lau, K. M.; Lau, T. W.; Lam, F. C.; Law, W. T.; Che, C. T.; Leung, P. C.; Fung, K. P.; Ho, Y. Y.; Lau, C. B. S. *Phytomedicine* **2007**, *14*, 778.
17. Lin, H. C. *J. Nat. Prod.* **1998**, *61*, 343.
18. Yoshikawa, M.; Uchida, E.; Kawaguchi, A.; Kitagawa, I.; Yamahara, J. *Chem. Pharm. Bull.* **1992**, *40*, 2248.
19. Yoshikawa, M.; Uchida, E.; Kawaguchi, A.; Kawaguchi, A.; Yamahara, J.; Murakami, N.; Kitagawa, I. *Chem. Pharm. Bull.* **1993**, *41*, 630.
20. Lin, H. C.; Ding, H. Y.; Wu, T. S.; Wu, P. L. *Phytochemistry* **1996**, *41*, 237.
21. Matsuda, H.; Ohta, T.; Kawaguchi, A.; Yoshikawa, M. *Chem. Pharm. Bull.* **2001**, *49*, 69.
22. Wang, X.; Cheng, C.; Sun, Q.; Li, F.; Liu, J.; Zheng, C. *J. Chromatogr., A* **2005**, *127*, 1075.
23. Wu, Y. T.; Huang, W. Y.; Lin, T. C.; Sheu, S. J. *J. Sep. Sci.* **2003**, *26*, 1629.
24. *Extraction and isolation of active compounds*. Moutan Cortex (P. *suffruticosa*) was purchased from traditional herbal market in Daejeon, Korea, in January 2007. A specimen of the plant (CNU-1554) has been verified by Professor K. Bae and deposited at the Herbarium of the College of Pharmacy. The Moutan Cortex (20 kg) was extracted three times with MeOH at 60 °C. The combined filtrates were taken to dryness in vacuo (40 °C) and residue (3000 g) was stirred with 1000 mL of 95% MeOH and suspended in $H_2O$, then successively partitioned with *n*-hexane, EtOAc, *n*-BuOH, and $H_2O$. The hexane layer (400 g) was chromatographed over silica gel using *n*-hexane–EtOAc (80:1, 20:1, and 10:2) to obtain seven fractions (Fr. **1**–**7**). Fr. 3 (2,5 g) was subjected to a silica gel column chromatography (CC) and eluted with *n*-hexane–EtOAc (6:1) gave a crystal compound (β-sitosterol (**6**), 200 mg). Fr. 4 (45 g) was subjected to a silica gel CC, eluted with *n*-hexane–EtOAc (6:1) and combination of similar eluates after TLC comparison afforded 8 fractions (Fr.4.1–4.8). Fr.4.1 (4.5 g) was chromatographed on an YMC gel column (ODS) using MeOH–$H_2O$ (2:1) to give ursolic acid (**2**, 300 mg) and betulinic acid (**3**, 200 mg). Fr. 4.4 (3.2 g) was purified by using a silica gel CC (*n*-hexane–EtOAc, 5:1) to yield daucosterol (**4**, 110 mg) and oleanolic acid (**5**, 110 mg).The EtOAc layer (600 g) was subjected to a silica gel column chromatography (CC) of increasing polarity ($CHCl_3$–MeOH, 100:0 and 97:3) to give 5 fractions (1–5). Repeat CC of Fr. 4 (11.2 g) with a silica gel CC (*n*-hexane–EtOAc, 6:1) gave 5 subfractions (Fr. 4.1–4.5). Then, Fr. 4.4 was separated by a silica gel CC ($CHCl_3$–MeOH, 30:1) and then purification by an YMC gel column (ODS) (MeOH–$H_2O$, 1:1) to afford palbinone (**1,** 300 mg) and 30-norderagenin (**7**, 17 mg).
25. Kadota, S.; Terashima, S.; Basnet, P.; Kikuchi, T.; Namba, T. *Chem. Pharm. Bull.* **1992**, *33*, 255.
26. Nes, W. D.; Norton, R. A.; Benson, M. *Phytochemistry* **1992**, *31*, 805.
27. Ikuta, A.; Itokawa, H. *Phytochemistry* **1988**, *27*, 2813.
28. Zang, M. W.; Zuccollo, A. *J. Biol. Chem.* **2004**, *279*, 47898.
29. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55.
30. Sakoda, H.; Gotoh, Y.; Katagiri, H.; Kurokawa, M.; Ono, H.; Onishi, Y.; Anai, M.; Ogihara, T.; Fujishiro, M.; Fukushima, Y.; Abe, M.; Shojima, N.; Kikuchi, M.; Oka, Y.; Hirai, H.; and Asano, T. *J. Biol. Chem.* **2003**, *278*, 25802. Phosphorylation of the AMPK and GSK-3β in HepG2 cells was detected in the cells exposed to the triterpenes (**1**–**7**) at the concentration of 10 μM; And the phosphorylation of AMPK and its downstream targets P-ACC and P-GSK-3β in HepG2 cells were detected in the cells exposed to 1 at the increasing concentrations (1, 2, 5, and 10 μM) under high glucose conditions. The cells lysates were analyzed via Western blotting for anti-phospho-AMPK, anti-phospho-GSK-3β (Ser9), and anti-phospho-ACC antibodies. Parallel immunoblots were analyzed for total kinase levels with anti-AMPK, anti-ACC, and β-actin antibodies were conducted as protein loading controls.
31. Lee, E. S.; Uhm, K. O.; Lee, Y. M.; Han, M. S.; Lee, M. S.; Park, J. M.; Suh, P. G.; Park, S. H.; Kim, H. S. *Biochem. Biophys. Res. Commun.* **2007**, *361*, 854.
32. Lin, C. L.; Lin, J. K. *Mol. Nutr. Food Res.* **2008**, *52*, 930.
33. Yamashita, R.; Saito, T.; Satoh, S.; Aoki, K.; Kaburagi, Y.; Sekihara, H. *Endocrinology* **2005**, *52*, 727.
34. Wu, S.; Wu, D.; Chen, Y.; Qian, P. *Zhongcaoyao* **2005**, *36*, 648.
35. Kadota, S.; Shigetoshi, S.; Kikuchi, T.; Namba, T. *Tetrahedron Lett.* **1992**, *33*, 255.
36. Kadora, S.; Terashima, S.; Li, J. X.; Namba, T.; Kageyu, A. *Phytother. Res.* **1995**, *9*, 379.
37. Carlson, C. A.; Kim, K. H. *J. Biol. Chem.* **1973**, *248*, 378.
38. Winder, W. W. *J. Appl. Physiol.* **2001**, *91*, 1017.
39. Zhang, W.; Hong, D.; Zhou, Y.; Zhang, Y.; Shen, Q.; Li, J. Y.; Hu, L. H.; Li, J. *Biochim. Biophys. Acta* **2006**, *1760*, 1505.
40. Hwang, S. L.; Kim, L. N.; Jung, H. H.; Kim, J. E.; Choi, D. K.; Hur, J. M.; Lee, J. Y.; Song, H.; Song, K. S.; Huh, T. L. *Biochem. Biophys. Res. Commun.* **2008**, *177*, 1253.