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Glucose uptake enhancing activity of puerarin and the role of *C*-glucoside suggested from activity of related compounds

Eisuke Kato *, Jun Kawabata

Laboratory of Food Biochemistry, Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Kita-9 Nishi-9, Kita-ku, Sapporo, Hokkaido 060-8589, Japan

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ABSTRACT

Chemical treatment of diabetes mellitus is widely studied and controlling of blood glucose level is the main course of therapy. In type 2 diabetes mellitus, insulin resistance is the major problem. An isoflavone *C*-glucoside, puerarin (**1**), is known to enhance glucose uptake into the insulin sensitive cell and is thought to be a candidate for treatment of diabetes mellitus. We synthesized **1** and several derivatives to apply for the structure–activity relationship study. The result against 3T3-L1 adipocyte indicated that the *C*-glucoside part of **1** is unconcerned in its activity when tested in vitro and the main structure responsible for its activity was the isoflavone moiety.

Diabetes mellitus (DM) is a currently rising problem in nations worldwide. Type 2 DM (T2DM) is the main type of this disease accounting to lifestyle like dietary habitat together with insufficient exercise and highly relates to obesity state of a patient. One of the major problems of T2DM is the insulin resistance. Weak response against insulin leaves the patients' blood glucose at high levels which eventually cause other symptoms like cardiovascular disease, renal failure, blindness and neurological disorders.[1] As the insulin resistance refuses treatment using exogenously administrated insulin to control blood glucose level, treatment of T2DM is performed by controlled food intake and exercise. And chemical treatment other than insulin is often accommodates with them. Chemical treatments of T2DM are mainly focused on the suppression of blood glucose rise or improvement of insulin resistance. α-Glucosidase inhibitors are used to suppress rapid elevation of blood glucose level after food intake, and synthetic peroxisomal proliferator-activated receptor γ (PPARγ) agonists are used to improve insulin resistance. Along with these, recent reports about glycosylated flavonoids controlling blood glucose level of model animal have gained attention on these kinds of plant originated natural compounds.[2–4]

Puerarin (**1**) is one of these glycosylated flavonoids isolated from *Pueraria iobata*.[5] The effect of **1** is shown by decreasing blood glucose level of DM model rat and by enhancing glucose uptake of muscle tissue.[3] Furthermore, the study indicates these glucose uptake enhancing activity is partially owed by a PPARγ agonist activity of **1**. Though working as a PPARγ agonist, structure of **1** that *C*-glucoside attached to 8-*C* position of daidzein (**19**), an isoflavone moiety, is unique compared to other synthetic PPARγ agonists such as rosiglitazone, pioglitazone or endogenous ligand 15-deoxy-$\Delta^{12,14}$-prostagrandin J2. To investigate the structure–activity relationships of **1** on the glucose uptake enhancing activity, especially on a role of the *C*-glucoside, we synthesized **1** and several related compounds, and tested them against cultured cell.

Of the whole structure of **1**, *C*-glucoside is the most unique structure, that is, different from common PPARγ agonists. To investigate about the *C*-glucoside, we synthesized **10** and **17** along with **1**. Although synthesis of **1** is already reported,[6] the procedure was not suited for a synthesis of **10** and several other related derivatives. So we chose to employ the C-glycosylation procedure using a glucosyl imidate **2** and an acetophenone **3**. Treatment of TMSOTf to the mixture of **2** and **3** afforded a *C*-glucoside **4**. After selectively protecting 6-OH of **4** by a benzyl group, aldol condensation with an aldehyde **6** was followed to give a chalcone **7a**. Then, **7a** was treated with $Tl(NO_3)_3$ and heated in an acidic medium to form isoflavone structure.[7] During this reaction, the benzyl group protecting 5-OH was selectively removed to give **8a** as the product. As this hydroxyl group needs to be removed for synthesis of **1**, **8a** was subjected to trifluoromethanesulfonylation and subsequent reaction

Abbreviation: DIAD, diisopropyl azodicarboxylate; G6PDH, glucose-6-phosphate dehydrogenase; NMO, *N*-methylmorpholine *N*-oxide; TBAF, tetrabutylammonium fluoride.

* Corresponding author. Tel./fax: +81 11 706 2496.
E-mail address: eikato@chem.agr.hokudai.ac.jp (E. Kato).

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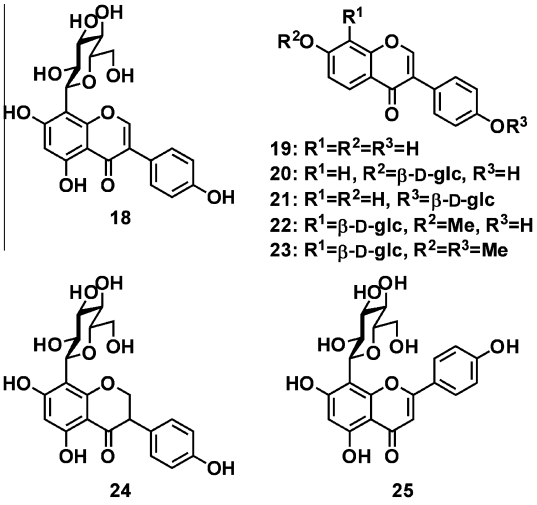
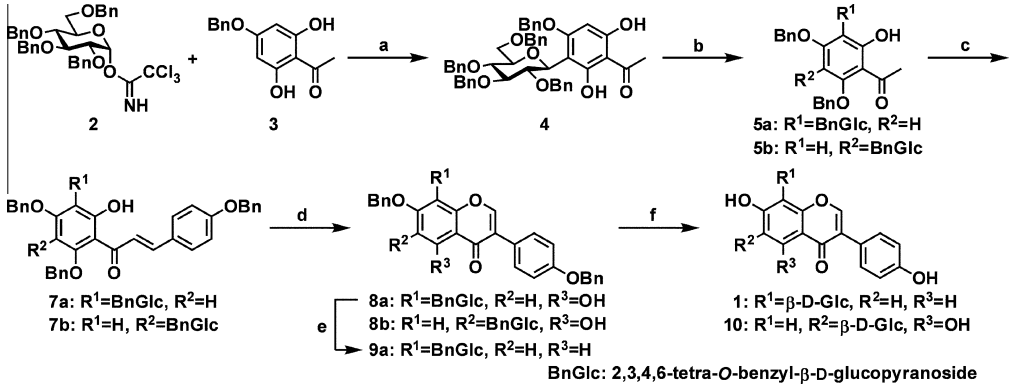
with $Pd(OAc)_2$ to give **9a**. Finally, benzyl groups were removed from **9a** by treatment with BBr_3 to give puerarin (**1**) in 13% overall yield (Scheme 1). Together with **1**, synthetic intermediates of **1** were converted to obtain **18** and **22–25**. A relative regioisomer of **1**, genistein 6-*C*-glucoside (**10**) was synthesized following puerarin's synthesis except for protecting 2-OH of **4**. 6-OH of **4** was temporarily protected by a TBS group, then 2-OH was protected by a benzyl group and the TBS group was removed to give **5b**. Aldol condensation of **5b** and **6**, reaction with $Tl(NO_3)_3$ and deprotection by BBr_3 were sequentially preformed to give **10**.[8] Unnecessary hydroxyl group 5-OH of **10** removed in the synthesis of **1** was ignored in this case as from the preliminary study it was shown to be ineffective in glucose uptake enhancing activity. Next, a derivative possessing a dihydroxypropyl group as a hydrophilic moiety instead of the *C*-glucoside, 8-(2,3-dihydroxypropyl)daidzein (**17**), was synthesized (Scheme 2). Starting from 2,4-dihydroxyacetophenone (**11**), an allyl group was incorporated in three steps by 4-O-methylation, 2-O-allylation and subsequent Claisen rearrangement of the allyl group to give **13**. Oxidation of the allyl group of **13** by OsO_4 and protection of the resulting diol gave **14**. Then, **14** was subjected to aldol condensation with **6**, followed by reaction with $Tl(NO_3)_3$ to form the isoflavone structure. Since the acetonide group protecting the diol was partially removed in this reaction, the mixture was re-protected to the acetonide **16** for the enhanced solubility in dichloromethane which was necessary for the next benzyl-removing step. At last, successive removal of protective groups from **16** by BBr_3 and NaSEt, respectively, gave the desired product **17**.[9]

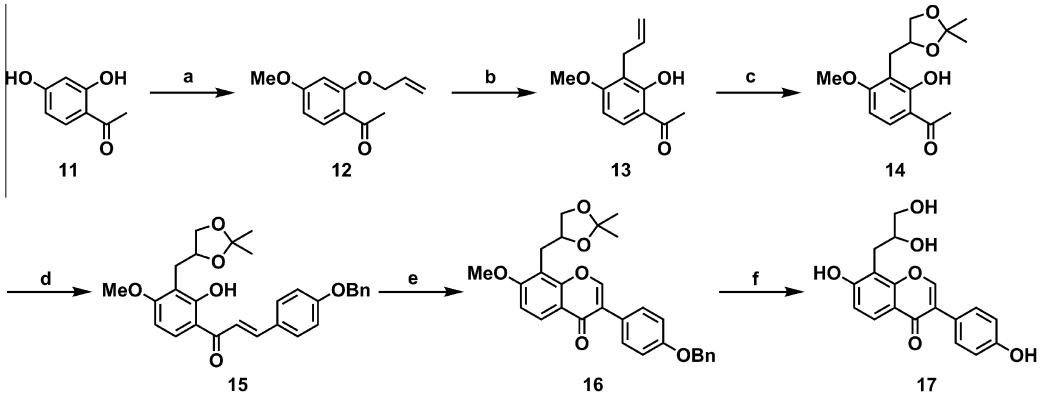


Compounds **1**, **10** and **17** along with several derivatives, either synthesized or purchased, were examined for their glucose uptake enhancing activity. The activity was measured according to the modified procedure of Yamamoto et al.[10] 3T3-L1 cell, a model cultured cell for studying adipocyte, was chosen to test the activity.[11] Although the previous report on the activity of **1** employed muscle tissue, experiments using cultured muscle model L6 cell was not applicable as the glucose uptake appeared inconstant when stimulated by **1**. Incubation of 3T3-L1 adipocyte with 10 μM of **1** enhanced the glucose uptake 1.5 times upon insulin stimulation compared to control experiment (Fig. 1). This was comparable to the result previously reported using muscle tissue of rat. Higher concentration also enhanced uptake at the similar ratio, but the result was rather inconstant and lower concentration decreased enhancement. So in the following experiment, 10 μM of each compound was tested against 3T3-L1 adipocyte for their activity.

Tested compounds were classified to three groups according to their activity, that is, (A) compounds exhibiting a comparable activity to **1** with significant difference (p <0.03) to control (1.4–1.5 times enhancement, Fig. 2, compounds **1**, **10**, **17**, **18** and **19**), (B) compounds showing a weaker activity compared to group A and has no significant difference to control (1.15–1.25 times enhancement, Fig. 2. compounds **20**, **21** and **22**), (C) compounds with a slightly to no activity (bellow 1.1 times enhancement, Fig. 2, compounds **23**, **24** and **25**). Group A mainly consists of the *C*-glucoside derivatives except for **18** suggesting that the most characteristic structure of **1**, *C*-glucoside may not be necessary for the activity itself. The 6-*C*-glucoside **10**, besides of the additional hydroxyl group at 5-*C* position which is shown to be ineffective for the activity from the result of **18**, differs only at the C-glucosylation position. 8-(2,3-Dihydroxypropyl)daidzein (**17**) has the diol group as a hydrophilic moiety instead of *C*-glucoside. And most of all, daidzein (**19**), an aglycon of **1**, lacks the *C*-glucoside itself. Thus, *C*-glucoside of **1** may attach to 6-*C* position, or can be modified to other simple hydrophilic groups, or simply removed without reducing its activity. Therefore, we can conclude that the *C*-glucoside of **1** is unconcerned in the glucose uptake enhancing activity. In contrast, compounds belonging to groups B and C clearly indicate the importance of aglycon, including the isoflavone structure and the phenolic hydroxyl group. *O*-Glucosides **20** and **21** retain the isoflavone structure common to group A compounds, but they showed a weaker enhancement of glucose uptake comparing to **1**. As concluded above, the *C*-glucoside of **1** is unconcerned in the glucose uptake enhancing activity. Concerning this, the decrease in activity of **20** and **21** should not be the result of the *C*-glucoside replacement from 8-*C* position. Instead, glucosylation of the phenolic hydroxyl group should contribute to this. This



Scheme 1. Synthesis of puerarin (**1**) and **10**. Reagent and conditions: (a) TMSOTf, CH_2Cl_2, 30 °C (70%); (b) **5a**: BnBr, K_2CO_3, acetone, 50 °C (90%); **5b**: (1) TBSCl, imid., DMF; (2) BnOH, Ph_3P, DIAD, THF then TBAF (86% in two steps); (c) *p*-benzyloxybenzaldehyde (**6**), NaOMe, MeOH, 1,4-dioxane (**7a**: 60%, **7b**: 74%); (d) (1) $Tl(NO_3)_3$, $(MeO)_3CH$, MeOH, 40 °C; (2) 10% HCl aq, MeOH, 1,4-dioxane, 80 °C (**8a**: 72%, **8b**: 30%); (e) (1) Tf_2O, pyr., CH_2Cl_2; (2) $Pd(OAc)_2$, Ph_3P, HCOOH, TEA, DMF (86% in two steps); (f) BBr_3, CH_2Cl_2, −78 °C (**1**: 71%, **10**: 51%).



Scheme 2. Synthesis of **17**. Reagent and conditions: (a) (1) MeI, K_2CO_3, acetone; (2) allyl bromide, K_2CO_3, KI, acetone (92% in two steps); (b) *N*,*N*-dimethylaniline, 210 °C (69%); (c) (1) OsO_4, NMO, MeCN, H_2O; (2) 2,2-dimethoxypropane, TsOH, DMF (90% in two steps); (d) **6**, NaOMe, MeOH (79%); (e) (1) $Tl(NO_3)_3$, $(MeO)_3CH$, MeOH, 40 °C; (2) 10% HCl aq, MeOH, 1,4-dioxane, 80 °C; (3) 2,2-dimethoxypropane, TsOH, DMF (86% in three steps); (f) (1) BBr_3, CH_2Cl_2, −78 °C; (2) NaSEt, DMF, 80 °C (78% in two steps).

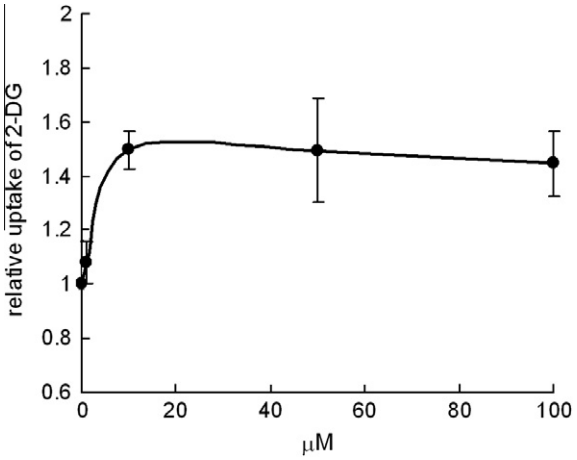


Figure 1. Glucose uptake enhancing activity of puerarin (**1**). 3T3-L1 adipocytes were incubated with each concentration of **1** and 2-DG uptake was measured upon stimulation with 100 nM of insulin. Control cells were treated in the same manner without the sample and the activity is expressed as relative value of 2-DG uptake against control.

was confirmed from the result of 7-*O*-methylpuerarin (**22**) and also from 4′,7-di-*O*-methylpuerarin (**23**). These two compounds apparently demonstrated the importance of the phenolic hydroxyl group as **22** exhibiting weaker activity than **1**, which is the same as *O*-glucoside **20** or **21**, and **23** classified to group C showing no activity. Consequently, the decreased activity of **20** and **21** should be the result of masking the phenolic hydroxyl group by O-glucosylation. Additionally, a slight difference in the activity of **20**, **21**, **22** and **23** may indicate a higher contribution of the 4′-OH group than the 7-OH group. Importance of the isoflavone structure is indicated by the rest of group C compounds, **24** and **25**. These compounds possess modified isoflavone structure, and both showed no activity at all, indicating clear result that the isoflavone itself is the most important structure for the activity.

Contribution of flavonoids to glucose uptake enhancing activity is also apparent from several reports. Several homoisoflavonoids without glucose in the structure are reported to exhibit glucose uptake enhancing activity.[13] Two flavonoids, kaempferol and quercetin, are also known to improve glucose uptake.[14] Moreover, many of synthetic PPARγ agonists are composed of a hydrophobic moiety. Puerarin (**1**), as well as kaempferol or quercetin is known to

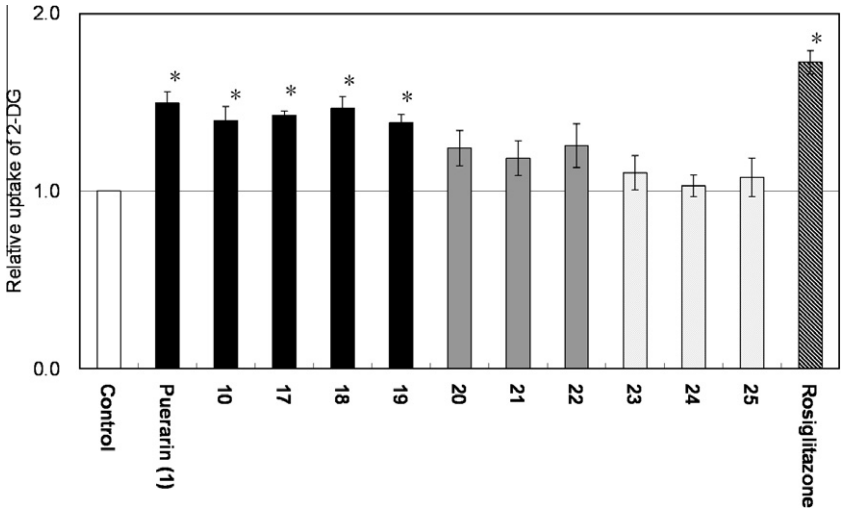


Figure 2. Glucose uptake enhancing activity of tested compounds. 3T3-L1 adipocytes were incubated with 10 μM of each samples and 2-DG uptake was measured upon stimulation with 100 nM of insulin. Control cells were treated without the sample and rosiglitazone (1 μM) was used as the positive control.[12] Activity is expressed as relative value of 2-DG uptake against control and each samples were tested at least three times with $n = 3$ for each experiment. $^*p < 0.03$ when compared to control. Groups A, B and C defined in the main text is colored individually.

elevate glucose uptake, at least partially, by the PPARγ agonist activity.[14,15] Also several isoflavones are reported to possess a PPARγ agonist activity.[16] Therefore, it is readily accepted that the activity of **1** largely owes to its aglycon daidzein (**19**). Regarding the importance of isoflavone, then what does the *C*-glucoside of **1** participates for? Although it is shown not participating in activity itself in this experiment, from compounds contained in *P. iobata*, **1**, not **19** is the content reported to be active for glucose uptake enhancement.[17] Therefore, *C*-glucoside may give an advantage when tested in vivo by giving solubility to scarcely water-soluble isoflavone and raising the absorbability into the body.

In conclusion, we have synthesized puerarin (**1**) and several derivatives to study their glucose uptake enhancing activity against 3T3-L1 adipocyte. The structure responsible for the glucose uptake enhancing activity was shown to be the isoflavone moiety daidzein (**19**) and the *C*-glucoside of **1** was not much involved in the activity. Although principal structure responsible for the activity of **1** was isoflavone moiety, the *C*-glucoside may involves in physical properties of the compound and raises availability by live body.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.06.077.

References and notes

1. World Health Organization (WHO). Fact sheet No. 312 'Diabetes', http://www.who.int/mediacentre/factsheets/fs312/en/ (accessed Apr 2010).
2. Jorge, A. P.; Horst, H.; De Sousa, E.; Pizzolatti, M. G.; Silva, F. R. M. B. *Chem. Biol. Interact.* **2004**, *149*, 89.
3. Hsu, F. L.; Liu, I. M.; Kuo, D. H.; Chen, W. C.; Su, H. C.; Cheng, J. T. *J. Nat. Prod.* **2003**, *66*, 788.
4. Zanatta, L.; Rosso, Â.; Folador, P.; Figueiredo, M. S. R. B.; Pizzolatti, M. G.; Leite, L. D.; Silva, F. R. M. B. *J. Nat. Prod.* **2008**, *71*, 532.
5. Murakami, T.; Nishikawa, Y.; Ando, T. *Chem. Pharm. Bull.* **1960**, *8*, 688.
6. Lee, D. Y. W.; Zhang, W.-Y.; Karnati, V. V. R. *Tetrahedron Lett.* **2003**, *44*, 6857.
7. Eade, R. A.; McDonald, F. J.; Pham, H. P. *Aust. J. Chem.* **1978**, *31*, 2699.
8. *Spectral data of* **10**: ^{1}H NMR (500 MHz, DMSO-d_6, rt): 10.86 (1H, s, OH), 10.06 (1H, s, OH), 9.36 (1H, s, OH), 7.76 (2H, d, J = 8.7 Hz), 6.87 (2H, d, J = 8.7 Hz), 6.58 (1H, s), 6.32 (1H, s), 4.65 (1H, d, J = 9.9 Hz), 3.65 (1H, dd, J = 2.0, 12.0 Hz), 3.61 (1H, dd, J = 9.1, 9.5 Hz), 3.54 (1H, dd, J = 4.6, 12.0 Hz), 3.3–3.2 (3H, m) ppm; ^{13}C NMR (125 MHz, DMSO-d_6, rt): 179.7, 165.7, 165.5, 158.9, 156.2, 145.7, 132.9, 123.2, 116.0, 109.7, 107.7, 103.1, 90.9, 81.2, 78.2, 74.3, 71.6, 69.6, 60.5 ppm; HR FD MS (positive): $[M]^+$ Found m/z 432.1040, $C_{21}H)20O_{10}^+$ requires m/z 432.1057.
9. *Spectral data of* **17**: ^{1}H NMR (270 MHz, CD_3OD, rt): 8.19 (1H, s), 7.96 (1H, d, J = 8.8 Hz), 7.37 (2H, d, J = 8.8 Hz), 6.97 (1H, d, J = 8.8 Hz), 6.84 (2H, d, J = 8.8 Hz), 3.99 (1H, tt, J = 6.0, 6.2 Hz), 3.53 (2H, d, J = 6.0 Hz), 3.06 (2H, d, J = 6.2 Hz) ppm; ^{13}C NMR (67.5 MHz, CD_3OD, rt): 178.6, 162.4, 158.7, 157.9, 154.7, 131.4, 126.1, 125.5, 124.3, 118.4, 116.2, 115.6, 114.0, 73.0, 67.1, 28.3 ppm; HR FD MS (positive): $[M]^+$ Found m/z 328.0913, $C_{18}H_{16}O_6^+$ requires m/z 328.0947.
10. Yamamoto, N.; Sato, T.; Kawasaki, K.; Murosaki, S.; Yamamoto, Y. *Anal. Biochem.* **2006**, *351*, 139.
11. *Glucose uptake enhancing activity assay:* The tested compounds were dissolved in DMSO and diluted 10 times with deionized water to the appropriate concentration resulting 10% DMSO aq solution. The sample solution was then sterilized by passing through 0.2 μm filter. 3T3-L1 cells were cultured in 24- or 48-well plates following the standard procedure for differentiation and the cells were used 8 days after inducing differentiation. Sample solution was added to the differentiated 3T3-L1 cells, the control cells were treated with the same amount of 10% DMSO aq and positive control cells were treated with 1 μM of rosiglitazone, a concentration enough to maximally activate PPARγ.[12] In every experiment, final concentration of DMSO was kept below 0.1%. After four days of incubation, cells were rinsed with serum-free Dulbecco's modified Eagle's medium (DMEM) and incubated for two hours in serum-free DMEM. The cells were washed twice with Krebs-Ringer-Phosphate-HEPES (KRPH) buffer (20 mM HEPES, 136 mM NaCl, 4.7 mM KCl, 1 mM NaH_2PO_4, 1 mM $MgSO_4$, 1 mM $CaCl_2$, pH 7.4). After washing, the cells were incubated in KRPH buffer with or without (blank) 100 nM insulin for 30 min. Then the cells were incubated in KRPH buffer with 1 mM 2-deoxyglucose (2-DG) for 30 min. After incubation, the cells were washed three times with saline and then 0.1 M NaOH aq (150 μL for 24-well plate, 75 μL for 48-well plate) was added. Cell lysate was frozen once and then heated at 90 °C for 40 min. The lysate was neutralized by 1 M HCl aq and triethanolamine buffer (200 mM, pH 8.1, 50 μL for 24-well plate, 25 μL for 48-well plate) was added. The amount of 2-DG or 6-phospho-2-DG contained in the lysate was then measured by the modified method of Yamamoto et al.[10] Briefly, 50 μL of the cell lysate or standard sample containing various concentration of 2-DG, 50 μL of assay cocktail (50 mM KCl, 0.5 mM $MgCl_2$, 50 μM resazurin sodium salt, 1.3 mM ATP, 0.1 mM $NADP^+$, 12 units/mL hexokinase, 32 units/mL G6PDH, 4 units/mL diaphorase in 50 mM triethanolamine buffer, pH 8.1) was mixed and incubated for 90 min. at 37 °C. The fluorescence of resulting resorfin (E_x 530, E_m 590) was measured by microplate reader (Bio-tech Instruments Inc., Synergy™ MX) for quantification of the amount of 2-DG uptake of the cells. Protein concentration of the lysate was quantified by Quickstart protein assay (Bio-Rad Laboratories Inc.) with BSA as the standard and the 2-DG uptake values were calculated as nmol/mg·protein. The blank amount was subtracted as a basal glucose uptake, and the relative amount against the control value was calculated. Each sample was tested at least three times of individual experiment and average value with standard error is shown as a result.
12. Lehmann, J. M.; Moore, L. B.; Smith-Oliver, T. A.; Wilkison, W. O.; Willson, T. M.; Kliewer, S. A. *J. Biol. Chem.* **1995**, *270*, 12953.
13. Zhang, H.; Yang, F.; Qi, J.; Song, X.-C.; Hu, Z.-F.; Zhu, D.-N.; Yu, B.-Y. *J. Nat. Prod.* **2010**, *73*, 548.
14. Fang, X.-K.; Gao, J.; Zhu, D.-N. *Life Sciences* **2008**, *82*, 615.
15. Xu, M.; Xiao, S.; Sun, Y.; Zheng, X.; Ou-yang, Y.; Guan, C. *Life Sciences* **2005**, *77*, 3183.
16. Chacko, B. K.; Chandler, R. T.; D'Alessandro, T. L.; Mundhekar, A.; Khoo, N. K. H.; Botting, N.; Barnes, S.; Patel, R. P. *J. Nutr.* **2007**, *137*, 351.
17. Fan, L. L.; O'Keefe, D. D.; Powell, W. J., Jr. *Acta Pharm. Sin.* **1984**, *19*, 801.