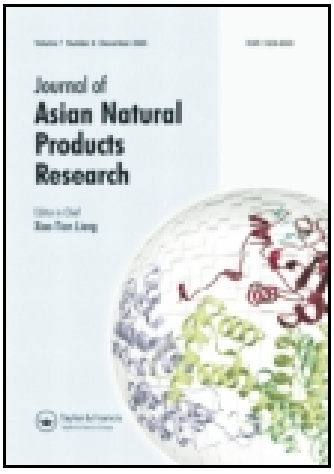

Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ganp20

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Published online: 28 Sep 2010.

To cite this article: Zhi-Qiang Feng , Ying-Yu Wang , Zong-Ru Guo , Feng-Ming Chu & Piao-Yang Sun (2010) The synthesis of puerarin derivatives and their protective effect on the myocardial ischemia and reperfusion injury, Journal of Asian Natural Products Research, 12:10, 843-850, DOI: 10.1080/10286020.2010.505563

To link to this article: http://dx.doi.org/10.1080/10286020.2010.505563

Journal of Asian Natural Products Research
Vol. 12, No. 10, October 2010, 843–850

ORIGINAL ARTICLE

The synthesis of puerarin derivatives and their protective effect on the myocardial ischemia and reperfusion injury

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(*Received 24 May 2010; final version received 28 June 2010*)

Puerarin is a naturally occurring isoflavone and is frequently used for the treatment of cardiovascular symptoms in China. By the structural modification of the puerarin molecule at different positions, seven new puerarin derivatives were obtained, and their cardioprotective activities (*in vitro* and *in vivo*) were respectively evaluated. The finding that the activities of **3** and **8** markedly exceeded puerarin suggested that the acylated modification of phenolic hydroxyl at C-7 in the puerarin molecule may improve the cardioprotective activity, which will be an important reference for further structural optimization.

Keywords: puerarin derivatives; myocardial ischemia; reperfusion injury

1. Introduction

Acute myocardial ischemia and myocardial infarction are the leading cause of morbidity and mortality in the Western World, and according to the World Health Organization, it will be the major cause of death in the world by the year 2020 [1]. Early restoration of blood flow (reperfusion) is an absolute prerequisite for the survival of ischemic myocardium. However, reperfusion has been referred by Braunwald and Kloner [2] as the 'double edged sword' because reperfusion itself may lead to accelerated and additional myocardial injury beyond that generated by ischemia alone, and this results in a spectrum of reperfusion-associated pathologies, ranging from reversible dysfunction ('myocardial stunning') to irreversible necrosis [2], collectively called reperfusion injury [3]. However, the reperfusion through thrombolysis, percutaneous coronary angioplasty, or bypass surgery is the standard treatment in impending acute myocardial infarction, meaning that reperfusion injury will be a major clinical problem in the near future [4].

This reperfusion injury involves the activation of an inflammatory cascade and is manifested as functional impairment, arrhythmia, and accelerated progression of cell death in certain critically injured myocytes. The major mediators of reperfusion injury are oxygen radicals, calcium loading, and neutrophils [5]. The oxygen radicals are generated by injured myocytes and endothelial cells in the ischemic zone, as well as by neutrophils that enter the ischemic zone, and become activated on reperfusion. These oxygen radicals exacerbate membrane damage, which leads to

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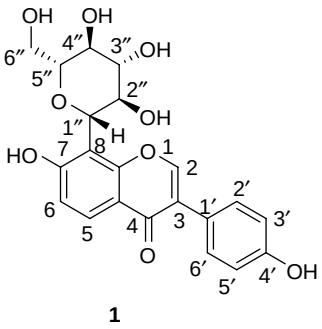
ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020.2010.505563
http://www.informaworld.com



1

Figure 1. The structure of puerarin (**1**).

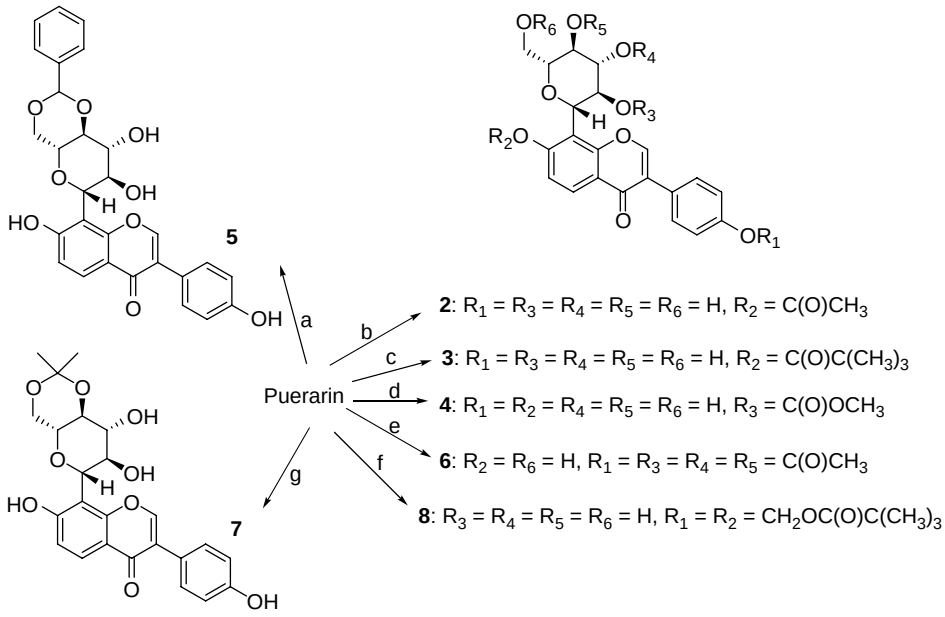
calcium loading. The neutrophils accumulate in the microcirculation, release inflammatory mediators, and contribute to microvascular obstruction and the no-reflow phenomenon in the reperfused myocardium [5].

Puerarin (**1**, daidzein-8-C-glucoside) (Figure 1) is a naturally occurring isoflavone, isolated from the root of *Pueraria lobata* (Willdenow) Ohwi, one of the most popular Chinese herbal medicines. Studies showed that **1** is an effective scavenger of active oxygen radicals such as hydroxyl and superoxide radicals, and this may result from the isoflavone's polyphenolic structure [6]. The results of laboratory and clinical studies [7] demonstrated that **1** can attenuate ischemia–reperfusion (I/R) injury and promote recovery of myocardial function by mechanisms such as inhibiting myocardial apoptosis [8], increasing SOD activity [9], downregulating Bax expression, upregulating Bcl-2 [10], inhibiting the production of proinflammatory cytokines [11], improving the myocardial ultramicrostucture [12], refraining the calcium overload [13], etc. It has also been reported that **1** protects the myocardium against ischemia and reperfusion injury via inhibiting mitochondrial permeability transition pore opening and activating the mitochondrial ATP-sensitive potassium channel [14]. Although **1** has beneficial effects in cases of myocardial injury, the specific bioactivity in the laboratory and the efficacy in clinical trials remain low, and the solubility and bioavailability need to be improved. Therefore, optimizing the pharmacological activities against myocardial ischemia and reperfusion injury by modifying the structure of **1** is essential to improve its efficacy in clinical trials. Herein, seven derivatives of **1** were synthesized and their cardioprotective effects on the myocardial ischemia and reperfusion injury were evaluated.

2. Results and discussion

There are two phenolic hydroxyl groups and four alcoholic hydroxyl groups in the structure of **1**, which could respectively be modified according to the reaction activity distinction resulting from different positions in the structure. In order to probe the key part of the molecular structure that results in the protective effect on myocardial ischemia and reperfusion injury, search for the relevance between the activity in reducing myocardial I/R injury and the structure modified for the phenolic hydroxyl and alcoholic hydroxyl located at different positions in the molecule, four acylates of different alcoholic hydroxyls (**4**–**7**) and three acylates of different phenolic hydroxyls (**2**, **3** and **8**) were designed and synthesized (Scheme 1).

The phenolic hydroxyl group at C-7 could selectively be acylated at an alkaline medium such as the solution of anhydrous K_2CO_3 in acetone, on the basis of the unequal acidity (the acidity of phenolic hydroxyl at C-7 is larger than at C-4′). When the molar ratio of **1**, K_2CO_3, and acylating agent was 1:1:1, the acylate (**2** and **3**) of phenolic hydroxyl at C-7 was afforded as the main acylate. However, when the molar ratio was 1:2:2, the two phenolic hydroxyls at C-7 and C-4′ were together acylated, such as **8**. It is interesting that two sets of signals are observed in the ^{1}H NMR measurements of the acylates of phenolic hydroxyl at C-7 at room



Scheme 1. The synthesis of derivatives **2**–**8**. Reagent and conditions: (a) benzoic aldehyde, tosic acid, acetone, rt; (b) acetyl chloride, K_2CO_3, acetone, rt; (c) pivalyl chloride, K_2CO_3, acetone, rt; (d) methylchloroformate, K_2CO_3, acetone, rt; (e) acetic anhydride, boron trifluoride, acetone, rt; (f) chloromethyl pivalate, NaI, K_2CO_3, acetone, rt; (g) 2-methoxyprop-1-ene, tosic acid, acetone, rt.

temperature, which suggested that the acylation of phenolic hydroxyl at C-7 leads to the formation of two main conformational isomers, resulting from the crowding between the acylate group and the glucose group [15]. The alcoholic hydroxyl could also selectively be acylated at an acidic medium such as the solution of BF_3 in acetone. When a suitable reaction condition was selected, the acylate of alcoholic hydroxyl groups located at different positions could be obtained, such as **4**–**7**. In order to discover the structure that will show the more protective effect on myocardial ischemia and reperfusion injury, seven compounds synthesized were respectively evaluated at the Langendorff perfusion model of isolated rat heart and the models of the SD rat myocardial ischemia induced by posterior pituitary hormones. Some of them showed pronounced cardioprotective activity.

At the Langendorff perfusion model, the isolated hearts were prepared from SD rat. The experimental groups consist of model group, sham group, and medication group. The level of lactate dehydrogenase (LDH) in the effluent from the perfused heart was used to monitor the damage of myocardial tissues. The statistical significance was compared between the medication groups and the model group by Student's *t*-test. The results are presented in Table 1.

LDH, one of important metabolic enzymes in cardiomyocytes, has been regarded as reliable indices with which to evaluate myocardial I/R injury. As shown in Table 1, I/R caused a significant increase in the level of LDH compared with the sham group. The release of LDH was reduced by treatment with **1** and its six derivatives except for **8**, and, especially, **3** and **2** among them exhibit a significant decrease in the level of LDH compared with the positive control, which showed that the cardioprotective activities of **3** and **2** markedly exceed **1**. This indicated that

Table 1. The effect of puerarin and its derivatives on I/R injury in the model of isolated rat heart.

Group	C, 10^{-4} mol/l	N	LDH activity (U/l g wet wt) Pre-I	LDH activity (U/l g wet wt) Post-RP	LDH active ratio Post-RP/Pre-I
Blank control		5	273 ± 77	216 ± 68	0.80 ± 0.13
Ischemic model		12	222 ± 69	638 ± 164	3.04 ± 0.83
2	2.18	5	318 ± 64	677 ± 102	2.21 ± 0.62
3	2.00	5	256 ± 120	418 ± 174	1.74 ± 0.89*
3	0.20	5	210 ± 32	634 ± 124	3.01 ± 0.26
4	2.11	5	193 ± 39	507 ± 96	2.67 ± 0.49
5	1.98	5	204 ± 50	542 ± 9	2.32 ± 0.46
7	2.19	5	199 ± 102	494 ± 136	2.75 ± 0.98
8	1.55	5	180 ± 42	536 ± 109	3.21 ± 1.26
Puerarin	2.40	5	273 ± 34	752 ± 222	2.85 ± 1.03

Notes: C, concentration; LDH, lactate dehydrogenase; Pre-I, pre-ischemia; Post-RP, post-reperfusion. $*P < 0.05$ vs. Ischemic model.

the acylated modifications of phenolic hydroxyl at C-7 in **1** may improve the cardioprotective activity, and the larger the bulk of the acylating agent, the greater the effect on activity. The reason could be that the bulky volume of the group linking with phenolic hydroxyl at C-7 leads to the larger change of conformation of **1** due to the crowding between the acylating group and the glucose group.

In the model of myocardial ischemia induced by pituitrin, the SD rats were anesthetized and their normal electrocardiogram (ECG) II leads were recorded. Then, hypophysin was administered i.v., and ECGs were continuously recorded for 5 min, and the ST-segment changes were observed. In two medication groups, the SD rats were, respectively, administered by hypodermic injection of 20 and 40 mg/kg derivatives of **1**, everyday once, lasting for 7 days, and after 30 min of the eventual time, pituitrin (0.5 U/kg) was administered i.v. to induce myocardial ischemia. The ST-segment elevation (ST-E) in the ECG was used to detect and quantify myocardial ischemia. The results are presented in Tables 2 and 3.

Assessment of the ST-segment of the ECG plays a pivotal role in the diagnosis and management of ischemic heart disease. The presence of ST-E is a diagnostic

Table 2. The effect of puerarin derivatives (20 mg/kg, hypo. 7 days) on the ST elevation in ECG in SD rats of myocardial ischemia induced by pituitrin.

Compound	N	ST-E (mV) Pre-ischemia	ST-E (mV) Post-ischemia	ST-E change
Model	15	0.091 ± 0.015	0.26 ± 0.05	0.167 ± 0.053
2	5	0.093 ± 0.015	0.21 ± 0.03	0.118 ± 0.018
3	5	0.1 ± 0	0.206 ± 0.017	0.106 ± 0.017*
4	5	0.096 ± 0.01	0.263 ± 0.03	0.172 ± 0.028
5	5	0.086 ± 0.018	0.23 ± 0.019	0.147 ± 0.034
6	5	0.0864 ± 0.019	0.22 ± 0.038	0.131 ± 0.023
7	5	0.093 ± 0.015	0.21 ± 0.021	0.115 ± 0.03
8	5	0.1 ± 0	0.17 ± 0.04	0.072 ± 0.036**
Puerarin	5	0.104 ± 0.009	0.198 ± 0.023	0.098 ± 0.023*

Note: $*P < 0.05$, $**P < 0.01$ vs. blank control.

Table 3. The effect of puerarin derivatives (40 mg/kg, hypo. 7 days) on the ST elevation in ECG in SD rats of myocardial ischemia induced by pituitrin.

Compound	*N*	ST-E (mV)		
		Pre-ischemia	Post-ischemia	ST-E change
Model	15	0.091 ± 0.015	0.26 ± 0.05	0.167 ± 0.053
2	5	0.093 ± 0.015	0.264 ± 0.052	0.171 ± 0.048
3	5	0.1 ± 0.004	0.19 ± 0.028	0.092 ± 0.028*
4	5	0.086 ± 0.018	0.231 ± 0.04	0.145 ± 0.037
5	5	0.093 ± 0.015	0.231 ± 0.023	0.138 ± 0.027
6	5	0.080 ± 0.018	0.21 ± 0.019	0.13 ± 0.024
7	5	0.096 ± 0.009	0.27 ± 0.05	0.175 ± 0.05
8	5	0.089 ± 0.017	0.178 ± 0.018	0.092 ± 0.015**
Puerarin	5	0.1 ± 0	0.198 ± 0.028	0.099 ± 0.028*

Note: $*P < 0.05$, $**P < 0.01$ vs. blank control.

criterion of acute myocardial ischemia. In this study, the impact of the preconditioning by derivatives of **1** on the amounts of elevation of the ST-segment was assessed in a model of SD rat myocardial ischemia induced by pituitrin. As shown in Tables 2 and 3, the myocardial ischemia induced by pituitrin caused a significant increase in the degree of ST-E compared with pre-ischemia, and the degree of elevation of the ST-segments was markedly reduced in rats pretreated by some derivatives of **1**; especially **3** and **8**, at both 20 and 40 mg/kg doses, exhibited a significant decrease in the degree of ST-E, which showed the potential cardioprotective activity of **3** and **8**. For **3**, the results evaluated in the cardioprotective activity in a model of SD rat myocardial ischemia induced by pituitrin were conformed to the results at the Langendorff perfusion model of isolated rat heart. However, as for **8**, the results evaluated at two models were inconsistent, which may be explained by the metabolite of **8**. In the molecular structure of **8**, two phenolic hydroxyls at C-7 and C-4′ had been acylated together by chloromethyl pivalate. In the presence of the esterolytic enzyme, the pivalate group at C-4′ in the molecule of **8** was easily hydrolyzed, but the hydrolysis of the pivalate group at C-7 was difficult due to the barrier shield from the bulky glucosyl unit. In other words, the cardioprotective activity of **8** at the ischemia model of SD rat may result from the metabolite of **8**, not from **8** itself. The hypothesis needs to be further confirmed by the study on the metabolite of **8**.

3. Conclusion

In summary, by the structural modification of **1** at different positions, seven new derivatives of **1** were obtained, and their cardioprotective activities were, respectively, evaluated in the model of myocardial I/R injury of Langendorff isolated rat heart and the model of SD rat myocardial ischemia induced by pituitrin. According to the results, the cardioprotective activities of **3** and **8** markedly exceeded **1**, which suggested that the acylated modification of phenolic hydroxyl at C-7 in **1** may improve the cardioprotective activity, and the larger the bulk of the acylating agent, the greater the effect on activity, which will be an important reference for further structural optimization.

4. Experimental

4.1 General experimental procedures

Melting points were recorded on a Yanaco melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian Oxford 300, using TMS as an

internal standard. Chemical shifts (δ) are expressed in ppm. Mass spectra were obtained on an Autospec-Ultima ETOF mass spectrometer for FAB-MS. HR-MS was recorded on Agilent LC/MSD TOF.

4.2 Compound 2

To a solution of puerarin (0.416 g, 1 mmol) in acetone (50 ml), anhydrous K_2CO_3 (0.138 g, 1 mmol) was added. After stirring for 10 min, acetyl chloride (1 mmol) was added dropwise at room temperature, and the mixture was stirred until the reaction was complete (monitored by TLC). After the removal of the solvent, the residue was diluted with ethyl acetate, and washed, respectively, with water and sat. aqueous solution of NaCl. The isolated organic layer was dried with anhydrous Na_2SO_4. The evaporation of the organic solvent gave the crude product, which was purified by chromatography on a column of silica gel (ethyl acetate/methanol = 10/1) to afford 7-*O*-acetyl puerarin (0.289 g, 63%), a white solid, mp 194–196°C. ^{1}H NMR (300 MHz, CD_3OD, ppm) δ: 8.28 and 8.26 (1H, s, H-2), 8.18 (1H, d, $J = 8.7$ Hz, H-5), 7.36 (2H, d, $J = 7.8$ Hz, H-2′, 6′), 7.18 and 7.15 (1H, d, $J = 8.7$ Hz, H-6), 6.80 (2H, d, $J = 7.8$ Hz, H-3′, 5′), 1.37 (3H, s, $-CH_3$), 5.05 and 4.67 (1H, d, $J = 9.9$ Hz, H-1″), 3.30–4.15 (6H, m, H-2″-H-6″). FAB-MS: *m/z* 459 $[M + H]^+$. HR-ESI-MS: *m/z* 459.1277 $[M + H]^+$ (calcd for $C_{23}H_{23}O_{10}$, 459.1285).

4.3 Compound 3

To a solution of puerarin (0.416 g, 1 mmol) in acetone (50 ml), anhydrous K_2CO_3 (0.138 g, 1 mmol) was added. After stirring for 10 min, the pivalyl chloride (1 mmol) was added dropwise at room temperature, and the mixture was stirred until the reaction was complete (monitored by TLC). After the removal of the solvent, the residue was diluted with ethyl acetate, and washed, respectively, with water and sat. aqueous solution of NaCl. The isolated organic layer was dried with anhydrous Na_2SO_4. The evaporation of the organic solvent gave the crude product, which was purified by chromatography on a column of silica gel (ethyl acetate/methanol = 10/1) to afford 7-*O*-pivalyl puerarin (0.345 g, 69%), a white solid, mp 216–218°C. ^{1}H NMR (300 MHz, CD_3OD, ppm) δ: 8.26 and 8.25 (1H, s, H-2), 8.17 (1H, d, $J = 9.0$ Hz, H-5), 7.35 (2H, d, $J = 8.4$ Hz, H-2′, 6′), 7.17 and 7.10 (1H, d, $J = 9.0$ Hz, H-6), 6.80 (2H, d, $J = 8.4$ Hz, H-3′, 5′), 1.37 (9H, s, $-CH_3$), 5.05 and 4.66 (1H, d, $J = 9.9$ Hz, H-1″), 3.22–4.16 (6H, m, H-2″-H-6″). FAB-MS: *m/z* 50 $[M + H]^+$. HR-ESI-MS: *m/z* 501.1743 $[M + H]^+$ (calcd for $C_{26}H_{29}O_{10}$, 501.1755).

4.4 Compound 4

To a solution of puerarin (0.416 g, 1 mmol) in acetone (50 ml), anhydrous K_2CO_3 (0.138 g, 1 mmol) was added. After stirring for 10 min, methyl chloroformate (1 mmol) was added dropwise at room temperature, and the mixture was stirred until the reaction was complete (monitored by TLC). After the removal of the solvent, the residue was diluted with ethyl acetate, and washed, respectively, with water and sat. aqueous solution of NaCl. The isolated organic layer was dried with anhydrous Na_2SO_4. The evaporation of the organic solvent gave the crude product, which was purified by chromatography on a column of silica gel (ethyl acetate/methanol = 10/1) to afford 10-*O*-methoxy formyl puerarin (0.25 g, 53%), a white solid, mp 180–182°C. ^{1}H NMR (300 MHz, CD_3OD, ppm) δ: 8.16 (1H, s, H-2), 7.98 (1H, d, $J = 8.7$ Hz, H-5), 7.33 (2H, d, $J = 8.4$ Hz, H-2′, 6′), 6.89 (1H, d, $J = 8.7$ Hz, H-6), 6.78 (2H, d, $J = 8.4$ Hz, H-3′, 5′), 3.39 (3H, s, $-OCH_3$), 5.38 (1H, dd, $J = 9.9$, 9.0 Hz, H-2″), 5.15 (1H, d, $J = 9.9$ Hz, H-1″), 3.42–3.85 (5H, m,

H-3″-H-6″). FAB-MS: *m/z* 475 $[M + H]^+$. HR-ESI-MS: *m/z* 475.1226 $[M + H]^+$ (calcd for $C_{23}H_{23}O_{11}$, 475.1234).

4.5 Compound 5

To a solution of puerarin (0.416 g, 1 mmol) and *p*-toluenesulfonic acid (two grains) in acetone (50 ml), benzaldehyde (1.2 mmol) was added dropwise at 0°C under stirring. The mixture was stirred at room temperature until the reaction was complete (monitored by TLC). Then, 5 ml solution of $NaHCO_3$ was added for the termination of the reaction. After the removal of the solvent, the residue was diluted with ethyl acetate, and washed, respectively, with water and aqueous solution of $NaHCO_3$. The isolated organic layer was dried with anhydrous Na_2SO_4. The evaporation of the organic solvent gave the crude product, which was purified by chromatography on a column of silica gel (ethyl acetate/methanol = 10/1) to afford **5** (0.24 g, 48%), a solid, mp 199–201°C. ^{1}H NMR (300 MHz, CD_3OD, ppm) δ: 8.16 (1H, s, H-2), 8.00 (1H, d, $J = 9.0$ Hz, H-5), 7.31 (2H, d, $J = 8.4$ Hz, H-2′, 6′), 6.95 (1H, d, $J = 9.0$ Hz, H-6), 6.79 (2H, d, $J = 8.4$ Hz, H-3′, 5′), 7.48 (2H, m, ArH), 7.29 (3H, m, ArH), 5.59 (1H, s, $PhCH(O)_2$), 4.32 (1H, dd, $J = 10.2$, 8.1 Hz, H-2″), 5.15 (1H, d, $J = 10.2$ Hz, H-1″), 4.22 (1H, dd, $J = 10.5$, 4.8 Hz, H-6″), 3.76 (1H, dd, $J = 10.5$, 6.6 Hz, H-6″), 3.69 (1H, dd, $J = 8.1$, 7.8 Hz, H-3″), 3.63 (1H, dd, $J = 9.3$, 7.8 Hz, H-4″), 3.57 (1H, m, H-5″). FAB-MS: *m/z* 505 $[M + H]^+$. HR-ESI-MS: *m/z* 505.1479 $[M + H]^+$ (calcd for $C_{28}H_{25}O_9$, 505.1493).

4.6 Compound 6

To a solution of puerarin (0.416 g, 1 mmol) in acetone (50 ml), acetic anhydride (5 mmol) and a solution of boron trifluoride in ether (0.2 ml) were added at 0°C under stirring. The mixture was stirred at room temperature until the reaction was complete (monitored by TLC). Ethanol (5 ml) was added and the mixture was stirred for 1 h. Then, a solution of $NaHCO_3$ was added. After the removal of the solvent, the residue was diluted with ethyl acetate, and washed, respectively, with water and a sat. aqueous solution of $NaHCO_3$. The isolated organic layer was dried with anhydrous Na_2SO_4. The evaporation of the organic solvent gave the crude product, which was purified by chromatography on a column of silica gel (ethyl acetate/methanol = 10/1) to afford **6** (0.210 g, 36%), a solid, mp 126–128°C. ^{1}H NMR (300 MHz, CD_3OD, ppm) δ: 8.34 (1H, s, H-2), 7.99 (1H, d, $J = 9.0$ Hz, H-5), 7.55 (2H, d, $J = 8.4$ Hz, H-2′, 6′), 6.92 (1H, d, $J = 9.0$ Hz, H-6), 7.12 (2H, d, $J = 8.4$ Hz, H-3′, 5′), 5.80 (1H, d, $J = 10.2$ Hz, H-1″), 5.31 (1H, m, H-2″), 5.25 (1H, m, H-3″), 3.98 (1H, m, H-4″), 3.54–3.84 (3H, m, H-5″-H-6″), 2.23 (3H, s, $COCH_3$), 1.96 (3H, s, $COCH_3$), 1,49 (3H, s, $COCH_3$), 1.32 (3H, s, $COCH_3$). FAB-MS: *m/z* 585 $[M + H]^+$. HR-ESI-MS: *m/z* 585.1586 $[M + H]^+$ (calcd for $C_{29}H_{29}O_{13}$, 585.1602).

4.7 Compound 7

To a solution of puerarin (0.416 g, 1 mmol) and *p*-toluenesulfonic acid (two grains) in acetone (50 ml), 2-methoxyl propylene (1.2 mmol) was added dropwise at 0°C under stirring. The mixture was stirred at room temperature until the reaction was complete (monitored by TLC). Then, 5 ml solution of $NaHCO_3$ was added. After the removal of the solvent, the residue was diluted with ethyl acetate, and washed, respectively, with water and sat. aqueous solution of $NaHCO_3$. The isolated organic layer was dried with anhydrous Na_2SO_4. The evaporation of the organic solvent gave the crude product, which was purified by chromatography on a column of silica gel (ethyl acetate/methanol = 10/1) to afford **7** (0.27 g, 59%), a solid, mp 230–232°C. ^{1}H NMR (300 MHz, CD_3OD, ppm)

δ: 8.17 (1H, s, H-2), 8.01 (1H, d, $J = 9.0$ Hz, H-5), 7.33 (2H, d, $J = 8.7$ Hz, H-2′, 6′), 6.94 (1H, d, $J = 9.0$ Hz, H-6), 6.79 (2H, d, $J = 8.7$ Hz, H-3′, 5′), 4.26 (1H, t, $J = 9.6$ Hz, H-2″), 5.06 (1H, d, $J = 9.6$ Hz, H-1″), 3.80 (2H, m, H-6″), 3.69 (1H, dd, $J = 9.6$, 9.3 Hz, H-3″), 3.54 (1H, dd, $J = 9.3$, 9.0 Hz, H-4″), 3.39 (1H, m, H-5″), 1.51 (3H, s, CH_3), 1.36 (3H, s, CH_3). FAB-MS: *m/z* 457 $[M + H]^+$. HR-ESI-MS: *m/z* 457.1479 $[M + H]^+$ (calcd for $C_{24}H_{25}O_9$, 457.1493).

4.8 Compound 8

To a solution of chloromethyl pivalate (2.05 g, 13.6 mmol) in anhydrous acetone (30 ml), anhydrous NaI (2.04 g, 13.6 mmol) was added, and the mixture was sealed overnight, then was added to a solution of puerarin (2.8 g, 6.8 mmol) and anhydrous K_2CO_3 (1.9 g, 13.6 mmol) in acetone (150 ml) under stirring, and the mixture was stirred until the reaction was complete (monitored by TLC). After the removal of the solvent, the residue was diluted with ethyl acetate (150 ml), and washed, respectively, with water and sat. aqueous solution of NaCl. The isolated organic layer was dried with anhydrous Na_2SO_4. The evaporation of the organic solvent gave the crude product, which was purified by chromatography on a column of silica gel (ethyl acetate/methanol = 10/1) to afford **8** (1.37 g, 31.3%), a white solid, mp 176–178°C. ^{1}H NMR (300 MHz, CD_3OD, ppm) δ: 8.19 (1H, s, H-2), 8.21 (1H, d, $J = 7.5$ Hz, H-5), 7.36 (2H, d, $J = 8.4$ Hz, H-2′, 6′), 7.38 (1H, d, $J = 7.5$ Hz, H-6), 6.80 (2H, d, $J = 8.4$ Hz, H-3′, 5′), 6.01 (2H, s, —OCH_2-O—), 5.90 (2H, m, —OCH_2O—), 5.02 and 4.96 (1H, d, $J = 9.9$ Hz, H-1″), 3.25–4.17 (6H, m), 1.10 (9H, s, —CH_3), 1.16 (9H, s, —CH_3). FAB-MS: *m/z* 645 $[M + H]^+$. HR-ESI-MS: *m/z* 645.2531 $[M + H]^+$ (calcd for $C_{33}H_{41}O_{13}$, 645.2541).

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