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Design, synthesis and biological evaluation of glucose-containing scutellarein derivatives as neuroprotective agents based on metabolic mechanism of scutellarin in vivo

Nian-Guang Li [a,b], Min-Zhe Shen [a], Zhen-Jiang Wang [a], Yu-Ping Tang [a,*], Zhi-Hao Shi [a,c], Yi-Fan Fu [a], Qian-Ping Shi [a,b], Hao Tang [a], Jian-Ao Duan [a,*]

[a] Jiangsu Key Laboratory for High Technology Research of TCM Formulae, Nanjing University of Chinese Medicine, Nanjing 210046, China
[b] Department of Medicinal Chemistry, Nanjing University of Chinese Medicine, Nanjing 210046, China
[c] Department of Organic Chemistry, China Pharmaceutical University, Nanjing 211198, China

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ABSTRACT

Based on metabolic mechanism of scutellarin in vivo that scutellarin could be hydrolyzed into scutellarein by β-glucuronide enzyme, some glucose-containing scutellarein derivatives were designed and synthesized through the introduction of glucose moiety at C-7 position of scutellarein via a glucosidic bond. Biological activity evaluation showed that these glucose-containing scutellarein derivatives exhibited potent DPPH radical scavenging activities. Furthermore, the improvement of physicochemical properties such as anticoagulant and neuroprotective activities alongside with the water solubility was achieved by introducing glucose. These findings suggest that the introduction of the glucose moiety to scutellarein wattants further development of this kind of compounds as neuroprotective agents.

Traditional Chinese medicines (TCMs) have been used clinically for many years and can be regarded as potential sources for drug discovery. Scutellarin (**1**) (Fig. 1), which is the main effective constituent of breviscapine (>85%), a clinic natural drug consisting of total flavonoids of *Erigeron breviscapus* (Vant.) Hand-Mazz. (Compositae), has been used for the treatment of cerebral infarction, coronary heart disease, and angina pectoris in China.[1] Due to the distinguished efficacy of scutellarin in the clinical therapy, the research of scutellarin has become a hot topic in China in recent years. Pharmacological studies have demonstrated that scutellarin is associated with a wide range of benefits to brain injury caused by cerebral ischemia/reperfusion, these benefits are due to its antioxidant and anticoagulant activities to attenuate neuronal damage.[2–4]

Pharmacokinetic studies on scutellarin have been investigated in rats,[5–7] dogs[8] and humans[9] after oral administration, and the results showed that the oral bioavailability of scutellarin was quite poor.[10] One reason was its poor aqueous solubility and low lipophilicity,[11] its poor ability to penetrate cell membranes has long been a major impediment to its overall effectiveness as an oral drug. The other reason was that scutellarin is readily converted into scutellarein (**2**) (Fig. 2) before absorption, the latter is relatively easily absorbed into the blood and can metabolite into glucuronidated, sulfated or methylated forms.[12] Due to the low bioavailability after oral administration, direct administration of scutellarin by injection is the most common route of administration in clinical settings. Nonetheless, therapeutic effects elicited by breviscapine require repeated injection daily for a long time, this is highly inconvenient and results in low patient compliance.

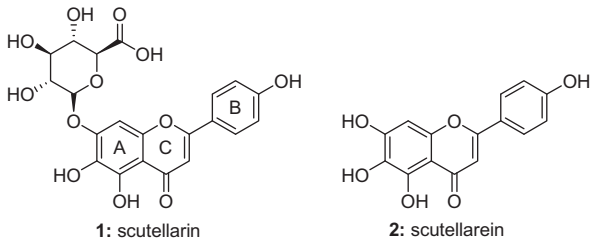

Figure 1. Chemical structures of scutellarin (**1**) and scutellarein (**2**).

* Corresponding authors. Tel./fax: +86 25 85811916.
E-mail addresses: yupingtang@njutcm.edu.cn (Y.-P. Tang), dja@njutcm.edu.cn (J.-A. Duan).

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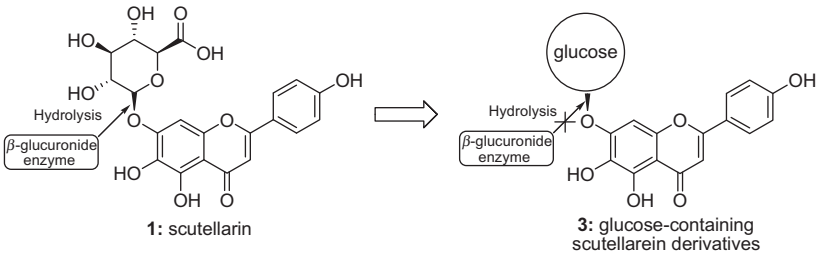
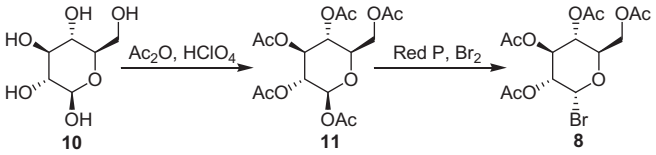


Figures 2. Design of glucose-containing scutellarein derivatives.

It has been reported that scutellarin (**1**) is readily hydrolyzed into scutellarein (**2**) by β-glucuronide enzyme and the microbial population in the intestine prior to absorption.[12] Since the glucose connected to scutellarein via a glucosidic bond is unlikely to be cleaved by β-glucuronide enzyme and the microbial population in the intestine, in the present study, the glucose moiety was introduced to scutellarein at C-7 position via a glucosidic bond (**3**) (Fig. 2). We assessed the anti-oxidation and anticoagulant activities, as well as the neuroprotective activities of **3a–3c** to investigate whether the biological activities of parent scutellarin **1** was retained in these glucose-containing scutellareins. Furthermore, the solubility of these glucose-containing scutellarein derivatives was also evaluated.

The regioselective D-glucose-containing scutellarein derivative (**3a**) was achieved using the Mitsunobu method,[13] which involved the coupling of protected scutellarein (**7**) with protected glucose (**8**) (Scheme 1). Scutellarin (**1**) was firstly hydrolyzed by refluxing with 6 N HCl in ethanol under a N_2 atmosphere to generate scutellarein (**4**) in 17.0% yield. Compound **4** was then converted into **5** (78.9% yield) after it was reacted with acetic anhydride and catalytic 4-*N*,*N*-dimethylaminopyridine (DMAP) in pyridine. Interestingly, when compound **5** reacted with BnBr using K_2CO_3 and catalytic KI in dry acetone, the desired intermediate **6** was obtained in 70% yield. Deprotection of the benzyl group in **6** was accomplished under hydrogenation conditions with 10% palladium on carbon as the catalyst in EtOH/CH_2Cl_2 gave **7** in 95% yield. Glycosylation of hydroxyl group took place completely at the position 7 using 2.5 equiv of K_2CO_3 and 2.0 equiv of AgO as bases in quinoline that led to **9**.[14] Finally, the hydrolysis of acetyl groups in **9** with a solution of sodium hydroxide afforded **3a** in 41% yield.

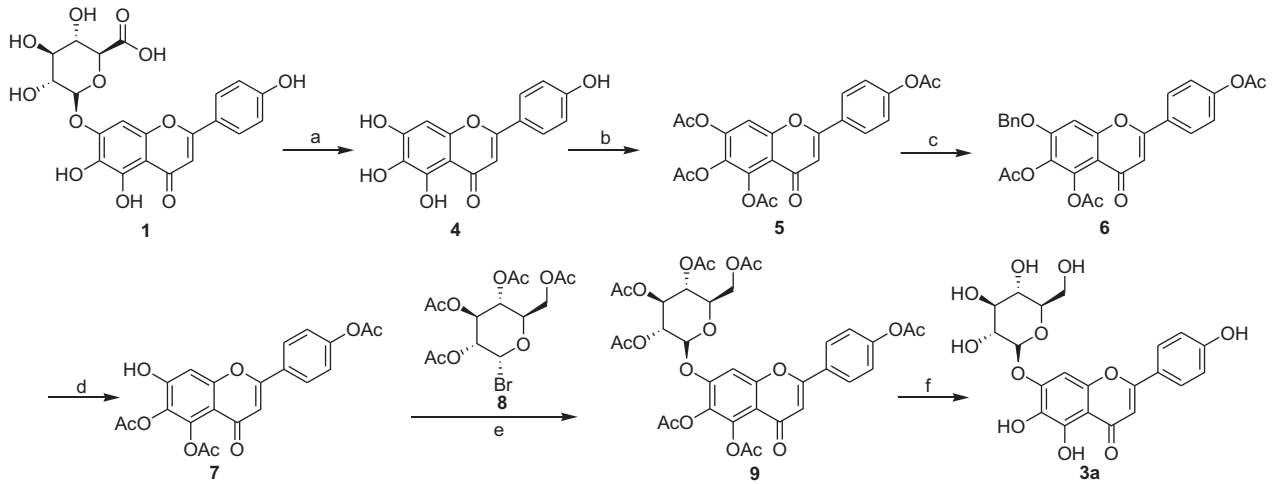


Scheme 2. Reagents and conditions: (a) Ac_2O (10.0 equiv, $HClO_4$ (0.05 equiv); (b) red phosphorus (0.2 equiv), Br_2 (10 equiv), H_2O, 25 °C, 40% over two steps.

O-Acetyl-D-glucosyl bromide **8** was synthesized from D-glucose (**10**) in two steps as shown in Scheme 2, firstly, penta-*O*-acetyl-D-glucose (**11**) was synthesized after D-glucose (**10**) was reacted with acetic anhydride under the catalyst of perchloric acid, then the reaction mixture was reacted with red phosphorus and liquid bromine directly without separation, and the target *O*-acetyl-D-glucosyl bromide **8** was obtained in 40% yield over these two steps.

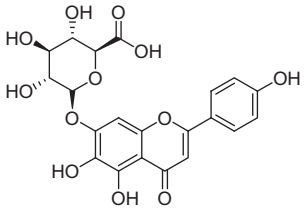
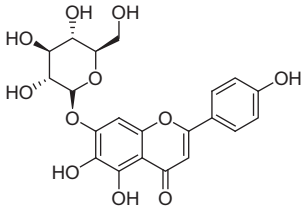
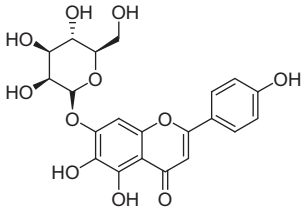
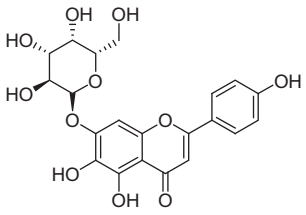
The formation of **3b** and **3c** were obtained through the introduction of *O*-acetyl-D-manmosyl bromide and *O*-acetyl-D-galactopyranosyl bromide, respectively, using a procedure similar to that described above.

The antioxidant activities of the synthesized glucose-containing scutellarein derivatives **3a–3c** were evaluated by examining DPPH radical scavenging according to our previous procedure.[15] For comparison purposes, the antioxidant activity of scutellarin (**1**) was included as positive controls. As shown in Table 1, the synthesized compounds showed DPPH radical scavenging activities (IC_{50} = 25.31–34.86 μM) that were similar to those of the scutellarin (**1**) (IC_{50} = 26.78 μM), which indicated that the introduction of the glucose moiety had little effect on the change of DPPH radical



Scheme 1. Reagents and conditions: (a) 6 N Concentrated hydrochloric acid, EtOH, N_2, 120 °C, 17.0%; (b) Ac_2O (10.0 equiv), pyridine (10.0 equiv), DMAP (0.1 equiv), 25 °C, 12 h, 78.9%; (c) $PhCH_2Br$ (3.0 equiv), K_2CO_3 (7.0 equiv), KI (1.0 equiv), acetone, reflux, 6 h, 70%; (d) Pd/C (10 wt %), H_2 (1 atm), CH_2Cl_2/Et_2OH, 25 °C, 24 h, 95%; (e) **8** (4.0 equiv), $CuSO_4$ (2.5 equiv), AgO (2.0 equiv), quinoline, 25 °C, 12 h, 40%; (f) NaOH (2.5 M), N_2, $CHCl_3$, 0 °C, 41%.

Table 1
In vitro antioxidatant activity in DPPH assay (IC_{50} in μM) and water solubility of glucose-containing scutellarein derivatives

Compd	Structure	IC_{50} (μM)	Solubility (μM)
1		26.78	7.53
3a		25.31	7.85
3b		34.86	8.19
3c		28.42	8.93

scavenging activity. Furthermore, the results showed that the DPPH radical scavenging activities did not appear to be altered significantly by the type of glucose linkage in these three glucose-containing scutellarein derivatives.

PC12 cells can adopt a neuronal phenotype and have been used extensively as a model for catecholamine-secreting neuronal cells.[16] Active mitochondria of living cells can cleave MTT to produce formazan, the amount of which it directly related to the number of living cell. So the neuroprotective effects of the synthesized glucose-containing scutellarein derivatives **3a–3c** were evaluated by protective effects on H_2O_2-induced cytotoxicity in PC12 cells using MTT assay method.[17] As shown in Table 2, PC12 cells viability markedly decreased after PC12 cell were exposed to H_2O_2.

Table 2
Attenuation of H_2O_2-induced PC12 cell damage by glucose-containing scutellarein derivatives (mean ± S.D., $n = 4$)

Compd (μM)	A517	Inhibiting rate (%)
Normal	0.728 ± 0.081	—
H_2O_2	0.464 ± 0.033	—
1/50	0.672 ± 0.022	78.79
1/25	0.603 ± 0.051	52.65
3a/50	0.675 ± 0.026	79.92
3a/25	0.637 ± 0.035	65.53
3b/50	0.613 ± 0.073	56.44
3b/25	0.526 ± 0.046	23.48
3c/50	0.684 ± 0.043	83.33
3c/25	0.625 ± 0.066	60.98

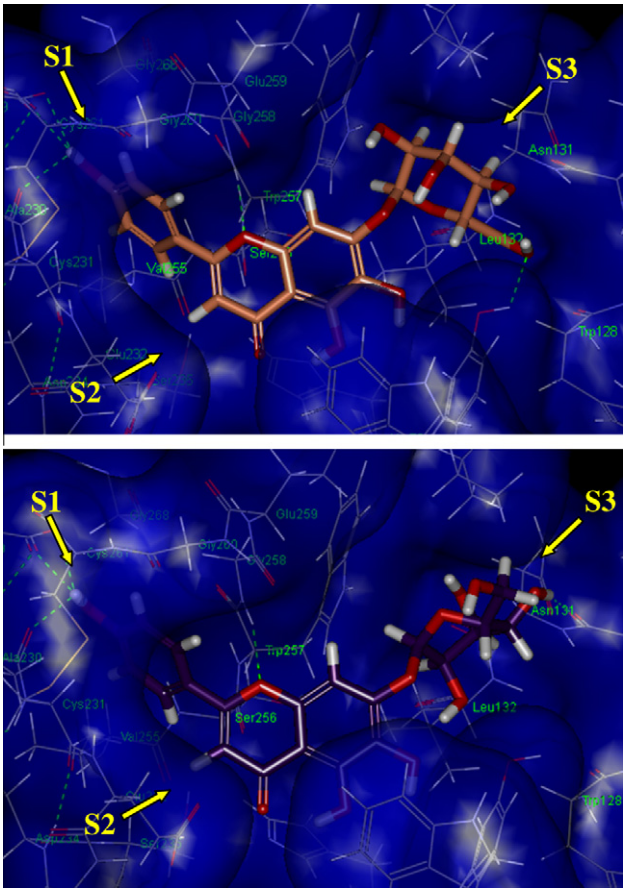
However, when the PC12 cells were co-incubated with scutellarin (**1**) and glucose-containing scutellarein derivatives **3a–3c**, H_2O_2-induced cell toxicity was significantly attenuated, and the protective effect of these compounds was resulted in concentration-dependent increases in cell survival (Table 2). Of the compounds tested, although no clear structure–activity relationship was found, **3a** and **3c** were more effective at protecting these neuronal cells against H_2O_2-induced oxidative injury than scutellarin (**1**). **3c** showed the most potency in the inhibition of oxidative injury, with inhibiting rates were 83.33% and 60.98% at 50 and 25 μM, respectively (Table 2), the inhibiting rates of the parent compounds **1** were 78.79% and 52.65% at 50 and 25 μM, respectively. Based on these findings, we conclude that glucose addition to the parent structures had some positive effect on the protective actions of the parent compounds against H_2O_2-induced oxidative neuronal damage.

Because the anticoagulant activities can be assessed through the inhibition of thrombin activities, which can be evaluated through the analyzation of the prolongation of the plasma clotting time of thrombin time (TT), activated partial thromboplastin time (APTT), prothrombin time (PT), and reduction of fibrinogen (FIB) content according to our previous studies,[18] so the anticoagulant activities of these glucose-containing scutellarein derivatives were investigated for TT, PT, APTT and FIB and the results were shown in Table 3. The most active compound was **3c**, it significantly prolonged TT (29.17 s) and APTT (39.62 s), increased PT (9.93 s) and decreased FIB content (20.81 g/L) compared to scutellarin (**1**). In addition, **3a** had stronger anticoagulant activity than scutellarin (**1**), although it decreased TT (18.13 s), however, it prolonged APTT (43.06 s) and PT (7.81 s), decreased FIB content (32.43 g/L) compared to scutellarin (**1**). **3b** showed no more active effect on plasma coagulation parameters than **1**, despite it prolonged APTT (38.20 s), decreased FIB content (26.68 s), but TT (19.54 s) and PT (6.57 s) decreased compared to **1**. These results indicated that 7-hydroxyl position can be modified by glucosyl group without reducing the anticoagulant activity.

In the thrombin inhibition activity tests, **3c** showed the strongest inhibitory activity on thrombin, so scutellarin (**1**) and **3c** was selected for the subsequent molecular docking experiment with thrombin (2R2M) (Fig. 3). There were three pockets (S1, S2, S3) in the thrombin as suggested by molecular modeling augured well for anticipating in vitro activity as well according to our previous studies.[18] In the binding mode of the scutellarin (**1**) with thrombin (2R2M), as shown in the up part of Figure 3, the B ring in scutellarin (**1**) mainly interacted with S1 pocket, the C ring mainly interacted with S2 pocket, and the glucuronic acid group in A ring mainly interacted with the S2 pocket. In the binding mode of the **3c** with thrombin (2R2M), as shown in the down part of Figure 3, the B ring in **3c** mainly interacted with S1 pocket, the C ring mainly interacted with S2 pocket, and the galactopyranosyl group in A ring mainly interacted with the S2 pocket. As displayed in the up part of Figure 4, scutellarin (**1**) formed five hydrogen bonds with the active site residues of 2R2M in the binding mode, and the active site residues were Tyr83, Asp229, Ala230 and

Table 3
The anticoagulant activities of glucose-containing scutellarein derivatives (mean ± S.D., $n = 4$)

Compd (100 μM)	Plasma coagulation parameters			
	TT (s)	PT (s)	APTT (s)	FIB (g/L)
1	21.35 ± 1.45	6.91 ± 0.17	33.06 ± 1.79	37.96 ± 1.48
3a	18.13 ± 1.59	7.81 ± 0.76	43.06 ± 1.78	32.43 ± 1.05
3b	20.54 ± 1.25	6.57 ± 0.62	38.20 ± 1.96	26.68 ± 1.32
3c	29.17 ± 1.78	9.93 ± 0.52	39.62 ± 1.58	20.81 ± 1.20



Figures 3. Scutellarin (**1**) (up) and **3d** (down) docked into the pockets of thrombin (2R2M).

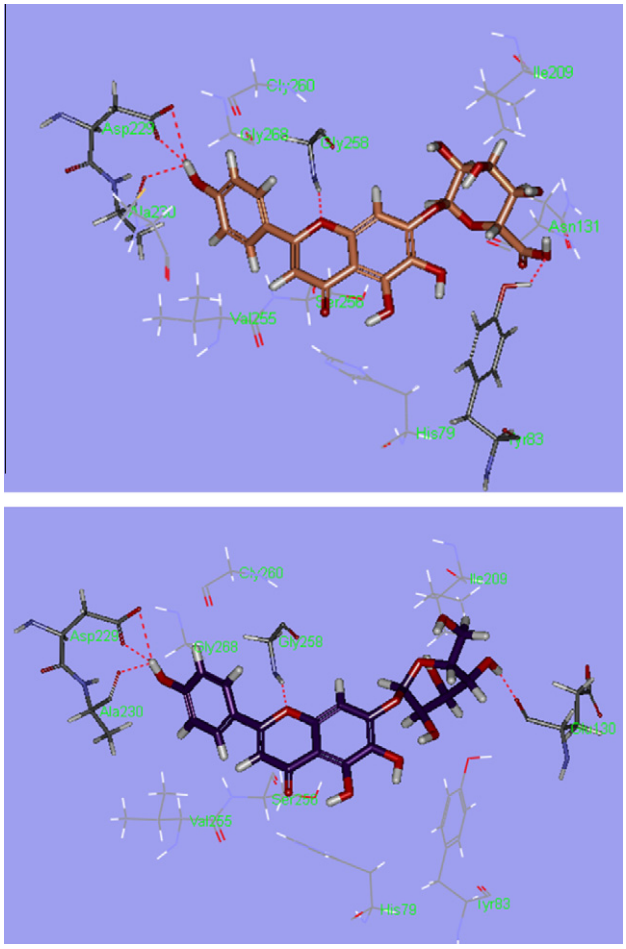


Figure 4. Scutellarin (**1**) (up) and **3d** (down) docked into residues of 2R2M, hydrogen bonding interactions are shown as red dashed lines.

Gly258, respectively. **3c** also formed five hydrogen bonds with the active site residues Glu130, Asp229, Ala230 and Gly258 of 2R2M (the down part of Fig. 4). These results confirmed our strategy for designing glucose-containing scutellarein derivatives as thrombin-inhibitors.

The aqueous solubility of the synthesized glucose-containing scutellarein derivatives has been determined using ultraviolet (UV) spectrophotometer method.[19,20] As presented in Table 1, the water solubitily of the glucose-containing scutellarein derivatives **3a**–**3c** was better compared to scutellarin (**1**). The best soluble compound was compound **3c** with its solubility in water was 8.93 μg/mL, which was greater than that of scutellarein (**2**). Compound **3a** showed similar water solubility compared with scutellarin (**1**), with its value was 7.85 μg/mL, and compound **3b** exhibited good water solubility with its value was 8.19 μg/mL. These results indicated that the introduction of glucosyl group at 7-hydroxyl position could help to improve the water solubility.

In conclusion, we synthesized glucose-containing scutellarein derivatives based on metabolic mechanism of scutellarin in vivo, and evaluated their antioxidant, anticoagulant and neuroprotective activities as well as their water solubility. The results showed these glucose-containing scutellarein derivatives exhibited potent DPPH radical scavenging activities. Furthermore, the improvement of physicochemical properties such as anticoagulant and neuroprotective activities alongside with the water solubility was achieved by introducing glucose. Taken together, these findings suggest that the introduction of the glucose moiety to scutellarein wattants further development this kind of compounds as neuroprotective agents.

Acknowledgments

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