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Synthesis of scutellarein derivatives to increase biological activity and water solubility

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ABSTRACT

In order to improve the biological activity and water solubility of scutellarin (**1**), some derivatives of its main metabolite (scutellarein) were designed and synthesized. All the compounds were tested for their thrombin inhibition activity through the analyzation of thrombin time (TT), activated partial thromboplastin time (APTT), prothrombin time (PT) and fibrinogen (FIB). Their antioxidant activities were assessed by measuring their scavenging capacities toward 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and the ability to protect PC12 cells against H_2O_2-induced cytotoxicity, their water solubility were also assessed by ultraviolet (UV) spectrophotometer. The results showed that compound **8b** demonstrated stronger anticoagulant and antioxidant activity, better water solubility compared with scutellarein (**2**), which warrants it as a promising agent for the treatment of ischemic cerebrovascular disease.

1. Introduction

As one of the leading causes of disability and death worldwide,[1] ischemic cerebrovascular disease has been a common and frequently-occurring disease that seriously endangers human health nowadays. Firstly, the increasing evidence[2] suggests that thrombin play a critical role in ischemic cerebrovascular disease, it acts as a multifunctional serine protease and catalyzes the proteolytic cleavage of the soluble plasma-protein fibrinogen to form insoluble fibrin, thus leading to clot formation. In addition, thrombin also serves as a potent platelet agonist and amplifies its own generation by feedback activation of several steps in the coagulation cascade.[3] Secondly, oxidative stresses are major causes of ischemic cerebrovascular disease,[4,5] some reactive oxygen species (ROS) including the superoxide anion radical ($O_2^{-\cdot}$), hydroxyl radical ($^{\cdot}OH$), nitric oxide ($NO^{\cdot}$), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) are constantly generated in the human body by various physiological functions.[6] These excessive ROS may result in increased levels of low-density lipoprotein (LDL), oxidative modification of LDL, and an impairment of endothelial derived relaxing factor (EDRF, nitric oxide)-mediated bioactions.[7]

On this basis, some researchers have recently proposed and searched some natural products with antithrombotic capacity and antioxidant activity for the treatment of ischemic cerebrovascular disease.[8] Traditional Chinese medicines 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 (>85%) of breviscapine, a natural drug consisting of total flavonoids of Erigeron breviscapus (Vant.) Hand-Mazz. (Compositae), has been used for the treatment of cerebral infarction, angina pectoris and coronary heart disease with a large market share in China.[9] Nowadays, the research on scutellarin (**1**) has become a hot topic in China due to its distinguished efficacy in the clinical therapy. Pharmacological studies found that scutellarin (**1**) exhibited antithrombotic and antioxidant activities to attenuate neuronal damage, thus had a wide range of benefits to brain injury caused by cerebral ischemia/reperfusion.[10–12]

However, scutellarin (**1**) has low water-solubility,[13] and the bioavailability of scutellarin (**1**) was very low with the absolute oral bioavailability in Beagle dog was rarely 0.4%.[14] Interestingly, some researchers found that scutellarin (**1**) was mainly absorbed in the form of its hydrolyzed product scutellarein (**2**) (Fig. 1) by

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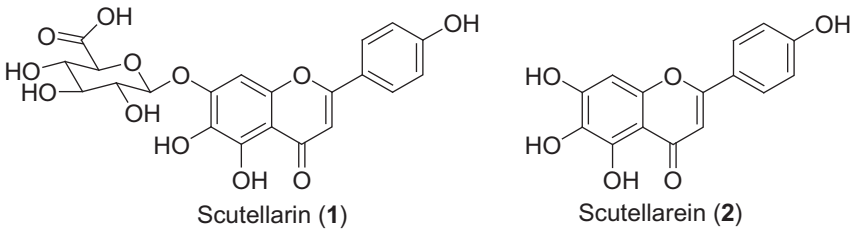


Figure 1. Chemical structures of scutellarin (**1**) and scutellarein (**2**).

intestinal,[15] furthermore, in the clinical trials,[14] a large amount of scutellarein (**2**) was found in urine and plasma after oral administration of breviscapine, indicating that scutellarin (**1**) was firstly hydrolyzed into aglycone when reaching colon and was then absorbed as scutellarein (**2**) as the real bioactive component in the body. Pharmacodynamics confirmed that scutellarein (**2**) had better protective effect than scutellarin (**1**) in rat cerebral ischemia,[16] scutellarein (**2**) can prevent thrombosis and platelet aggregation, and improve the characteristics of hemorheology in rats.[17] In our previous studies, we found that scutellarein (**2**) had stronger scavenging capacities toward DPPH, $ABTS^{+\cdot}$, ·OH free radicals than scutellarin (**1**), and had better protective effect on H_2O_2-induced cytotoxicity in PC12 cells.[18,19] These results suggested that scutellarein (**2**) can be a promising lead compound for the discovery of potent agent with thrombin-inhibitory and antioxidant activities for the treatment of ischemic cerebrovascular disease.

Amination of the substrates has considerable importance for the synthesis and modification of biologically active compounds.[20] This technique provides convenient access to many useful synthetic building blocks, because the resulting amino group can be easily converted to various functionalities, particularly, to quaternary ammonium salts to increase water solubility. In our previous studies, we synthesized some scutellarein derivatives where the amino side chain was introduced at the C-7 hydroxyl position.[19] Herein, we describe the synthesis and in vitro biological evaluation of scutellarein derivatives with electrophilic substitution at C-4′, together with a discussion of structure–activity relationships. The thrombin inhibition activity of all the new derivatives were evaluated through the analyzation of thrombin time (TT), activated partial thromboplastin time (APTT), prothrombin time (PT) and fibrinogen (FIB). The antioxidant activities of these target products were assessed by measuring their scavenging capacities toward 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and the ability to protect PC12 cells against H_2O_2-induced cytotoxicity, and the water solubility were assessed by ultraviolet (UV) spectrophotometer.

2. Results and discussion

2.1. Chemistry

The 4′-*O*-amide-scutellarein derivatives **8a**–**8e** were synthesized as shown in Scheme 1. Under nitrogen protection, scutellarein (**2**) was obtained by refluxing scutellarin (**1**) with 6N HCl in EtOH solution.[20,21] Then at 175 °C and using diphenyl ether as solvent according to our developed method,[22–24] **2** was treated with dichlorodiphenylmethane to produce the desired compound **3** in high yield after reacting for 30 min. Subsequently, **3** was converted into **7a**–**7e** after the reaction with acyl chlorides **6a**–**6e** using K_2CO_3 and KI as the catalysts in *N*,*N*-dimethylformamide (DMF).[19] The side chains **6a**–**6e** were synthesized from **4a**–**4e** and bis(trichloromethyl) carbonate (**5**). Under hydrogenation conditions in THF/EtOH using the catalyst of 10% palladium on carbon at the room temperature, the benzophenone ketal group in **7a**–**7e** was removed to afford the target compounds **8a**–**8e**.

Subsequently, we want to synthesize the other three series of scutellarein derivatives **16a**–**16c**, **21a**–**21c** and **26a**–**26b** based on Scheme 1, however, it did not work, so these compounds were obtained as shown in Scheme 2, considering that the different reaction conditions could be used to remove benzyl and ketal protecting groups. Firstly, benzyl bromide was used to protect the hydroxyl group selectively at C-4′ position in compound **3** to afford **9**. Fortunately, the benzophenone ketal group was removed under the hydrolysis condition with AcOH in H_2O to give **10** in 96% yield. Taken into account that the methoxymethyl and benzyl groups could be deprotected by different reaction conditions, methoxymethyl group can be removed in acidic condition whilst benzyl group is labile under hydrogenation, chloromethyl methyl ether was then selected to protect the two *ortho* phenolic hydroxyl groups at C-6 and C-7 positions in **10**. Compound **10** reacted with chloromethyl methyl ether and K_2CO_3 in acetone solution affording **11** with free phenolic hydroxyl group at C-5 position.[25] The benzyl group at C-4′ position in **11** could be selectively removed under hydrogenation condition, this method gave **12** in 91% yield with the catalyst of 10% Pd/C in a mixed solution of EtOH and THF. TLC analysis showed that this reaction was finished after 8 h and no side product was detected. The reaction of **12** with RCl **14a**–**14c**, **19a**–**19c** and **24a**–**24b** using K_2CO_3 and KI in DMF led to **15a**–**15c**, **20a**–**20c** and **25a**–**25b**, respectively. The side chains **14a**–**14c** were obtained by the reaction of **4a**–**4c** with chloroacetyl chloride **13** in refluxing benzene. **19a**–**19c** were produced from **18a**–**18c** by $SOCl_2$ catalyzed chlorination, which were obtained after alkylation of **4a**–**4c** and 2-chloro-1-ethanol (**17**), **24a**–**24b** were synthesized from 1-chloro-3-hydroxypropan (**22**) in the same way of **19a**–**19c**. Finally, HCl catalyzed removing of the methoxymethyl protecting group afforded the scutellarein derivatives **16a**–**16c**, **21a**–**21c** and **26a**–**26b** in high yield.[25]

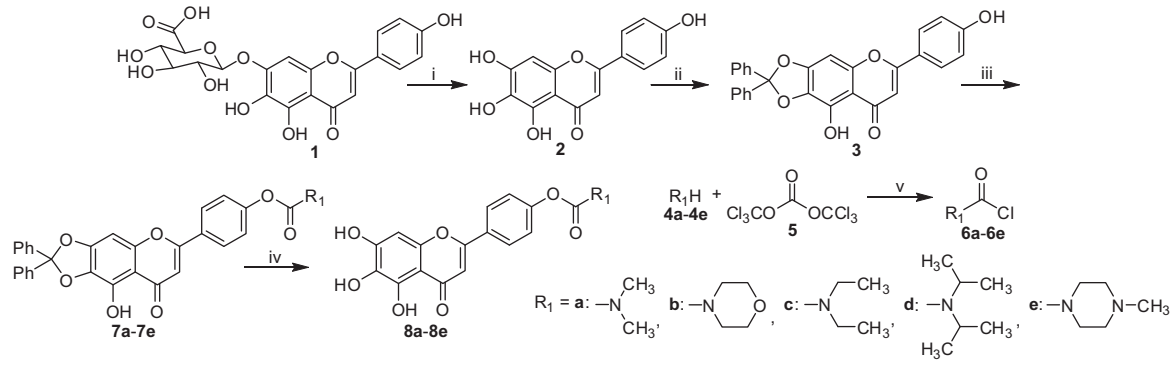
2.2. Biological activity

2.2.1. Anti-thrombic activity

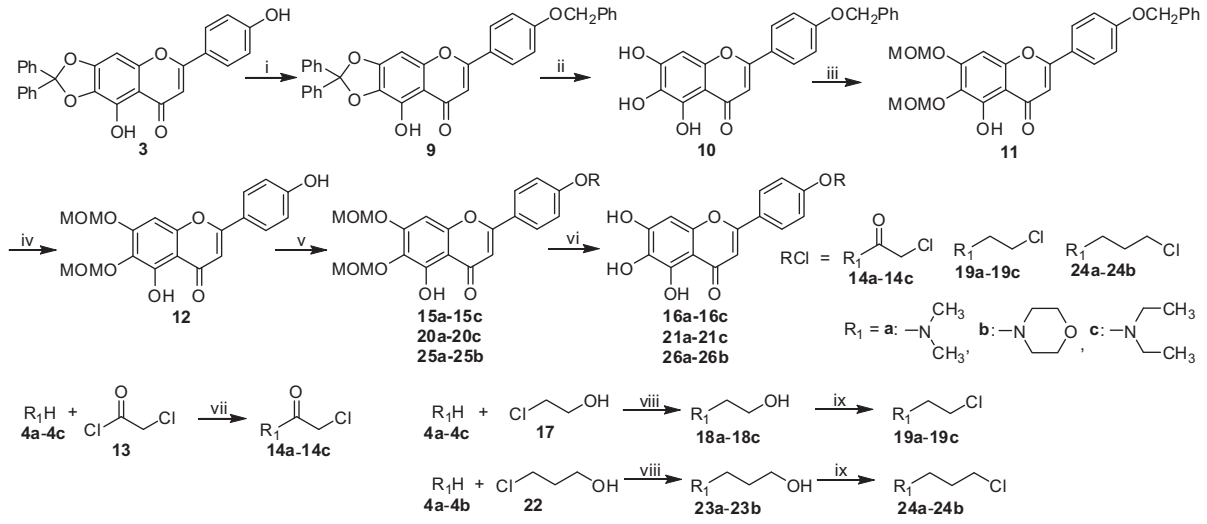
Because the antithrombotic activity can be assessed by measuring the prolongation of the plasma clotting time of thrombin time (TT), activated partial thromboplastin time (APTT), INR increasement of prothrombin time (PT), and reduction of fibrinogen (FIB) content according to our previous studies,[25] so the thrombin activity of different compounds was investigated for TT, APTT, PT and FIB, and the results were shown in Table 1.

From the results we could see that the antithrombotic activity remained when the glucuronyl group in scutellarin (**1**) was hydrolyzed to produce scutellarein (**2**), although the PT decreased and the FIB content increased in scutellarein (**2**), the TT and APTT in scutellarein (**2**) increased compared to those in scutellarin (**1**), this result indicated that the glucuronyl group was not important for the antithrombotic activity.

In the series of scutellarein derivatives **8a**–**8e**, the most active compound was **8b**, it prolonged TT and PT, decreased FIB content



Scheme 1. Reagents and conditions: (i) HCl, EtOH, N_2, reflux, 36 h, 17%; (ii) dichlorodiphenylmethane, diphenyl ether, 175 °C, 30 min, 85%; (iii) **6a**–**6e**, K_2CO_3, KI, DMF, 25 °C, 12 h, 78.3–86.2%; (iv) H_2, 10% Pd/C, THF/EtOH, 8 h, 91.5–93.4%; (v) DMF, CCl_4, reflux, 6 h.



Scheme 2. Reagents and conditions: (i) $PhCH_2Br$, K_2CO_3, DMF, 25 °C, 12 h, 93%; (ii) AcOH/H_2O 4:1, reflux, 1.5 h, 96%; (iii) chloromethyl methyl ether (MOMCl), K_2CO_3, acetone, reflux, 6 h, 89%; (iv) 10% Pd/C, H_2, THF/EtOH, 8 h, 91%; (v) RCl, K_2CO_3, KI, DMF, 25 °C, 12 h, 61.8–68.1%; (vi) HCl, Et_2O/CH_2Cl_2 1:1, 25 °C, 6 h, 83.8–89.3%; (vii) benzene, reflux, 1 h; (viii) toluene, reflux, 3 h; (ix) $SOCl_2$, toluene, reflux, 2 h.

Table 1
The thrombin inhibition activity of scutellarein derivatives

Compd (100 μM)	Plasma coagulation parameters			
	TT (s)	APTT (s)	PT (s)	FIB (g/l)
1	23.08 ± 0.87	34.18 ± 2.25	6.28 ± 0.08	6.13 ± 0.15
2	24.40 ± 1.59	36.65 ± 3.60	5.93 ± 1.21	6.84 ± 0.10
8a	24.30 ± 1.15	27.78 ± 1.21	8.10 ± 0.27	5.56 ± 0.67
8b	24.62 ± 1.97	28.28 ± 1.59	8.55 ± 0.15	5.52 ± 0.18
8c	24.36 ± 0.16	28.20 ± 1.40	7.67 ± 0.19	5.21 ± 0.22
8d	24.57 ± 0.81	27.97 ± 2.31	7.97 ± 0.19	5.65 ± 0.49
8e	24.52 ± 0.94	26.87 ± 3.36	7.03 ± 0.41	5.42 ± 0.63
16a	22.27 ± 2.20	29.25 ± 0.70	7.17 ± 0.17	5.14 ± 0.22
16b	19.90 ± 1.20	27.10 ± 1.86	7.33 ± 0.07	5.49 ± 0.25
16c	20.92 ± 0.72	26.40 ± 2.72	7.45 ± 0.11	5.37 ± 0.29
21a	22.97 ± 2.80	29.97 ± 1.12	6.82 ± 0.45	5.11 ± 0.15
21b	19.70 ± 0.35	29.05 ± 1.35	6.70 ± 0.25	5.23 ± 0.28
21c	22.03 ± 0.86	30.45 ± 0.63	6.90 ± 0.17	5.72 ± 0.22
26a	19.90 ± 2.20	26.58 ± 1.19	6.15 ± 0.26	5.28 ± 0.23
26b	19.60 ± 1.71	28.25 ± 3.10	6.63 ± 0.08	5.42 ± 0.29

Data represent mean ± SD. $n = 4$.

compared to scutellarein (**2**). Interestingly, when the morpholine ring in **8b** was replaced by 4-methylpiperazine to afford **8e**, the antithrombotic activity decreased significantly, this result could be seen that the TT, APTT and PT of **8e** decreased compared with those indicators in **8b**. Furthermore, when the morpholine ring in **8b** was replaced by fatty amine groups such as dimethylamine and diisopropylamine to produce **8a** and **8d**, all the four indicators were shown to be less active than **8e**, this result indicated that the morpholine ring in the side chain was very important for the antithrombotic activity.

In the series of scutellarein derivatives **16a**–**16c**, in which there was one more methylene group compared to **8a**–**8c**, **16a** showed the same antithrombotic activity compared to **8a**, **16a** only increased APTT and decreased FIB. **16b** and **16c** showed less antithrombotic activity compared to **8b** and **8c**, respectively, three indicators in **16b** which were TT, APTT and PT were shown to be less active than **8b**, and no indicator in **16c** showed stronger activity compared to **8c**.

When the carbonyl group in **16a**–**16c** was changed into methylene group, three compounds **21a**–**21c** were obtained, these compounds showed equal antithrombotic activity to **16a**–**16c**, **21a** and **21c** only showed increased TT and APTT compared to **16a** and **16c**, and **21b** only showed increased APTT and decreased FIB compared to **16b**.

When the carbon chain in **21a** and **21b** extended to produce **26a** and **26b**, the antithrombotic activity decreased significantly, which could be seen that no one indicator showed stronger activity compared to **21a** and **21b**.

The above antithrombotic activity indicated that using formyl group as a linker between the scutellarein ring and the side chain

was very important to keep the antithrombotic activity, when the formyl group was changed into acetyl, ethyl and propyl groups, the antithrombotic activity decreased, respectively.

2.2.2. Antioxidants

The in vitro antioxidant activities[26] of these scutellarein derivatives were assessed by measuring their scavenging capacities toward DPPH assay and the ability to protect PC12 cells against H_2O_2-induced cytotoxicity.

2.2.2.1. DPPH radical-scavenging activity. DPPH assay[27] measured the hydrogen-donating ability of antioxidants to convert the stable DPPH free radical into 1,1-diphenyl-2-picrylhydrazine. The reaction was accompanied by a change in color from deep-yellow to light-yellow and was monitored spectrophotometrically.

The data obtained in the DPPH assay was depicted in Table 2. From the results we can infer that, the scutellarein derivatives **8a**–**8e** were very active in the DPPH assay, with three compounds **8b**, **8d** and **8e** showed stronger antioxidant activities compared to scutellarin (**1**) (IC_{50} = 27.93 μM) and scutellarein (**2**) (IC_{50} = 22.58 - μM), with their IC_{50}s against DPPH were 19.27 μM, 17.03 μM and 18.87 μM, respectively. In the series of **16a**–**16c**, in which there was one more methylene group compared to **8a**–**8c**, the antioxidant activity decreased significantly, with their IC_{50}s were 80.70 μM, 83.26 μM and 81.28 μM, respectively. In the two series of **21a**–**16c** and **26a**–**26b**, in which there was no carbonyl group in the amide side chain, the antioxidant activity was very low with their IC_{50}s were above 200 μM.

2.2.2.2. Inhibitory effect on PC12 cells induced by oxidative stress. PC12 cells can adopt a neuronal phenotype and have been used extensively as a model for catecholamine-secreting neuronal cells.[28] Active mitochondria of living cells can cleave MTT to produce formazan, the amount of which directly related to the number of living cells. So the neuroprotective effects of the synthesized scutellarein derivatives were evaluated by the ability to protect PC12 cells against H_2O_2-induced cytotoxicity using MTT assay method.[18]

The results of the inhibition rate against cell damage were summarized in Table 2. Comparison of the data reported in Table 2 indicated compounds **8b**, **8d** and **8e** showed stronger inhibitory effect than scutellarein (**2**) against H_2O_2-induced cytotoxicity in PC12 cells, in a concentration of 50 μM, **8b**, **8d** and **8e** showed 94.25%, 93.36% and 92.48% inhibition rate against cell damage, and in a concentration of 25 μM, **8b**, **8d** and **8e** showed 73.89%, 71.68% and 68.58% inhibition rate against cell damage, respectively, while the inhibiting rates of scutellarein (**2**) were 91.15% and 64.60% at 50 and 25 μM, respectively. The less active compounds were **8c** and **8a**, these two compounds exhibited 75.22% and 71.23% inhibition rate against cell damage in a concentration of 50 μM, 51.33% and 48.67% inhibition rate against cell damage in a concentration of 25 μM, respectively. The protective activity of **16a**–**16c** against H_2O_2-induced cytotoxicity on PC12 cells decreased, with their inhibition rates were below 70% in a concentration of 50 μM. Unfortunately, compounds **21a**–**21c** and **26a**–**26b** lost its protective activity against cell damage with its inhibition rate below 60% in a concentration of 50 μM and below 40% in a concentration of 25 μM.

These antioxidant activities showed the similar structure–activity relationship compared to the antithrombotic biology, for example, the presence of the methanamide group in the side chain was very important to protect the PC12 cells against H_2O_2-induced oxidative injury, while the introduction of ethanamide, ethylamine and propylamine groups would decrease the protective activity against H_2O_2-induced cytotoxicity on PC12 cells.

Table 2
The in vitro antioxidatant activity in DPPH assay, protective effect against H_2O_2-induced cytotoxicity in PC12 cells, and water solubility of the scutellarein derivatives

Compd	DPPH assay IC_{50} (μM)	PC12 cells assay		Water solubility	
		Concentrations of compd (μM)	Inhibiting rate (%)	Solubility (μg/ml)	Fold increase[a]
1	27.93	50	79.20	7.62	
		25	53.10		
2	22.58	50	91.15	6.85	1.00
		25	64.60		
8a	37.17	50	71.23	9.56	1.40
		25	48.67		
8b	19.27	50	94.25	15.34	2.34
		25	73.89		
8c	32.60	50	75.22	9.23	1.35
		25	51.33		
8d	17.03	50	93.36	8.67	1.27
		25	71.68		
8e	18.87	50	92.48	15.06	2.20
		25	68.58		
16a	80.70	50	68.14	9.32	1.36
		25	46.46		
16b	83.26	50	64.60	9.45	1.38
		25	43.36		
16c	81.28	50	66.37	8.95	1.31
		25	44.69		
21a	214.8	50	56.19	9.28	1.35
		25	35.84		
21b	263.6	50	48.23	9.40	1.37
		25	32.30		
21c	205.1	50	58.41	8.68	1.27
		25	38.50		
26a	231.1	50	52.21	8.50	1.24
		25	33.63		
26b	274.4	50	44.25	8.56	1.25
		25	28.32		

[a] Fold increase in water solubility relative to scutellarein (**2**).

2.2.3. Solubility

The aqueous solubility of the synthesized scutellarein derivatives was determined using UV spectrophotometer.[29–31] As presented in Table 2, the introduction of morpholinyl ring showed the best water solubility among the four series of scutellarein derivatives, the values of **8b**, **16b**, **21b** and **26b** in water solubility were 15.34 μg/ml, 9.45 μg/ml, 9.40 μg/ml and 8.56 μg/ml, and showed 2.34-fold, 1.38-fold, 1.37-fold and 1.25-fold compared to scutellarein (**2**) respectively. Furthermore, the introduction of methyl piperazinyl ring in **8e** also showed remarkable increase in solubility with its value was 15.06 μg/ml and exhibited 2.20-fold. The replacement of alicyclic amine by the aliphatic amine showed good water solubility, such as the dimethyl amine substituted derivative **8a**, **16a**, **21a** and **26a**, their values in water solubility were 9.56 μg/ml, 9.32 μg/ml, 9.28 μg/ml and 8.50 μg/ml, and only showed 1.40-fold, 1.36-fold, 1.35-fold and 1.24-fold compared to scutellarein (**2**), respectively. These results indicated that the introduction of the alicyclic amine such as the morpholinyl ring and the methyl piperazinyl ring would increase the aqueous solubility of the synthesized scutellarein derivatives.

3. Conclusion

Scutellarin (**1**) has been used clinically to treat acute cerebral infarction and paralysis induced by cerebrovascular diseases such as hypertension, cerebral thrombosis, cerebral haemorrhage for many years in China,[9] it has showed the effectiveness on dilating blood vessels, improving microcirculation, increasing cerebral blood flow, and inhibiting platelet aggregation.[32] However, scutellarin (**1**) has pharmacokinetic problems such as low solubility in water and fast metabolism which results in its low bioavailability. Amination of the substrates has considerable importance for the

synthesis and modification of biologically active compounds,[20] because the resulting amino group can be easily converted to quaternary ammonium salts to increase water solubility. In this study, we took scutellarein (**2**) as a promising lead compound, which is one major metabolite of scutellarin (**1**) in vivo, designed and synthesized new scutellarein derivatives with electrophilic substitution at C-4′, to improve biological activities of scutellarein (**2**). In particular, compound **8b**, which there was a morpholine ring in the side chain at the C-4′ position, demonstrated stronger anticoagulant and antioxidant activity, better water solubility compared with scutellarein (**2**), which warrants it as a promising agent for the treatment of ischemic cerebrovascular disease.

4. Experimental

4.1. General methods

Scutellarin (**1**) was purchased from Sichuan Mianning Jiexiang Materials Co. Ltd (Chengdu, China). Reagents and solvents were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). All non-aqueous reactions were carried out under nitrogen protection using flame-dried glassware, the anhydrous solvents were transferred via syringe or stainless steel cannula. Organic solvents were concentrated below 45 °C by Büchi rotary evaporator at about 20 mm Hg. Using UV light of 254 nm as the detection wavelength, all reactions were monitored by TLC, performed on 0.15–0.20 mm silica gel plates. Chromatographic separation was carried out on silica gel (160–200 mesh) using mixtures of petroleum ether (60–90) and ethyl acetate as eluent. The melting points (Mp) were measured on a WRS-1B apparatus and were not corrected. The ^{1}H NMR and ^{13}C NMR spectra were carried out on Bruker AV-300 (300 MHz) in DMSO-d_6, chemical shifts were expressed as δ values in ppm relative to tetramethylsilane as standard, and peak abbreviations were expressed as follows: s, singlet; d, doublet; m, multiplet. Mass spectra (MS) were performed on Waters Synapt HDMS spectrometer equipped with an electrospray ionization source (ESI).

4.2. Synthesis of scutellarein derivatives

4.2.1. 5,6,7-Trihydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one (2)

Concentrated HCl (120 mL) was added into a stirring mixture of **1** (10 g, 21.65 mmol) and water (10 mL) in ethanol (120 mL), the reaction mixture was refluxed for 36 h under nitrogen, and then was allowed to cool to room temperature. Water (1000 mL) was added, the solid appeared was filtered, and then purified by column chromatography on silica gel with 50% ethyl acetate in petroleum ether as eluent to afford **2** (1.05 g, 17% yield) as a yellow solid, mp 160–162 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 6.57 (s, 1H, C_3-H), 6.74 (s, 1H, C_8-H), 6.91 (d, J = 8.8 Hz, 2H, $C_{3'}C_{5'}$-H), 7.90 (d, J = 8.8 Hz, 2H, $C_{2'}C_{6'}$-H), 8.71 (s, 1H, C_6-OH), 10.29 (s, 1H, C_7-OH), 10.44 (s, 1H, $C_{4'}$-OH), 12.78 (s, 1H, C_5-OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 98.53 (C8), 102.27 (C3), 103.22 (C10), 115.76 (2 × C (C3′,C5′)), 121.37 (C1′), 124.91 (C6), 128.47 (2 × C(C2′,C6′)), 145.42 (C9), 152.98 (C5), 153.28 (C7), 160.99 (C4′), 163.47 (C2), 181.95 (C4). ESI-MS: m/z 287 $[M+H]^+$.

4.2.2. 9-Hydroxy-6-(4-hydroxyphenyl)-2,2-diphenyl-8*H*-1,3-dioxolo[4,5-*g*][1]benzopyran-8-one (3)

Scutellarein (**2**) (10 g, 34.94 mmol) was added into a solution of dichlorodiphenylmethane (12.4 g, 52.29 mmol, 1.5 equiv) in diphenyl ether (200 mL), the reaction mixture was heated at 175 °C under nitrogen, and then was allowed to cool to room temperature after 30 min. Petroleum ether (1000 mL) was added dropwise, the solid appeared was filtered, and then purified by column chromatography on silica gel with 25% ethyl acetate in petroleum ether as eluent to afford **3** (13.35 g, 85%) as a yellow solid, mp 284–286 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 6.69 (s, 1H, C_3-H), 6.82 (s, 1H, C_8-H), 6.96 (d, J = 8.7 Hz, 2H, $C_{3'}C_{5'}$-H), 7.47–7.50 (m, 6H, ArH), 7.58–7.61 (m, 4H, ArH), 7.94 (d, J = 8.7 Hz, 2H, $C_{2'}C_{6'}$-H), 10.41 (s, 1H, $C_{4'}$-OH), 12.99 (s, 1H, C_5-OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 94.50 (C8), 103.32 (C3), 104.93 (C10), 116.45 (2 × C (C3′,C5′)), 119.16 (C), 121.21 (C1′), 126.29 (C6), 126.34 (2 × C(C2′, C6′)), 126.50 (2 × C), 129.05 (4 × C), 129.14 (4 × C), 139.26 (2 × C), 141.94 (C9), 152.77 (C5), 152.95 (C7), 161.80 (C4′), 164.00 (C2), 182.32 (C4). ESI-MS: m/z 449 $[M-H]^-$.

4.2.3. Synthesis of 7a–7e

DMF (7.73 ml, 0.1 mol) was added into a solution of imine (**4a**–**4c**) (1 mol) in CCl_4 (500 ml) at 25 °C, 30 min later, a solution of triphosgene (119.1 g, 0.4 mol) in CCl_4 (200 ml) was added dropwise. This mixture was refluxed gently for 6 h and then allowed to cool to room temperature. After concentration under reduced pressure, the crude residue was purified by vacuum distillation to afford the imino chlorides (**6a**–**6e**) which were then used directly in the next step.

Compound **3** (100 mg, 0.27 mmol) was added into anhydrous DMF (20 mL) at 0 °C, treated with K_2CO_3 (44 mg, 0.32 mmol) and KI (11 mg, 0.07 mmol) followed by imino chlorides (**6a**–**6e**) (0.32 mmol), then the reaction mixture was warmed to room temperature. After 12 h, the mixture was poured into water (50 mL), and then extracted with ethyl acetate (50 mL × 3), the organic layer was washed with brine (100 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The appeared crude material was then purified by column chromatography on silica gel using 50% ethyl acetate in petroleum ether as eluent to afford **7a**–**7e** as yellow solids.

4.2.3.1. 4-(9-Hydroxy-8-oxo-2,2-diphenyl-8*H*-[1,3]dioxolo[4,5-*g*]chromen-6-yl)phenyl dimethylcarbamate (7a). 78.3% yield, yellow solid, mp 211–213 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.94 (s, 3H, CH_3), 3.07 (s, 3H, CH_3), 7.04 (s, 1H, C_3-H), 7.12 (s, 1H, C_8-H), 7.33–7.38 (m, 4H, ArH), 7.47–7.59 (m, 10H, ArH), 8.11 (d, J = 8.7 Hz, 2H, $C_{2'}C_{6'}$-H), 12.98 (s, 1H, C_5-OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 39.05 (2 × C(CH_3)), 94.52 (C8), 103.31 (C3), 104.95 (C10), 119.15 (C), 121.46 (2 × C(C3′,C5′)), 126.21 (C1′), 126.30 (C6), 126.49 (2 × C), 128.33 (2 × C(C2′,C6′)), 129.04 (4 × C), 129.13 (4 × C), 139.27 (2 × C), 141.95 (C9), 152.76 (C5), 152.94 (C7), 155.06 (CO), 155.77 (C4′), 164.01 (C2), 182.33 (C4). ESI-MS: m/z 522 $[M+H]^+$.

4.2.3.2. 4-(9-Hydroxy-8-oxo-2,2-diphenyl-8*H*-[1,3]dioxolo[4,5-*g*]-chromen-6-yl)phenyl morpholine-4-carboxylate (7b). 85.4% yield, yellow solid, mp 193–195 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.12–3.15 (m, 4H, morpholine CH_2NCH_2), 3.54–3.57 (m, 4H, morpholine CH_2OCH_2), 7.04 (s, 1H, C_3-H), 7.13 (s, 1H, C_8-H), 7.38 (d, J = 8.7 Hz, 2H, $C_{3'}C_{5'}$-H), 7.45–7.49 (m, 6H, ArH), 7.54–7.58 (m, 4H, ArH), 8.11 (d, 2H, J = 8.7 Hz, $C_{2'}C_{6'}$-H), 12.97 (s, 1H, C_5-OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 47.96 (2 × C(CH_2)), 67.11 (2 × C(CH_2)), 94.53 (C8), 103.30 (C3), 104.94 (C10), 119.16 (C), 121.45 (2 × C (C3′,C5′)), 126.22 (C1′), 126.31 (C6), 126.48 (2 × C), 128.32 (2 × C (C2′,C6′)), 129.05 (4 × C), 129.14 (4 × C), 139.26 (2 × C), 141.96 (C9), 152.77 (C5), 152.93 (C7), 155.14 (CO), 155.75 (C4′), 164.00 (C2), 182.31 (C4). ESI-MS: m/z 564 $[M+H]^+$.

4.2.3.3. 4-(9-Hydroxy-8-oxo-2,2-diphenyl-8*H*-[1,3]dioxolo[4,5-*g*]chromen-6-yl)phenyl diethylcarbamate (7c). 82.1% yield, yellow solid, mp 196–198 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.18–1.21 (t, 6H, 2 × CH_3), 3.32–3.34 (m, 4H, 2 × CH_2), 7.03 (s, 1H, C_3-H), 7.12 (s, 1H, C_8-H), 7.33 (d, J = 8.7 Hz, 2H, $C_{3'}C_{5'}$-H),

7.46–7.49 (m, 6H, ArH), 7.54–7.58 (m, 4H, ArH), 8.11 (d, J = 8.7 Hz, 2H, $C_{2'}C_{6'}$-H), 12.98 (s, 1H, C_5-OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 12.98 (2 × C(CH_3)), 42.76 (2 × C(CH_2)), 94.52 (C8), 103.29 (C3), 104.93 (C10), 119.15 (C), 121.44 (2 × C(C3′,C5′)), 126.23 (C1′), 126.33 (C6), 126.47 (2 × C), 128.31 (2 × C(C2′,C6′)), 129.04 (4 × C), 129.15 (4 × C), 139.27 (2 × C), 141.95 (C9), 152.76 (C5), 152.94 (C7), 155.02 (CO), 155.76 (C4′), 164.01 (C2), 182.32 (C4). ESI-MS: m/z 550 $[M+H]^+$.

4.2.3.4. 4-(9-Hydroxy-8-oxo-2,2-diphenyl-8*H*-[1,3]dioxolo[4,5-*g*]chromen-6-yl)phenyl diisopropylcarbamate (7d). 86.2% yield, yellow solid, mp 205–207 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.26 (d, 12H, 4 × CH_3), 3.96–4.05 (m, 2H, CH), 7.03 (s, 1H, C_3-H), 7.13 (s, 1H, C_8-H), 7.33 (d, J = 8.7 Hz, 2H, $C_{3'}C_{5'}$-H), 7.44–7.49 (m, 6H, ArH), 7.53–7.58 (m, 4H, ArH), 8.09 (d, J = 8.7 Hz, 2H, $C_{2'}C_{6'}$-H), 12.98 (s, 1H, C_5-OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 21.16 (4 × C(CH_3)), 46.94 (2 × C(CH)), 94.50 (C8), 103.31 (C3), 104.94 (C10), 119.16 (C), 121.46 (2 × C(C3′,C5′)), 126.25 (C1′), 126.35 (C6), 126.48 (2 × C), 128.32 (2 × C(C2′,C6′)), 129.05 (4 × C), 129.16 (4 × C), 139.28 (2 × C), 141.97 (C9), 152.77 (C5), 152.93 (C7), 155.03 (CO), 155.77 (C4′), 164.02 (C2), 182.34 (C4). ESI-MS: m/z 578 $[M+H]^+$.

4.2.3.5. 4-(9-Hydroxy-8-oxo-2,2-diphenyl-8*H*-[1,3]dioxolo[4,5-*g*]-chromen-6-yl)phenyl 4-methylpiperazine-1-carboxylate (7e). 82.9% yield, yellow solid, mp 217–219 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.12–3.15 (t, 4H, 2 × CH_2), 3.54–3.57 (t, 4H, 2 × CH_2), 3.67 (s, 3H, CH_3), 7.04 (s, 1H, C_3-H), 7.13 (s, 1H, C_8-H), 7.38 (d, J = 8.7 Hz, 2H, $C_{3'}C_{5'}$-H), 7.44–7.49 (m, 6H, ArH), 7.53–7.58 (m, 4H, ArH), 8.11 (d, J = 8.7 Hz, 2H, $C_{2'}C_{6'}$-H), 12.97 (s, 1H, C_5-OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 44.56 (C(CH_3)), 48.95 (2 × C(CH_2)), 55.11 (2 × C(CH_2)), 94.49 (C8), 103.30 (C3), 104.93 (C10), 119.15 (C), 121.44 (2 × C(C3′,C5′)), 126.24 (C1′), 126.32 (C6), 126.45 (2 × C), 128.30 (2 × C(C2′,C6′)), 129.04 (4 × C), 129.15 (4 × C), 139.27 (2 × C), 141.98 (C9), 152.75 (C5), 152.92 (C7), 154.86 (CO), 155.78 (C4′), 164.03 (C2), 182.31 (C4). ESI-MS: m/z 577 $[M+H]^+$.

4.2.4. Synthesis of 8a–8e

Compound **7a**–**7e** (100 mg) was stirred with 10% Pd/C catalyst (2 mg) in EtOH (50 mL) and THF (50 mL) for 8 h under hydrogen at atmospheric pressure, then the Pd/C catalyst was removed by filtration over celite. After the filtrate was evaporated, the crude material was purified by column chromatography on silica gel using 50% ethyl acetate in petroleum ether as eluent to afford **8a**–**8e** as yellow solids.

4.2.4.1. 4-(5,6,7-Trihydroxy-4-oxo-4*H*-chromen-2-yl)phenyl dimethylcarbamate (8a). 92.3% yield, yellow solid, mp 263–265 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.93 (s, 3H, CH_3), 3.06 (s, 3H, CH_3), 6.61 (s, 1H, C_3-H), 6.90 (s, 1H, C_8-H), 7.32 (d, J = 8.7 Hz, 2H, $C_{3'}C_{5'}$-H), 8.07 (d, J = 8.7 Hz, 2H, $C_{2'}C_{6'}$-H), 8.78 (s, 1H, C_6-OH), 10.53 (s, 1H, C_7-OH), 12.65 (s, 1H, C_5-OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 39.01 (2 × C(CH_3)), 98.54 (C8), 102.26 (C3), 103.23 (C10), 121.66 (2 × C(C3′,C5′)), 124.92 (C6), 127.01 (C1′), 128.45 (2 × C(C2′,C6′)), 145.43 (C9), 153.29 (C7), 154.83 (C4′), 155.01 (CO), 155.98 (C5), 163.42 (C2), 181.96 (C4). ESI-MS: m/z 358 $[M+H]^+$.

4.2.4.2. 4-(5,6,7-Trihydroxy-4-oxo-4*H*-chromen-2-yl)phenyl morpholine-4-carboxylate (8b). 91.5% yield, yellow solid, mp 234–235 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.43–3.46 (m, 4H, 2 × CH_2), 3.63–3.66 (m, 4H, 2 × CH_2), 6.62 (s, 1H, C_3-H), 6.92 (s, 1H, C_8-H), 7.37 (d, J = 8.7 Hz, 2H, $C_{3'}C_{5'}$-H), 8.09 (d, J = 8.7 Hz, 2H, $C_{2'}C_{6'}$-H), 8.78 (s, 1H, C_6-OH), 10.54 (s, 1H, C_7-OH), 12.65 (s, 1H, C_5-OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 47.98 (2 × C(CH_2)), 67.13 (2 × C(CH_2)), 98.53 (C8), 102.26 (C3), 103.24 (C10), 121.65 (2 × C(C3′,C5′)), 124.93 (C6), 127.02 (C1′), 128.44 (2 × C(C2′,C6′)), 145.44 (C9), 153.28 (C7), 154.82 (C4′), 154.88 (CO), 155.97 (C5), 163.41 (C2), 181.96 (C4). ESI-MS: m/z 400 $[M+H]^+$.

4.2.4.3. 4-(5,6,7-Trihydroxy-4-oxo-4*H*-chromen-2-yl)phenyl diethylcarbamate (8c). 93.4% yield, yellow solid, mp 115–117 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.21–1.24 (t, 6H, 2 × CH_3), 3.41–3.44 (m, 4H, 2 × CH_2), 6.61 (s, 1H, C_3-H), 6.90 (s, 1H, C_8-H), 7.32 (d, J = 8.7 Hz, 2H, $C_{3'}C_{5'}$-H), 8.09 (d, J = 8.7 Hz, 2H, $C_{2'}C_{6'}$-H), 8.79 (s, 1H, C_6-OH), 10.51 (s, 1H, C_7-OH), 12.65 (s, 1H, C_5-OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 12.96 (2 × C(CH_3)), 42.78 (2 × C(CH_2)), 98.55 (C8), 102.25 (C3), 103.23 (C10), 121.67 (2 × C(C3′,C5′)), 124.91 (C6), 127.01 (C1′), 128.46 (2 × C(C2′,C6′)), 145.44 (C9), 153.28 (C7), 154.84 (C4′), 155.00 (CO), 155.98 (C5), 163.42 (C2), 181.95 (C4). ESI-MS: m/z 386 $[M+H]^+$.

4.2.4.4. 4-(5,6,7-Trihydroxy-4-oxo-4*H*-chromen-2-yl)phenyl diisopropylcarbamate (8d). 93.1% yield, yellow solid, mp 243–245 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.26 (d, 12H, 4 × CH_3), 3.96–4.05 (m, 2H, 2 × CH), 6.61 (s, 1H, C_3-H), 6.90 (s, 1H, C_8-H), 7.30 (d, J = 8.7 Hz, 2H, $C_{3'}C_{5'}$-H), 8.17 (d, J = 8.7 Hz, 2H, $C_{2'}C_{6'}$-H), 8.80 (s, 1H, C_6-OH), 10.53 (s, 1H, C_7-OH), 12.65 (s, 1H, C_5-OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 21.18 (4 × C(CH_3)), 46.96 (2 × C(CH)), 98.53 (C8), 102.25 (C3), 103.23 (C10), 121.66 (2 × C(C3′,C5′)), 124.91 (C6), 127.02 (C1′), 128.45 (2 × C(C2′,C6′)), 145.44 (C9), 153.29 (C7), 154.82 (C4′), 155.01 (CO), 155.99 (C5), 163.41 (C2), 181.95 (C4). ESI-MS: m/z 414 $[M+H]^+$.

4.2.4.5. 4-(5,6,7-Trihydroxy-4-oxo-4*H*-chromen-2-yl)phenyl 4-methylpiperazine-1-carboxylate (8e). 92.8% yield, yellow solid, mp 152–154 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.24 (s, 3H, CH_3), 2.36–2.39 (t, 4H, 2 × CH_2), 3.42–2.45 (t, 4H, 2 × CH_2), 6.28 (s, 1H, C_3-H), 6.91 (s, 1H, C_8-H), 7.35 (d, J = 8.7 Hz, 2H, $C_{3'}C_{5'}$-H), 8.18 (d, J = 8.7 Hz, 2H, $C_{2'}C_{6'}$-H), 8.77 (s, 1H, C_6-OH), 10.50 (s, 1H, C_7-OH), 12.25 (s, 1H, C_5-OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 44.57 (C(CH_3)), 48.98 (2 × C(CH_2)), 55.12 (2 × C(CH_2)), 98.54 (C8), 102.25 (C3), 103.23 (C10), 121.66 (2 × C(C3′,C5′)), 124.94 (C6), 127.01 (C1′), 128.43 (2 × C(C2′,C6′)), 145.45 (C9), 153.29 (C7), 154.82 (C4′), 154.89 (CO), 155.97 (C5), 163.42 (C2), 181.96 (C4). ESI-MS: m/z 413 $[M+H]^+$.

4.2.5. 6-(4-(Benzyloxy)phenyl)-9-hydroxy-2,2-diphenyl-8*H*-[1,3]dioxolo[4,5-*g*]chromen-8-one (9)

K_2CO_3 (107 mg, 0.77 mmol, 1.75 equiv) was added to a solution of **3** (200 mg, 0.44 mmol) in DMF (10 mL) followed by the addition of benzyl bromide (0.078 mL, 0.66 mmol, 1.5 equiv). This mixture was stirred at 0 °C for 2 h and then was warmed to room temperature. After 12 h, the mixture was dissolved in water (100 mL), extracted with ethyl acetate (50 mL × 3), then the organic layer was washed with brine (100 mL), dried over Na_2SO_4, and concentrated under reduced pressure to give the crude solid, which was then purified by column chromatography on silica gel using 33% ethyl acetate in petroleum ether as eluent to afford **9** (221 mg, 0.41 mmol, 93% yield) as a yellow solid, mp 226–228 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 5.22 (s, 2H, CH_2), 6.95 (s, 1H, C_3-H), 7.08 (s, 1H, C_8-H), 7.22 (d, J = 8.7 Hz, 2H, $C_{3'}C_{5'}$-H), 7.32–7.48 (m, 11H, ArH), 7.55–7.61 (m, 4H, ArH), 8.04 (d, J = 8.7 Hz, 2H, $C_{2'}C_{6'}$-H), 13.10 (s, 1H, C_5-OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 71.47 (C(CH_2)), 94.52 (C8), 103.33 (C3), 104.94 (C10), 114.83 (2 × C(C3′,C5′)), 119.17 (C), 119.92 (C1′), 125.95 (2 × C(C2′,C6′)), 126.30 (C6), 126.51 (2 × C), 127.15 (2 × C), 127.82 (C), 129.01 (2 × C), 129.06 (4 × C), 129.15 (4 × C), 139.27 (2 × C), 141.28 (C), 141.96 (C9), 152.79 (C5), 152.94 (C7), 163.99 (C4′), 164.02 (C2), 182.34 (C4). ESI-MS: m/z 541 $[M+H]^+$.

4.2.6. 2-(4-(Benzyloxy)phenyl)-5,6,7-trihydroxy-4*H*-chromen-4-one (10)

Compound **9** (150 mg, 0.28 mmol) was added to a solution of water (4 mL) in AcOH (16 mL), this solution was refluxed for 1.5 h under nitrogen and then allowed to cool. The mixture was poured into water (100 mL), extracted with ethyl acetate (50 mL × 3), then the organic layer was washed with brine (100 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The obtained crude material was purified by column chromatography on silica gel using 25% ethyl acetate in petroleum ether as eluent to afford **10** (100 mg, 0.27 mmol, 96% yield) as a yellow solid, mp 234–236 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 5.28 (s, 2H, CH_2), 6.81 (s, 1H, C_3-H), 6.92 (d, 2H, J = 8.7 Hz, $C_{3'}C_{5'}$-H), 7.00 (s, 1H, C_8-H), 7.35–7.45 (m, 3H, ArH), 7.51–7.54 (m, 2H, ArH), 7.94 (d, 2H, J = 8.7 Hz, $C_{2'}C_{6'}$-H), 8.74 (s, 1H, C_6-OH), 10.35 (s, 1H, C_7-OH), 12.69 (s, 1H, C_5-OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 71.46 (C(CH_2)), 98.54 (C8), 102.28 (C3), 103.24 (C10), 114.86 (2 × C(C3′,C5′)), 119.95 (C1′), 124.93 (C6), 125.97 (2 × C(C2′,C6′)), 127.14 (2 × C), 127.83 (C), 129.02 (2 × C), 141.28 (C), 145.43 (C9), 152.98 (C5), 153.27 (C7), 163.47 (C2), 163.97 (C4′), 181.96 (C4). ESI-MS: m/z 375 $[M-H]^-$.

4.2.7. 2-(4-(Benzyloxy)phenyl)-5-hydroxy-6,7-bis(methoxymethoxy)-4*H*-chromen-4-one (11)

Chloromethyl methyl ether (0.082 mL, 1.08 mmol) was added into a solution of **10** (100 mg, 0.27 mmol) in dry acetone (20 mL) followed by the addition of K_2CO_3 (156 mg, 1.13 mmol). This mixture was refluxed gently for 6 h and then allowed to cool to room temperature. The reaction mixture was filtered, washed with acetone, and then concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel using 20% ethyl acetate in petroleum ether as eluent to afford **11** (112 mg, 0.24 mmol, 89%) as a yellow solid, mp 174–176 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.45 (s, 3H, CH_3), 3.54 (s, 3H, CH_3), 5.17 (s, 2H, CH_2), 5.24 (s, 2H, CH_2), 5.37 (s, 2H, CH_2), 6.62 (s, 1H, C_3-H), 7.00 (s, 1H, C_8-H), 7.23 (d, J = 8.7 Hz, 2H, $C_{3'}C_{5'}$-H), 7.34–7.49 (m, 5H, ArH), 8.04 (d, J = 8.7 Hz, 2H, $C_{2'}C_{6'}$-H), 12.69 (s, 1H, C_5-OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 56.22 (C(CH_3)), 56.36 (C(CH_3)), 71.48 (C(CH_2)), 94.16 (C(CH_2)), 94.23 (C(CH_2)), 96.67 (C8), 102.25 (C3), 102.89 (C10), 114.87 (2 × C(C3′,C5′)), 119.96 (C1′), 125.98 (2 × C(C2′,C6′)), 127.13 (2 × C), 127.84 (C), 129.01 (2 × C), 129.87 (C6), 141.29 (C), 144.78 (C9), 150.76 (C5), 155.32 (C7), 163.45 (C2), 163.98 (C4′), 181.97 (C4). ESI-MS: m/z 465 $[M+H]^+$.

4.2.8. 5-Hydroxy-2-(4-hydroxyphenyl)-6,7-bis(methoxymethoxy)-4*H*-chromen-4-one (12)

Compound **11** (100 mg, 0.22 mmol) was stirred with 10% Pd/C catalyst (2 mg) in EtOH (50 mL) and THF (50 mL) for 8 h under hydrogen at atmospheric pressure, then the Pd/C catalyst was removed by filtration over celite. After the filtrate was evaporated, the crude material was purified by column chromatography on silica gel using 50% ethyl acetate in petroleum ether as eluent to afford **12** (76 mg, 0.20 mmol, 91%) as a yellow solid, mp 151–152 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.44 (s, 3H, CH_3), 3.54 (s, 3H, CH_3), 5.17 (s, 2H, CH_2), 5.37 (s, 2H, CH_2), 6.61 (s, 1H, C_3-H), 6.87 (s, 1H, C_8-H), 6.94 (d, J = 8.6 Hz, 2H, $C_{3'}C_{5'}$-H), 7.96 (d, J = 8.6 Hz, 2H, $C_{2'}C_{6'}$-H), 10.41 (s, 1H, $C_{4'}$-OH), 12.74 (s, 1H, C_5-OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 56.21 (C(CH_3)), 56.38 (C(CH_3)), 94.17 (C(CH_2)), 94.22 (C(CH_2)), 96.66 (C8), 102.26 (C3), 102.88 (C10), 115.77 (2 × C(C3′,C5′)), 121.36 (C1′), 128.48 (2 × C(C2′,C6′)), 129.88 (C6), 144.77 (C9), 150.78 (C5), 155.31 (C7), 160.98 (C4′), 163.46 (C2), 181.96 (C4). ESI-MS: m/z 375 $[M+H]^+$.

4.2.9. Synthesis of 15a–15c, 20a–20c and 25a–25b

Chloroacetyl chloride **13** (0.06 mol, 4.0 ml) was added dropwise into the solution of imine (**4a**–**4c**) (0.06 mol) in benzene (20 ml) at 10 °C. 30 min later, this mixture was refluxed gently for 1 h and then allowed to cool to room temperature. The organic layer was washed with brine (10 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was purified by vacuum distillation to afford the imino chlorides (**14a**–**14c**) which were then used directly in the next step.

2-Chloro-1-ethanol (**17**) (0.05 mol, 3.4 ml) was added dropwise into the solution of imine (**4a**–**4c**) (0.1 mol) in toluene (20 ml) at 25 °C, this mixture was refluxed gently for 3 h and then allowed to cool to room temperature. The mixture was filtered, washed with toluene. Then $SOCl_2$ (10 ml) was added into the filtrate, this mixture was refluxed gently for 2 h, and concentrated under reduced pressure. The residue was recrystallized from anhydrous alcohol to afford **19a**–**19c** as white solids which were then used directly in the next step. **24a**–**24b** were synthesized from 1-chloro-3-hydroxypropan (**22**) and imine (**4a**–**4c**) in the same way of **19a**–**19c**.

Compound **12** (100 mg, 0.27 mmol) was added into anhydrous DMF (20 mL) at 0 °C, treated with K_2CO_3 (44 mg, 0.32 mmol) and KI (11 mg, 0.07 mmol) followed by **14a**–**14c**, **19a**–**19c** and **24a**–**24b** (0.32 mmol), then the reaction mixture was warmed to room temperature. After 12 h, the mixture was poured into water (50 mL), and then extracted with ethyl acetate (50 mL × 3), the organic layer was washed with brine (100 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The appeared crude material was then purified by column chromatography on silica gel using 50% ethyl acetate in petroleum ether as eluent to afford **15a**–**15c**, **20a**–**20c** and **25a**–**25b** as yellow solids.

4.2.9.1. 2-(4-(5-Hydroxy-6,7-bis(methoxymethoxy)-4-oxo-4*H*-chromen-2-yl)phenoxy)-*N*,*N*-dimethylacetamide (15a).

68.1% yield, yellow solid, mp 186–187 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.01 (s, 6H, 2 × CH_3), 3.31 (s, 3H, CH_3), 3.54 (s, 3H, CH_3), 4.94 (s, 2H, CH_2), 5.18 (s, 2H, CH_2), 5.38 (s, 2H, CH_2), 6.62 (s, 1H, C_3-H), 6.96 (s, 1H, C_8-H), 7.10 (d, J = 8.7 Hz, 2H, $C_{3'}C_{5'}$-H), 8.06 (d, J = 8.7 Hz, 2H, $C_{2'}C_{6'}$-H), 12.70 (s, 1H, C_5-OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 38.13 (2 × C(CH_3)), 56.22 (C(CH_3)), 56.37 (C(CH_3)), 64.22 (C(CH_2)), 94.16 (C(CH_2)), 94.23 (C(CH_2)), 96.65 (C8), 102.25 (C3), 102.87 (C10), 115.03 (2 × C(C3′,C5′)), 121.00 (C1′), 127.12 (2 × C(C2′,C6′)), 129.89 (C6), 144.78 (C9), 150.77 (C5), 155.32 (C7), 163.33 (C4′), 163.45 (C2), 166.02 (CO), 181.97 (C4). ESI-MS: m/z 460 $[M+H]^+$.

4.2.9.2. 5-Hydroxy-6,7-bis(methoxymethoxy)-2-(4-(2-morpholino-2-oxoethoxy)phenyl)-4*H*-chromen-4-one (15b).

62.3% yield, yellow solid, mp 176–178 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.47 (s, 3H, CH_3), 3.48–3.52 (m, 4H, 2 × CH_2), 3.54 (s, 3H, CH_3), 3.58–3.63 (m, 4H, 2 × CH_2), 4.99 (s, 2H, CH_2), 5.18 (s, 2H, CH_2), 5.37 (s, 2H, CH_2), 6.63 (s, 1H, C_3-H), 6.96 (s, 1H, C_8-H), 7.13 (d, J = 8.7 Hz, 2H, $C_{3'}C_{5'}$-H), 8.03 (d, J = 8.7 Hz, 2H, $C_{2'}C_{6'}$-H), 12.69 (s, 1H, C_5-OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 44.44 (2 × C(CH_2)), 56.23 (C(CH_3)), 56.36 (C(CH_3)), 64.18 (C(CH_2)), 66.33 (2 × C(CH_2)), 94.18 (C(CH_2)), 94.21 (C(CH_2)), 96.65 (C8), 102.25 (C3), 102.89 (C10), 115.13 (2 × C(C3′,C5′)), 121.01 (C1′), 127.25 (2 × C(C2′,C6′)), 129.89 (C6), 144.78 (C9), 150.77 (C5), 155.32 (C7), 163.19 (C4′), 163.47 (C2), 167.03 (CO), 181.95 (C4). ESI-MS: m/z 502 $[M+H]^+$.

4.2.9.3. *N*,*N*-Diethyl-2-(4-(5-hydroxy-6,7-bis(methoxymethoxy)-4-oxo-4*H*-chromen-2-yl)phenoxy)acetamide (15c).

67.7% yield, yellow solid, mp 181–182 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.02–1.07 (t, 3H, CH_3), 1.15–1.20 (t, 3H, CH_3), 3.24–3.36 (m, 4H, 2 × CH_2), 3.44 (s, 3H, CH_3), 3.54 (s, 3H, CH_3), 4.94 (s, 2H, CH_2), 5.18 (s, 2H, CH_2), 5.37 (s, 2H, CH_2), 6.62 (s, 1H, C_3-H), 6.95 (s, 1H, C_8-H), 7.10 (d, J = 8.7 Hz, 2H, $C_{3'}C_{5'}$-H), 8.03 (d, J = 8.7 Hz, 2H, $C_{2'}C_{6'}$-H), 12.69 (s, 1H, C_5-OH). ^{13}C NMR (75 MHz, DMSO-d_6)

δ: 12.33 (2 × C(CH_3)), 42.02 (2 × C(CH_2)), 56.22 (C(CH_3)), 56.39 (C(CH_3)), 64.25 (C(CH_2)), 94.18 (C(CH_2)), 94.23 (C(CH_2)), 96.67 (C8), 102.27 (C3), 102.89 (C10), 114.01 (2 × C(C3′,C5′)), 121.02 (C1′), 127.99 (2 × C(C2′,C6′)), 129.87 (C6), 144.79 (C9), 150.78 (C5), 155.32 (C7), 163.02 (C4′), 163.47 (C2), 166.92 (CO), 181.97 (C4). ESI-MS: *m*/*z* 488 $[M+H]^+$.

4.2.9.4. 2-(4-(2-(Dimethylamino)ethoxy)phenyl)-5-hydroxy-6,7-bis(methoxymethoxy)-4*H*-chromen-4-one (20a). 64.5% yield, yellow solid, mp 191–193 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.29 (s, 6H, 2 × CH_3), 2.71–2.75 (t, 2H, CH_2), 3.45 (s, 3H, CH_3), 3.54 (s, 3H, CH_3), 4.16–4.21 (t, 2H, CH_2), 5.17 (s, 2H, CH_2), 5.36 (s, 2H, CH_2), 6.62 (s, 1H, C_3-H), 6.96 (s, 1H, C_8-H), 7.15 (d, *J* = 8.7 Hz, 2H, $C_{3'}C_{5'}$-H), 8.05 (d, *J* = 8.7 Hz, 2H, $C_{2'}C_{6'}$-H), 12.72 (s, 1H, C_5-OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 46.45 (2 × C(CH_3)), 56.23 (C(CH_3)), 56.37 (C(CH_3)), 58.32 (C(CH_2)), 66.88 (C(CH_2)), 94.19 (C(CH_2)), 94.23 (C(CH_2)), 96.67 (C8), 102.26 (C3), 102.86 (C10), 114.22 (2 × C(C3′,C5′)), 120.37 (C1′), 127.36 (2 × C(C2′,C6′)), 129.89 (C6), 144.76 (C9), 150.77 (C5), 155.33 (C7), 159.87 (C4′), 163.45 (C2), 181.97 (C4). ESI-MS: *m*/*z* 446 $[M+H]^+$.

4.2.9.5. 5-Hydroxy-6,7-bis(methoxymethoxy)-2-(4-(2-morpholinoethoxy)phenyl)-4*H*-chromen-4-one (20b). 61.8% yield, yellow solid, mp 198–199 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.71–2.74 (t, 4H, 2 × CH_2), 3.44 (s, 3H, CH_3), 3.53 (s, 3H, CH_3), 3.55–3.60 (m, 6H), 4.19–4.21 (t, 2H, CH_2), 5.17 (s, 2H, CH_2), 5.36 (s, 2H, CH_2), 6.62 (s, 1H, C_3-H), 6.95 (s, 1H, C_8-H), 7.15 (d, *J* = 8.7 Hz, 2H, $C_{3'}C_{5'}$-H), 8.05 (d, *J* = 8.7 Hz, 2H, $C_{2'}C_{6'}$-H), 12.69 (s, 1H, C_5-OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 53.79 (2 × C(CH_2)), 54.82 (C(CH_2)), 56.22 (C(CH_3)), 56.37 (C(CH_3)), 66.88 (2 × C(CH_2)), 66.92 (C(CH_2)), 94.16 (C(CH_2)), 94.22 (C(CH_2)), 96.67 (C8), 102.25 (C3), 102.89 (C10), 114.36 (2 × C(C3′,C5′)), 120.25 (C1′), 127.66 (2 × C(C2′,C6′)), 129.88 (C6), 144.78 (C9), 150.76 (C5), 155.33 (C7), 159.88 (C4′), 163.44 (C2), 181.94 (C4). ESI-MS: *m*/*z* 488 $[M+H]^+$.

4.2.9.6. 2-(4-(2-(Diethylamino)ethoxy)phenyl)-5-hydroxy-6,7-bis(methoxymethoxy)-4*H*-chromen-4-one (20c). 63.2% yield, yellow solid, mp 203–205 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.23–1.28 (t, 6H, 2 × CH_3), 2.51–2.54 (m, 4H, 2 × CH_2), 3.25–3.28 (t, 2H), 3.45 (s, 3H, CH_3), 3.54 (s, 3H, CH_3), 4.14–4.17 (t, 2H, CH_2), 5.09 (s, 2H, CH_2), 5.17 (s, 2H, CH_2), 6.63 (s, 1H, C_3-H), 6.96 (s, 1H, C_8-H), 7.14 (d, *J* = 8.7 Hz, 2H, $C_{3'}C_{5'}$-H), 8.05 (d, 2H, *J* = 8.7 Hz, $C_{2'}C_{6'}$-H), 12.70 (s, 1H, C_5-OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 14.02 (2 × C(CH_3)), 49.58 (2 × C(CH_2)), 54.13 (CH_2), 56.23 (C(CH_3)), 56.39 (C(CH_3)), 66.33 (C(CH_2)), 94.16 (C(CH_2)), 94.22 (C(CH_2)), 96.67 (C8), 102.25 (C3), 102.89 (C10), 114.21 (2 × C(C3′,C5′)), 120.25 (C1′), 127.36 (2 × C(C2′,C6′)), 129.87 (C6), 144.76 (C9), 150.77 (C5), 155.32 (C7), 159.83 (C4′), 163.45 (C2), 181.95 (C4). ESI-MS: *m*/*z* 474 $[M+H]^+$.

4.2.9.7. 2-(4-(3-(Dimethylamino)propoxy)phenyl)-5-hydroxy-6,7-bis(methoxymethoxy)-4*H*-chromen-4-one (25a). 66.9% yield, yellow solid, mp 199–200 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.03–2.06 (m, 2H), 2.49 (s, 6H, 2 × CH_3), 2.83–2.86 (m, 2H, CH_2), 3.45 (s, 3H, CH_3), 3.54 (s, 3H, CH_3), 4.13–4.17 (m, 2H, CH_2), 5.17 (s, 2H, CH_2), 5.37 (s, 2H, CH_2), 6.63 (s, 1H, C_3-H), 6.97 (s, 1H, C_8-H), 7.14 (d, *J* = 8.7 Hz, 2H, $C_{3'}C_{5'}$-H), 8.06 (d, *J* = 8.7 Hz, 2H, $C_{2'}C_{6'}$-H), 12.69 (s, 1H, C_5-OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 28.17 (C(CH_2)), 46.38 (2 × C(CH_3)), 54.99 (C(CH_2)), 56.22 (C(CH_3)), 56.37 (C(CH_3)), 67.01 (C(CH_2)), 94.18 (C(CH_2)), 94.21 (C(CH_2)), 96.67 (C8), 102.25 (C3), 102.87 (C10), 114.23 (2 × C(C3′,C5′)), 120.18 (C1′), 127.52 (2 × C(C2′,C6′)), 129.89 (C6), 144.76 (C9), 150.79 (C5), 155.32 (C7), 159.87 (C4′), 163.47 (C2), 181.95 (C4). ESI-MS: *m*/*z* 460 $[M+H]^+$.

4.2.9.8. 5-Hydroxy-6,7-bis(methoxymethoxy)-2-(4-(3-morpholinopropoxy)phenyl)-4*H*-chromen-4-one (25b). 62.4% yield, yellow solid, mp 203–205 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.91–1.93 (m, 2H, CH_2), 2.34–2.37 (m, 2H, CH_2), 2.38–2.41 (m, 4H, 2 × CH_2), 3.44–3.47 (m, 4H, 2 × CH_2), 3.48 (s, 3H, CH_3), 3.54 (s, 3H, CH_3), 4.11–4.13 (m, 2H, CH_2), 5.17 (s, 2H, CH_2), 5.36 (s, 2H, CH_2), 6.62 (s, 1H, C_3-H), 6.95 (s, 1H, C_8-H), 7.13 (d, *J* = 8.7 Hz, 2H, $C_{3'}C_{5'}$-H), 8.05 (d, *J* = 8.7 Hz, 2H, $C_{2'}C_{6'}$-H), 12.70 (s, 1H, C_5-OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 28.33 (C(CH_2)), 51.10 (C(CH_2)), 53.75 (2 × C(CH_2)), 56.23 (C(CH_3)), 56.39 (C(CH_3)), 66.85 (C(CH_2)), 66.89 (2 × C(CH_2)), 94.18 (C(CH_2)), 94.23 (C(CH_2)), 96.67 (C8), 102.27 (C3), 102.86 (C10), 114.38 (2 × C(C3′,C5′)), 120.18 (C1′), 127.57 (2 × C(C2′,C6′)), 129.87 (C6), 144.78 (C9), 150.76 (C5), 155.32 (C7), 159.87 (C4′), 163.45 (C2), 181.95 (C4). ESI-MS: *m*/*z* 502 $[M+H]^+$.

4.2.10. Synthesis of 16a–16c, 21a–21c and 26a–26b

HCl (1 mL) was added dropwise into a solution of **15a**–**15c**, **20a**–**20c** and **25a**–**25b** (1.00 mmol) in CH_2Cl_2 (5 mL) and Et_2O (5 mL) at 0 °C, then this mixture was warmed to room temperature. After 6 h, the mixture was poured into water (20 mL), and then extracted with ethyl acetate (20 mL × 3), the organic layer was washed with brine (20 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The appeared crude material was purified by column chromatography on silica gel using 50% ethyl acetate in petroleum ether as eluent to afford **16a**–**16c**, **21a**–**21c** and **26a**–**26b** as yellow solids.

4.2.10.1. *N,N*-Dimethyl-2-(4-(5,6,7-trihydroxy-4-oxo-4*H*-chromen-2-yl)phenoxy)acetamide (16a). 88.2% yield, yellow solid, mp 228–229 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.85 (s, 6H, 2 × CH_3), 4.96 (m, 2H, CH_2), 6.60 (s, 1H, C_3-H), 6.88 (s, 1H, C_8-H), 7.10 (d, *J* = 8.7 Hz, 2H, $C_{3'}C_{5'}$-H), 8.09 (d, *J* = 8.7 Hz, 2H, $C_{2'}C_{6'}$-H), 8.96 (s, 1H, C_6-OH), 10.63 (s, 1H, C_7-OH), 12.36 (s, 1H, C_5-OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 38.12 (2 × C(CH_3)), 64.23 (C(CH_2)), 98.67 (C8), 102.24 (C3), 103.55 (C10), 115.02 (2 × C(C3′,C5′)), 121.01 (C1′), 124.66 (C6), 127.11 (2 × C(C2′,C6′)), 145.57 (C9), 152.86 (C5), 153.87 (C7), 163.34 (C4′), 163.44 (C2), 166.03 (CO), 181.96 (C4). ESI-MS: *m*/*z* 372 $[M+H]^+$.

4.2.10.2. 5,6,7-Trihydroxy-2-(4-(2-morpholino-2-oxoethoxy)phenyl)-4*H*-chromen-4-one (16b). 86.8% yield, yellow solid, mp 231–232 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.42–3.46 (m, 4H, 2 × CH_2), 3.58–3.62 (m, 4H, 2 × CH_2), 4.99 (s, 2H, CH_2), 6.28 (s, 1H, C_3-H), 6.84 (s, 1H, C_8-H), 7.11 (d, *J* = 8.7 Hz, 2H, $C_{3'}C_{5'}$-H), 8.11 (d, *J* = 8.7 Hz, 2H, $C_{2'}C_{6'}$-H), 8.83 (s, 1H, C_6-OH), 10.49 (s, 1H, C_7-OH), 12.35 (s, 1H, C_5-OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 44.43 (2 × C(CH_2)), 64.17 (C(CH_2)), 66.34 (2 × C(CH_2)), 98.64 (C8), 102.26 (C3), 103.56 (C10), 115.14 (2 × C(C3′,C5′)), 121.00 (C1′), 124.67 (C6), 127.26 (2 × C(C2′,C6′)), 145.55 (C9), 152.87 (C5), 153.86 (C7), 163.18 (C4′), 163.48 (C2), 167.04 (CO), 181.96 (C4). ESI-MS: *m*/*z* 414 $[M+H]^+$.

4.2.10.3. *N,N*-Diethyl-2-(4-(5,6,7-trihydroxy-4-oxo-4*H*-chromen-2-yl)phenoxy)acetamide (16c). 89.1% yield, yellow solid, mp 235–237 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.16–1.23 (t, 6H, 2 × CH_3), 3.26–3.30 (m, 4H, 2 × CH_2), 4.94 (m, 2H, CH_2), 6.28 (s, 1H, C_3-H), 6.84 (s, 1H, C_8-H), 7.08 (d, *J* = 8.7 Hz, 2H, $C_{3'}C_{5'}$-H), 8.11 (d, *J* = 8.7 Hz, 2H, $C_{2'}C_{6'}$-H), 8.81 (s, 1H, C_6-OH), 10.51 (s, 1H, C_7-OH), 12.35 (s, 1H, C_5-OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 12.34 (2 × C(CH_3)), 42.03 (2 × C(CH_2)), 64.26 (C(CH_2)), 98.65 (C8), 102.26 (C3), 103.57 (C10), 114.02 (2 × C(C3′,C5′)), 121.01 (C1′), 124.66 (C6), 127.98 (2 × C(C2′,C6′)), 145.57 (C9), 152.86 (C5), 153.88 (C7), 163.03 (C4′), 163.46 (C2), 166.91 (CO), 181.96 (C4). ESI-MS: *m*/*z* 400 $[M+H]^+$.

4.2.10.4. 2-(4-(2-(Dimethylamino)ethoxy)phenyl)-5,6,7-trihydroxy-4*H*-chromen-4-one (21a). 85.3% yield, yellow solid, mp 143–145 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.87 (s, 6H, 2 × CH_3), 3.51–3.55 (t, 2H, CH_2), 4.44–4.47 (t, 2H, CH_2), 6.63 (s, 1H, C_3-H), 6.87 (s, 1H, C_8-H), 7.17 (d, J = 8.7 Hz, 2H, $C_{3'}C_{5'}$-H), 8.07 (d, J = 8.7 Hz, 2H, $C_{2'}C_{6'}$-H), 8.84 (s, 1H, C_6-OH), 10.58 (s, 1H, C_7-OH), 12.72 (s, 1H, C_5-OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 46.44 (2 × C(CH_3)), 58.35 (C(CH_2)), 66.87 (C(CH_2)), 98.66 (C8), 102.25 (C3), 103.58 (C10), 114.23 (2 × C(C3′,C5′)), 120.38 (C1′), 124.65 (C6), 127.37 (2 × C(C2′,C6′)), 145.58 (C9), 152.87 (C5), 153.86 (C7), 159.85 (C4′), 163.43 (C2), 181.96 (C4). ESI-MS: m/z 358 [M+H]$^+$.

4.2.10.5. 5,6,7-Trihydroxy-2-(4-(2-morpholinoethoxy)phenyl)-4*H*-chromen-4-one (21b). 83.8% yield, yellow solid, mp 248–250 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.58–2.61 (m, 4H, 2 × CH_2), 2.71–2.73 (t, 2H, CH_2), 3.56–3.59 (m, 4H, 2 × CH_2), 4.17–4.20 (t, 2H, CH_2), 6.18 (s, 1H, C_3-H), 6.74 (s, 1H, C_8-H), 7.12 (d, J = 8.7 Hz, 2H, $C_{3'}C_{5'}$-H), 8.07 (d, J = 8.7 Hz, 2H, $C_{2'}C_{6'}$-H), 12.38 (s, 1H, C_5-OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 53.77 (2 × C(CH_2)), 54.81 (C(CH_2)), 66.89 (2 × C(CH_2)), 66.91 (C(CH_2)), 98.67 (C8), 102.24 (C3), 103.57 (C10), 114.35 (2 × C(C3′,C5′)), 120.24 (C1′), 124.64 (C6), 127.67 (2 × C(C2′,C6′)), 145.57 (C9), 152.88 (C5), 153.85 (C7), 159.89 (C4′), 163.45 (C2), 181.95 (C4). ESI-MS: m/z 400 [M+H]$^+$.

4.2.10.6. 2-(4-(2-(Diethylamino)ethoxy)phenyl)-5,6,7-trihydroxy-4*H*-chromen-4-one (21c). 87.4% yield, yellow solid, mp 140–141 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.17–1.24 (t, 6H, 2 × CH_3), 3.21–3.25 (m, 4H, 2 × CH_2), 3.52–3.55 (t, 2H, CH_2), 4.42–4.45 (t, 2H, CH_2), 6.29 (s, 1H, C_3-H), 6.86 (s, 1H, C_8-H), 7.19 (d, J = 8.7 Hz, 2H, $C_{3'}C_{5'}$-H), 8.16 (d, J = 8.7 Hz, 2H, $C_{2'}C_{6'}$-H), 8.78 (s, 1H, C_6-OH), 10.51 (s, 1H, C_7-OH), 12.31 (s, 1H, C_5-OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 14.03 (2 × C(CH_3)), 49.59 (2 × C(CH_2)), 56.38 (C(CH_3)), 66.34 (C(CH_2)), 98.66 (C8), 102.26 (C3), 103.56 (C10), 114.22 (2 × C(C3′,C5′)), 120.24 (C1′), 124.65 (C6), 127.35 (2 × C(C2′,C6′)), 145.56 (C9), 152.87 (C5), 153.86 (C7), 159.84 (C4′), 163.44 (C2), 181.94 (C4). ESI-MS: m/z 386 [M+H]$^+$.

4.2.10.7. 2-(4-(3-(Dimethylamino)propoxy)phenyl)-5,6,7-trihydroxy-4*H*-chromen-4-one (26a). 89.3% yield, yellow solid, mp 153–155 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.21–1.24 (m, 2H, CH_2), 2.68–2.72 (m, 2H, CH_2), 2.75 (s, 6H, 2 × CH_3), 4.16–4.19 (m, 2H, CH_2), 6.32 (s, 1H, C_3-H), 6.85 (s, 1H, C_8-H), 7.15 (d, J = 8.7 Hz, 2H, $C_{3'}C_{5'}$-H), 8.12 (d, J = 8.7 Hz, 2H, $C_{2'}C_{6'}$-H), 8.84 (s, 1H, C_6-OH), 10.47 (s, 1H, C_7-OH), 12.33 (s, 1H, C_5-OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 28.16 (C(CH_2)), 46.37 (2 × C(CH_3)), 54.98 (C(CH_2)), 67.02 (C(CH_2)), 98.65 (C8), 102.24 (C3), 103.55 (C10), 114.22 (2 × C(C3′,C5′)), 120.17 (C1′), 124.66 (C6), 127.53 (2 × C(C2′,C6′)), 145.54 (C9), 152.88 (C5), 153.87 (C7), 159.85 (C4′), 163.46 (C2), 181.96 (C4). ESI-MS: m/z 372 [M+H]$^+$.

4.2.10.8. 5,6,7-Trihydroxy-2-(4-(3-morpholinopropoxy)phenyl)-4*H*-chromen-4-one (26b). 85.4% yield, yellow solid, mp 232–234 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.22–1.25 (m, 2H, CH_2), 2.12–2.16 (m, 6H), 3.61–3.65 (m, 4H, 2 × CH_2), 4.11–4.14 (m, 2H, CH_2), 6.28 (s, 1H, C_3-H), 6.86 (s, 1H, C_8-H), 7.13 (d, J = 8.7 Hz, 2H, $C_{3'}C_{5'}$-H), 8.06 (d, J = 8.7 Hz, 2H, $C_{2'}C_{6'}$-H), 8.82 (s, 1H, C_6-OH), 10.55 (s, 1H, C_7-OH), 12.34 (s, 1H, C_5-OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 28.34 (C(CH_2)), 51.11 (C(CH_2)), 53.74 (2 × C(CH_2)), 66.84 (C(CH_2)), 66.88 (2 × C(CH_2)), 98.66 (C8), 102.28 (C3), 103.54 (C10), 114.37 (2 × C(C3′,C5′)), 120.17 (C1′), 124.67 (C6), 127.56 (2 × C(C2′,C6′)), 145.53 (C9), 152.87 (C5), 153.88 (C7), 159.89 (C4′), 163.44 (C2), 181.96 (C4). ESI-MS: m/z 414 [M+H]$^+$.

4.3. Antithrombotic assay

4.3.1. Blood collection

Male New Zealand white rabbits, weighing 2–2.5 kg were obtained from the experimental animal center of Nanjing University of Chinese Medicine and were approved by Animal Ethics Committee of Nanjing University of Chinese Medicine. They were kept in plastic cages at 22 ± 2 °C with free access to pellet food and water and on a 12 h light/dark cycle. Animal welfare and experimental procedures were carried out in accordance with the guide for the care and use of laboratory animals (National Research Council of USA, 1996) and related ethical regulations of Nanjing University of Chinese Medicine. Rabbits were anesthetized with pentobarbital (50 mg/kg) and blood was drawn from the common carotid artery. Blood was collected into plastic tubes with 3.8% sodium citrate (citrate/blood: 1:9, v/v) for plasma anticoagulation. Platelet-poor plasma (PPP) was separated from blood by centrifugation at 3000 rpm for 10 min.

4.3.2. Plasma antithrombotic assay

TT, APTT, PT and FIB were examined with commercial kits following the manufacturer's instructions by a coagulometer (Model LG-PABER-I, Steellex Co., China). All the compounds were dissolved in 80% ethanol, and the concentration was 100 μM. TT was determined by incubating 40 μl PPP solution for 3 min at 37 °C, followed by addition of 40 μl thrombin solution and 20 μl sample for 3 min at 37 °C. APTT was determined by incubating 10 μl sample solution and 50 μl PPP solution with 50 μl APTT-activating agent for 3 min at 37 °C, followed by addition of 50 μl $CaCl_2$. PT was determined by incubating 40 μl PPP solution for 3 min at 37 °C, followed by addition of 40 μl thromboplastin agent and 20 μl sample. FIB was determined by incubating 10 μl PPP solution with 90 μl imidazole buffer for 3 min at 37 °C, followed by addition of 50 μl FIB agent and 10 μl sample solution. The antithrombotic activity was assessed by assaying the prolongation of the plasma clotting time of TT, APTT, INR increasement of PT, and reduction of FIB content.

4.4. Antioxidant assay

4.4.1. DPPH radical scavenging assay

The DPPH· radical scavenging activity was measured according to previous studies.[20] The solution of the sample (100 μL) in dimethylsulfoxide (DMSO) was added to 100 μL of DPPH radical in ethanol (0.2 mM) in 96-well plate. The sample solution refers to the tested compounds and the reference antioxidants at various concentrations, as well as DMSO as a control. The solutions of the tested compounds had concentrations ranging from 5 μM to 500 μM. The reaction leading to the scavenging of DPPH radical was completed within 30 min at 25 °C. The absorbance of the mixture was then measured at 517 nm with a microplate reader. The inhibition of DPPH radical was expressed as percentage: Scavenging rate % = $[1 - (A_1 - A_2)/A_0] \times 100\%$, where A_1 was the absorbance of the test substance acted with DPPH, A_2 was the absorbance of the test substance without DPPH and A_0 was the absorbance of the blank. The IC_{50} value was defined as the concentration of sample that causes 50% loss of the DPPH radical.

4.4.2. PC12 cells antioxidant assay

Cell viability was estimated by MTT assay. Briefly, PC12 cells were seeded in 96-well plates at a density of 5×10^4 cells/ml (100 μl per well), then they were cultured at 37 °C in humidified air containing 5% CO_2 for 24 h. The experiment was divided into control group and H_2O_2 damage model group, each set five wells. In the control group was serum-free DMEM medium. The H_2O_2 damage model group was exposure to 400 μmol L^{-1} H_2O_2 for 1 h

before the test drugs were added in. Drug concentrations were 50 μmol L^{-1} and 25 μmol L^{-1}. The cells were incubated with 20 μl of MTT solution for 4 h at 37 °C. The formazan crystals formed in intact cells were dissolved in 150 μl of DMSO, and then vigorously shaking for 10 min. Finally, the absorbance was assessed at 517 nm. The inhibiting rate of H_2O_2-induced cytotoxicity in PC12 cells was calculated as inhibiting rate (%) = $[(A_1 - A_2)/(A_0 - A_2)] \times 100\%$, where A_1 was the absorbance in the presence of sample and H_2O_2, A_2 was the absorbance in the presence of H_2O_2 (model), A_0 was the absorbance without sample and H_2O_2 (normal).

4.5. Solubility determination

The solubility of scutellarein derivatives in water was determined using the known method[29–31] with minor modifications. The UV/UV device is composed of a UV source (for UV photodegradation) and a UV absorption detector (for on-line UV measurement). The UV source is a low pressure mercury lamp and the UV detector is a UV–vis spectrophotometer Anthelie (Secomam) controlled by software Dathelie, version 4.1f. The pathlength of the Suprasil quartz cell is 10 mm and the scan speed is 2000 nm min^{-1}. Under the influence of UV radiation, scutellarein derivatives were monitored by UV absorption spectrophotometry at the wavelength of maximum absorbance (334 nm) from the whole spectrum using a multicomponent exploitation method. Each tested compound (250 μg) was dissolved in 25 ml CH_3OH. The solutions of the tested compounds had concentrations ranging from 0.1 μg/ml to 10 μg/ml. Different concentration solutions of each compound were determined by UV scanning, and the absorbances were obtained. The results showed a good linear relationship, then all the standard curves were completed. Each tested compound (150 μg) was ultrasound dissolved in 10 ml pure water for 1 h at room temperature. The solutions were stranded for 30 min and centrifuged at the speed of 30000 r/min. The aqueous solution of each compound was determined by UV scanning, and the absorbances were obtained. Then through the analyzation of standard curve, all the compounds in the water solubility were obtained.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2015.09.047. These data include MOL files and InChiKeys of the most important compounds described in this article.

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