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Synthesis, biological evaluation of prenylflavonoids as vasorelaxant and neuroprotective agents

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ABSTRACT

A series of prenylflavonoids with multiple hydroxyl groups were synthesized and evaluated for their vasorelaxant activities against rat aorta rings pre-contracted by phenylephrine (PE), as well as their neuroprotective effects against OGD induced PC12 cell injury. The results indicated that the prenyl group at A-ring of prenylflavonoids, as well as hydroxyl groups at B-ring was important for their activities. (±)Leachianone G **1b**, bearing 8-prenyl and 2′,4′-dihydoxyl groups, exhibited the most potent vasorelaxant and neuroprotective effects.

A number of large-scale epidemiological studies had validated the protective effects of flavonoids against cardiovascular diseases,[1,2] and several possible mechanisms including anti-inflammatory, antioxidant and improving vascular function effects were suggested.[3,4] Besides, the vascular relaxation responses and antihypertensive effects of flavonoids demonstrated in vitro and in vivo studies were presented recently.[5,6] Superior to traditional vasodilators such as nitrates and varapamil, flavonoids possessed additional antioxidant property, which was also very important for the treatment of cardiovascular diseases.[7,8] Furthermore, accumulated evidence indicated that some flavonoids (quercetin, baicalin and icariin) could remarkably protect against cerebral ischemia neuronal damage, mainly due to their antioxidant properties.[9,10] Therefore, the development of both vasorelaxant potent and neuroprotective prominent flavonoid derivatives would be very interest, and may result in new leads. This would be a good strategy for the treatment of cardiovascular, especially cerebrovascular diseases.

Prenylflavonoids, a unique class of naturally occurring flavonoids characterized by the presence of a prenylated side chain on the flavonoid skeleton, were mainly isolated from traditional medicinal plants.[11] It was reported that prenyl groups on flavonoids could remarkably improve various biological activities such as antioxidant activities.[11–13] Eleven prenylated flavonoids were designed and synthesized according to predictive results of SVM classification model in our previous studies. Some of the synthesized compounds (such as compound **1e**) were found to possess remarkable vasorelaxant activities.[14] With the aim of developing flavonoids with dual vasorelaxant and neuroprotective activities, herein, a series of prenylflavonoids with multiple hydroxyl groups were prepared and evaluated for their biological activities. Among them, compounds **1a**,[15] **1b**,[16,17] **1e**,[18] **3a**,[19] **4b**[20] and **4c**[21] had been reported as natural products, and their antioxidant, estrogen-like, anticancer and other bioactivities were investigated.

The synthetic method of prenylated flavonoids **1a–b**, **1d–g**, **2a–b**, **3a** and **4b–d** were outlined in Scheme 1. Compounds **1a** and **1e–g** were previously synthesized,[14,22] compounds **1b**, **1d**, **2a–b** and **3a** were prepared according to our previous studies.[14,23] Prenylflavones **4b–d** were obtained by dehydrogenation in the presence of I_2 in pyridine and successively demethoxymethylation in catalytic amount of 3 N HCl in MeOH/THF (1/1, v/v) using corresponding flavanones **7b–d**.

The vasorelaxant activities of synthesized prenylflavonoids were evaluated in the rat thoracic aorta rings with endothelium against PE-induced contractions model,[24] and the neuroprotective effects on oxygen-glucose deprivation (OGD) induced PC12 cell injury were also assayed.[9] The results are summarized in Table 1 and Figure 1. Among these compounds, some prenylflavanone and prenylflavone derivatives (**1b**, **1e** and **4b**) exhibited potent and dual activities, while prenylaurone (**2a–b**) and prenylchalcone (**3a**) derivatives only showed either neuroprotective or vasorelaxant activities. Comparing the vasorelaxant and neuroprotective activities between (±)Homoeriodictyol (without prenyl) and compound **1e** (with prenyl) which shared the same skeleton indicated that the prenyl group could significantly enhance both of the biological

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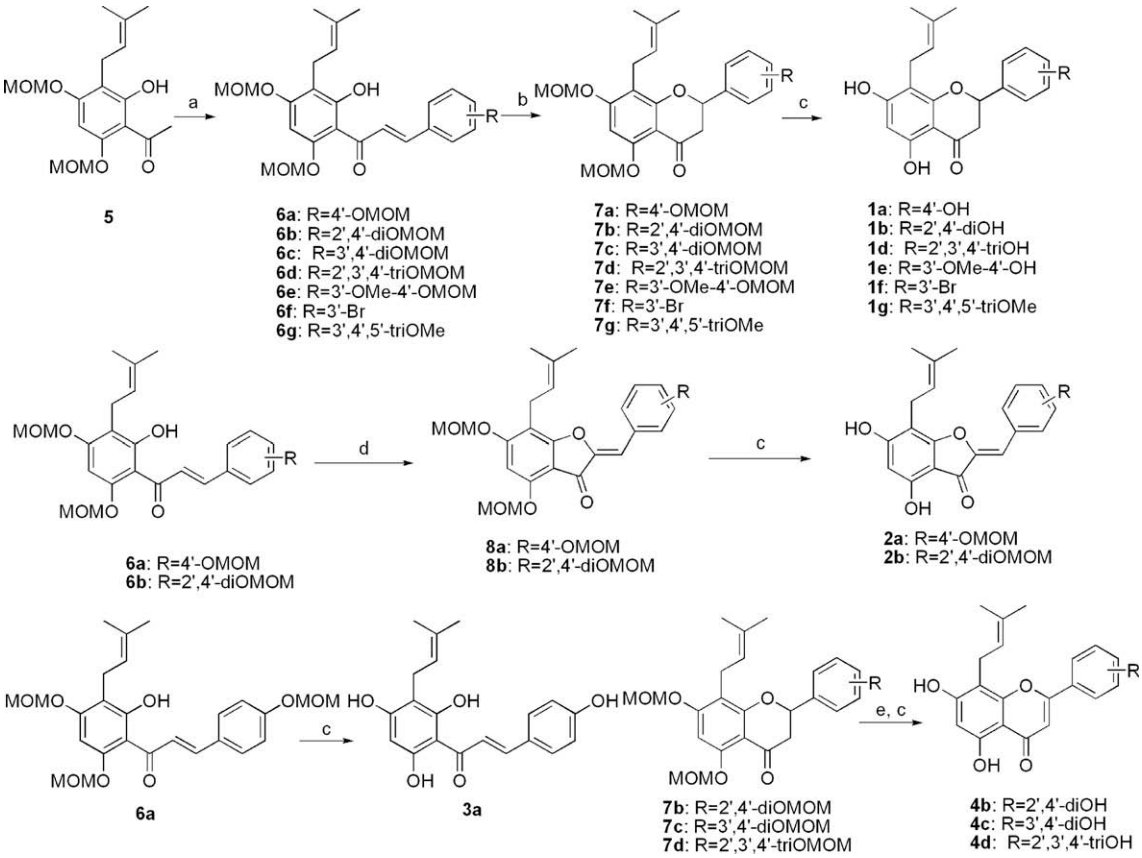
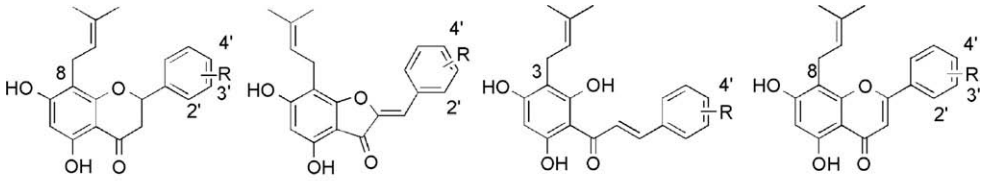


Scheme 1. Synthesis of prenylflavonoids **1a–b**, **1d–g**, **2a–b**, **3a** and **4b–d**. Reagents and conditions: (a) appropriate benzaldehyde, 10% KOH (H_2O/EtOH); (b) NaOAc, EtOH, reflux; (c) 3 N HCl, MeOH/THF, reflux; (d) $Hg(OAc)_2$, pyridine, 60 °C; (e) I_2, pyridine, 90 °C.

Table 1
Vasorelaxant and neuroprotective effects of prenylflavnoids



Compounds	R	Vasorelaxant effect[a]		Neuroprotective effect[b]
		EC_{50} (×10 μM)	E_{max} (%)	Cell viability (%)
Quercetin	/	24.4	91.3 ± 13.2	N.T.[c]
Edaravone	/	N.T.[c]	N.T.[c]	93.2 ± 2.9
(±)Homoeriodictyol	/	8.20	74.9 ± 10.9	89.1 ± 0.3
1a	4′-OH	7.27	100.7 ± 10.0	89.3 ± 4.2
1b	2′,4′-diOH	0.93	94.9 ± 13.4	94.8 ± 1.0
1d	2′,3′,4′-triOH	N.D.[d]	69.7 ± 4.4	90.3 ± 2.4
1e	3′-OMe-4′-OH	10.0[e]	95.8 ± 5.4[e]	94.0 ± 1.3
1f	3′-Br	1.6[e]	78.2 ± 4.7[e]	88.1 ± 1.8
1g	3′,4′,5′-triOMe	2.5[e]	66.3 ± 17.0[e]	89.1 ± 1.6
2a	4′-OH	N.D.[d]	74.5 ± 8.9	81.6 ± 1.7
2b	2′,4′-diOH	N.D.[d]	30.5 ± 15.3	90.4 ± 2.8
3a	4′-OH	0.85	81.4 ± 11.6	86.7 ± 2.4
4b	2′,4′-diOH	17.6	91.5 ± 8.1	93.5 ± 1.6
4c	3′,4′-diOH	N.D.[d]	62.5 ± 11.9	91.5 ± 2.4
4d	2′,3′,4′-triOH	N.T.[c]	N.T.[c]	88.6 ± 2.2

[a] Vasorelaxant effects of flavonoids on aortic rings with endothelium pre-contracted with 1 μM PE.
[b] The neuroprotective effects of flavonoids on OGD-induced PC12 cell injury. The cell viability in control was taken as 100%, and cell viability in OGD condition were 80.6%. Flavonoids (1 μM) and Edaravone (30 μM) significantly attenuated the reduced cell viability.
[c] N.T. means no tested.
[d] N.D. means not determined.
[e] The vasorelaxant activities have been reported in our previous studies.[14]

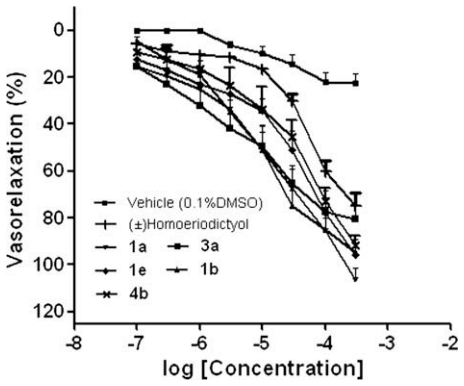


Figure 1. Effects of potent prenylflavonoids (**1a**, **1b**, **1e**, **3a** and **4b**) together with (±)Homoeriodictyol on relaxation in aortic rings with endothelium pre-contracted with 1 μM phenylephrine (PE) in concentration-dependent manner. Results are expressed as mean ± SD (n = 3–4).

activities. The results were consistent with previous studies which indicated prenyl groups contributed greatly to various biological activities of flavonoids.[11–13]

Probe on different substituent groups at B-ring of prenylflavonoids was followed after the discussion on the effects of molecular skeleton and prenyl groups. The results indicated that both of the optimal vasorelaxant and neuroprotective effects were also intensely associated with the presence of hydroxyl groups (E_{max} and cell viability: **1a**–**b**, **1e** > **1f**–**g**), while three contiguous hydroxyl groups at B-ring attenuated the activities (E_{max} and cell viability: **1b**, **4b** > **1d**, **4d**). For example, (±)Leachianone G **1b** which bear 8-prenyl and 2′,4′-$(OH)_2$ exhibited the most potent vasorelaxant (EC_{50}: 9.3 μM, E_{max}: 94.9%) and neuroprotective activities (cell viability for **1b**: 94.8%). In addition, 3′-OMe at B-ring of prenylflavanone could significantly increase the neuroprotective activities (cell viability for **1e** and **1a**: 94.0% and 89.3%, respectively).

Figure 2 presented that the cumulative addition of prenylflavonoids **1b**, **1e** and **4b** (0.01–1 μM) caused concentration-dependent neuroprotective effects with the maximal effect observed at 1 μM.

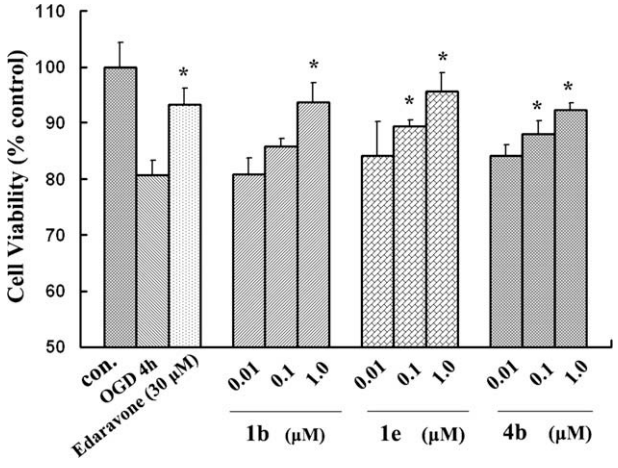


Figure 2. Neuroprotective effects of prenylflavonoids **1b**, **1e** and **4b** on OGD-induced PC-12 cells injury in concentration-dependent manner. Prenylflavonoids **1b**, **1e** and **4b** (1 μM) and Edaravone (30 μM) significantly attenuated the reduced cell viability. Date are reported as mean ± SD (n = 4–6), $^{*}P < 0.05$ versus OGD alone.

In summary, the synthesized prenylflavonoids had been characterized as agents with remarkable vasorelaxant and significant neuroprotective effects. Some common structural features shared in potent agents were found, such as the existence of prenyl group at A-ring and 2′,4′-$(OH)_2$ substitution at B-ring of prenylflavonoids. (±)Leachianone G **1b**, the most potent one, would be a promising structural template for the development of novel and more efficient vasorelaxant and neuroprotective agents.

Further studies of (±)Leachianone G **1b** are in progress in order to clarify the precise mechanism of its vasorelaxant and neuroprotective effects.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.04.120.

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