Full Paper

Design, Synthesis, and Biological Evaluation of Prenylated Chalcones as Vasorelaxant Agents

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Five prenylated chalcones and one allylated chalcone were prepared according to the analysis based on support vector machine (SVM) classification model. Most of the synthesized chalcones showed potent vasorelaxant activities through evaluation in aortic rings with the endothelium pre-contracted by phenylephrine (PE), indicating that the experimental activities were in good agreement with the theoretical ones. Structure-activity relationship of these compounds showed that the substituent pattern and number of hydroxyl groups were crucial for their vasorelaxant activities and that the replacement of prenyl group with allyl group retained the potent activity.

Keywords: Flavonoids / Prenylated chalcones / Support vector machine (SVM) / Vasorelaxant activity

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Introduction

Epidemiological studies suggested that the incidence of heart diseases can be lowered in humans if they have a high dietary intake of flavonoids [1, 2]. Cardioprotective effects of flavonoids are connected and might be explained by their anti-inflammatory, antioxidant, anti-platelet, and vascular dysfunction protecting activities [3, 4]. Recently, many flavonoids have been found to exhibit vasorelaxant effects in isolated vascular preparation and animal models [4–6]. Superior to traditional vasodilator, flavonoids also exhibited antioxidant activities [7]. All these facts prompt the strategy to develop novel and more potent flavonoids as vasorelaxant agents for the treatment of cardiovascular diseases. Chalcones, a member of the flavonoid family, have been identified as interesting compounds that bear antioxidant activity and could stabilize action on the vascular wall including vasodilatory and anti-aggregating effects [8, 9]. In our previous studies, a support vector machine (SVM) was employed to establish a good classification model using 111 vasodilators and 232 non-vasodilators. As shown in Fig. 1, the predictibe accuracy of the obtained classification model for the training, the test, and overall data sets were 93.0%, 82.6%, and 89.5%, respectively. In order to

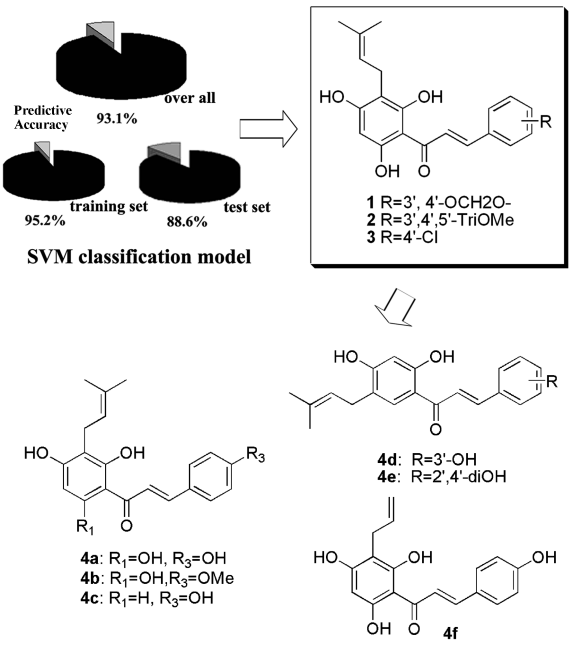

Figure 1. Results of SVM classification model and structures of prenylated chalcone derivatives synthesized.

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Abbreviations: phenylephrine (PE); support vector machine (SVM)

Table 1. The molecular descriptor values of compounds **4a – f**.

Comp.	D/D	Jhetp	TIC1	SEigp	SP20	RDF030m	Mor06u	ATS8v	JGI1
4a	210.25	2.19	157.52	−6.00	8.33	6.28	−2.11	3.02	0.23
4b	231.39	2.12	170.90	−6.00	10.24	7.19	−0.83	3.06	0.22
4c	193.98	2.14	151.91	−4.80	8.38	5.82	−2.42	2.91	0.22
4d	193.43	2.15	151.91	−4.80	10.96	8.05	−2.34	2.98	0.24
4e	210.71	2.14	157.52	−6.00	11.15	5.85	−1.35	3.03	0.25
4f	172.50	2.17	131.30	−6.00	9.15	6.84	−2.87	2.79	0.21

validate the model, three prenylated chalcones predicted as vasodilators by the classification model were synthesized and evaluated as moderate vasodilators (Fig. 1) [10].

In the present study, with the aim to discover more potent prenylated chalcones and to study the structure-activity relationship (SAR) of these compounds, five prenylated chalcones and one allylated chalcone were prepared according to the analysis based on the SVM classification model. Among these compounds, desmethylxanthohumol **4a** and isobavachalcone **4c** were natural products, and their antioxidant, anticancer, and other bioactivities had been reported [11 – 13]. However, few studies about their vasorelaxant activities were available. Herein, the vasorelaxant activities of all these synthesized compounds were evaluated against rat-aorta rings pretreated with 1 μM phenylephrine (PE). The structure-activity relationship was also discussed.

Table 2. The experimental and theoretical results of vasorelaxant activities of synthesized chalcones.

Compound	EC_{50} (10 μM)	E_{max} (%)[a]	Classification[b]
Quercetin	24.4	91 ± 13	Active
4a	0.85	80 ± 13	Active
4b	805	55 ± 5	Active[c]
4c	10.2	101 ± 5	Active
4d	12.3	92 ± 9	Active
4e	1.07	81 ± 5	Active
4f	2.40	108 ± 3	Active
1[d]	4.21	90 ± 13	Active
2[d]	N.D.[e]	79 ± 20	Active
3[d]	679	64 ± 11	Active[c]

[a] These compounds were reported in our previous studies [10].
[b] each value is the mean ± SD from four experiments.
[c] The classification of compounds was provided by SVM model.
[d] N.D.: not determined.
[e] The compounds misclassified by the SVM classification model could not significantly relax the PE-induced vascular contraction (E_{max} < 70% and EC_{50} > 1 mM) were classified as inactive vasodilators misclassified compounds.

Results and discussion

Data preparation and SVM prediction

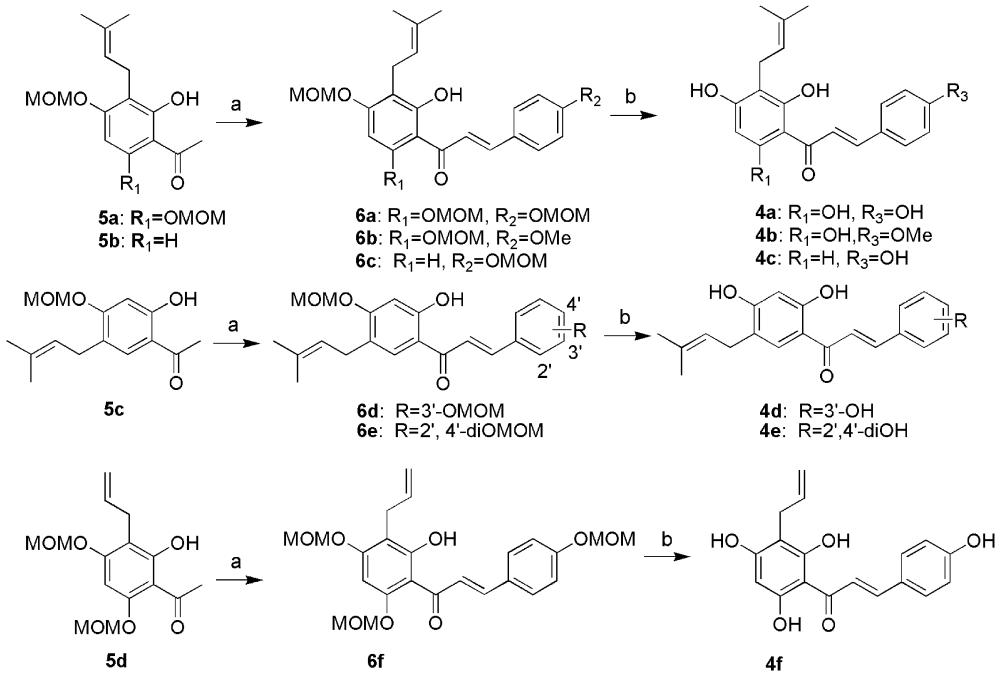
All the chalcone derivatives were sketched, optimized, and finally energy-minimized using CHARMm, and to obtain stable structures for further studies Discovery Studio 2.0 software (Accelrys, Inc. San Diego, CA) was used. The resulted geometry was transferred into Dragon software (TELETE, srl, Milano, Italy), which could calculate constitutional descriptors, topological descriptors, walk-and-path counts, information indices, 2-D autocorrelations, edge adjacency indices, Burden eigenvalue descriptors, etc. In addition, nine most relevant descriptors in the SVM classification model were calculated for the target compounds as shown in Table 1. The predicted classes (active or inactive) of these compounds were calculated as shown in Table 2 using the obtained SVM classification model.

Chemistry

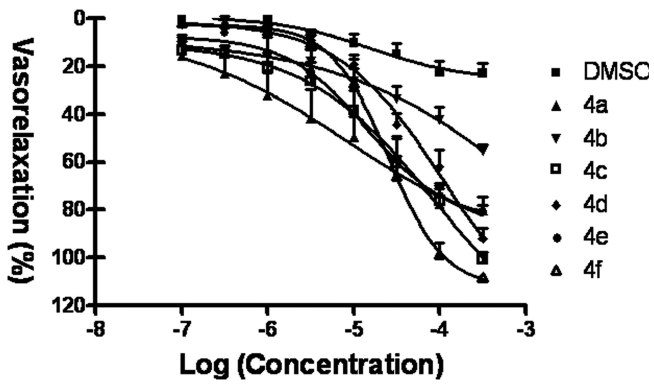
The synthetic route for prenylated and allylated chalcones **4a – f** is outlined in Scheme 1. Acetophenones **5a – d** were prepared according to the reported methods [14, 15]. Condensation of **5a – d** with corresponding benzaldehydes in aqueous alcoholic alkali solution afforded chalcones **6a – f**. Demethoxymethylation of **6a – f** was carried out in catalytic amounts of 3 N HCl in MeOH / THF (1 : 1, v/v) at reflux temperature, leading to the products **4a – f**. The structures of the synthesized compounds were elucidated by ^{1}H-NMR and ESI-MS.

Vasorelaxant activities

Vasorelaxant activities of compounds **4a – f** were investigated in aortic rings with the endothelium pre-contracted with 1 μM phenylephrine. Quercetin, a well known vasodilator, was used as the positive control. The vasorelaxant abilities of the tested compounds were based on their potency (EC_{50}) and efficacy (E_{max}). The results are shown in Table 2. All tested compounds **4a – f** promoted relaxation in a dose-dependent manner with the maximum effect observed at 300 μM (Fig. 2). All but compound **4b** showed potent vasorelaxant activities, which demonstrated that the predicted vasorelaxant activities were in good agreement with the experimental



Scheme 1. Synthesis of chalcone derivatives **4a**–**f**.



Chalcones were added cumulatively to achieve the appropriate concentrations. Results are expressed as means ± SD in terms of percentage relaxation of the contraction to PE (n = 4).

Figure 2. Effects of chalcones derivatives on relaxation in aortic rings with the endothelium pre-contracted by 1 μM phenylephrine.

results. Study on the effects of different substituents at the B-ring of prenylated chalcones revealed that the hydroxyl group commonly resulted in higher activity (**4a**, **4c**–**f** > **4b**, **2**, **3**), regardless of the presence of prenyl groups at the 3- or 5-position of the A-ring. Compounds **4c** and **4d** without 6-OH at the A-ring of chalcones (EC_{50} of **4c** and **4d**: 102 and 123 μM, respectively) exhibited a much weaker potency than compound **4a** (EC_{50} of **4a**: 8.5 μM), but showed more efficacy (E_{max} of **4c**, **4d** and **4a**: 101%, 92%, and 80%, respectively). However, compound **4e** without 6-OH at the A-ring of chalcones exhibited effects similar to **4a** rather than **4c** or **4d** (EC_{50} and E_{max} of **4e**: 10.7 μM, 81%, respectively), suggesting that the number of hydroxyl groups at the chalcone skeleton is also crucial for their vasorelaxant activity, as shown in the examples of **4a** and **4e**, which bear the same number of hydroxyl groups. In addition, compound **4f**, a chalcone with an allyl group replacing the prenyl group of **4a**, showed more efficacy (E_{max}: **4f** > **4a**) but less potency (EC_{50}: **4f** > **4a**), indicating that the replacement of the prenyl with an allyl group retained the potent activity.

Conclusion

A series of prenylated chalcones bearing different substituents were prepared and evaluated for their vasorelaxant activities according to the results of our previous studies. Most of the tested compounds showed potent vasorelaxant activities, which indicated that experimental activities were in good agreement with theoretical results. The preliminary structure-activity relationships showed that the hydroxyl group at the B-ring of chalcones usually resulted in better vasorelaxant activity; the substituent pattern and the number of hydroxyl groups are also crucial for their vasorelaxant activity, and the replacement of the prenyl with an allyl group retains the potent activity.

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The authors have declared no conflict of interest.

Experimental

Chemistry

Melting points were determined on a Büchi B-540 apparatus (Büchi Labortechnik, Flawil, Switzerland) and are uncorrected. All ^{1}H-NMR spectra were recorded on Bruker 400 MHz-spectrometer (Bruker Bioscience, Billerica, MA, USA) with $CDCl_3$ or acetone-d_6 as solvent. Chemical shifts were reported in δ values (ppm), relative to internal TMS, and *J* values were reported in Hertz (Hz). Mass spectra (ESI, positive ion) were recorded on an Esquire-LC-00075 spectrometer (Bruker Bioscience). Reagents and solvents were purchased from known commercial suppliers and were used without further purification. Compounds **5a – d** and **4f** were prepared according to the approaches in previous references [14, 15].

*General method for synthesis of compounds **6a – e***

To a cold solution of the acetophenone **5a – c** and the appropriate benzaldehyde in 3 mL of H_2O / EtOH (1 : 4, v/v), 600 mg KOH in 3 mL H_2O / EtOH (1 : 4, v/v) was added with stirring. The resulting mixture was stirred under N_2 atmosphere at room temperature for 36 h. Then, the reaction mixture was poured into ice-water, acidified to pH ~ 5 with 2 N HCl, and extracted with ethyl acetate. The organic phase was washed with brine, dried over anhydrous Na_2SO_4, and was concentrated *in vacuo*. The residue was purified to give chalcone **6a – e**.

*2-Hydroxy-4,4′,6-tri(methoxymethoxy)-3-(3,3-dimethylallyl)chalcone **6a***

Reagent: compound **5a** (500 mg, 1.54 mmol), 4-methoxymethoxy-benzaldehyde (269 mg, 1.62 mmol); purification: silica gel column chromatography (petroleum ether / ethyl acetate = 12 : 1, v/v). A yellow oil (548 mg, 70%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.67 (s, 3H,), 1.79 (s, 3H), 3.34 (d, *J* = 6.8 Hz, 2H), 3.49 (s, 3H), 3.50 (s, 3H), 3.52 (s, 3H), 5.21 (m, 1H), 5.22 (s, 2H), 5.25 (s, 2H), 5.27 (s, 2H), 6.40 (s, 1H,), 7.06 (d, *J* = 8.8 Hz, 2H), 7.55 (d, *J* = 8.8 Hz, 2H), 7.76 (d, *J* = 15.6 Hz, 1H), 7.83 (d, *J* = 15.6 Hz, 1H), 13.83 (s, 1H, OH). ESI-MS *m/z*: 473 $[M + H]^+$.

*2-Hydroxy-4,6-di(methoxymethoxy)-4′-methoxy-3-(3,3-dimethylallyl)chalcone **6b***

Reagent: compound **5a** (500 mg, 1.54 mmol), 4-methoxybenzaldehyde (220 mg, 1.62 mmol); purification: silica gel column chromatography (petroleum ether / ethyl acetate = 15 : 1, v/v). A yellow oil (557 mg, 65%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.68 (s, 3H,), 1.81 (s, 3H), 3.34 (d, *J* = 6.8 Hz, 2H), 3.50 (s, 3H), 3.53 (s, 3H), 3.87 (s, 3H), 5.21 (m, 1H), 5.26 (2H, s), 5.28 (2H, s), 6.41 (s, 1H), 6.94 (d, *J* = 8.4 Hz, 2H), 7.57 (d, *J* = 8.4 Hz, 2H), 7.78 (d, *J* = 16.0 Hz, 1H), 7.82 (d, *J* = 16.0 Hz, 1H), 13.90 (s, 1H, OH). ESI-MS *m/z*: 443 $[M + H]^+$.

*2-Hydroxy-4,4′-di(methoxymethoxy)-3-(3,3-dimethylallyl)chalcone **6c***

Reagent: compound **5b** (501 mg, 1.90 mmol), 4-methoxymethoxy-benzaldehyde (330 mg, 1.99 mmol); purification: silica gel column chromatography (petroleum ether / ethyl acetate = 15 : 1, v/v). A yellow oil (615 mg, 75%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.72 (s, 3H), 1.82 (s, 3H), 3.44 (d, *J* = 6.8 Hz, 2H), 3.51 (s, 6H), 5.25 (s, 2H), 5.27 (m, 1H), 5.31 (s, 2H), 6.70 (d, *J* = 8.4 Hz, 1H), 7.08 (d, *J* = 8.8 Hz, 2H), 7.51 (d, *J* = 15.6 Hz, 1H), 7.64 (d, *J* = 8.8 Hz, 2H), 7.80 (d, *J* = 8.4 Hz, 1H), 7.88 (d, *J* = 15.6 Hz, 1H), 12.80 (s, 1H, OH). ESI-MS *m/z*: 413 $[M + H]^+$.

*2-Hydroxy-3′,4-di(methoxymethoxy)-5-(3,3-dimethylallyl)chalcone **6d***

Reagent: compound **5c** (500 mg, 1.89 mmol), 3-methoxybenzaldehyde (329 mg, 1.99 mmol); purification: silica gel column chromatography (petroleum ether / ethyl acetate = 15 : 1, v/v). A yellow oil (557 mg, 70%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.80 (s, 3H), 1.82 (s, 3H), 3.34 (d, *J* = 6.8 Hz, 2H), 3.53 (s, 3H), 3.56 (s, 3H), 5.28 (s, 2H), 5.31 (s, 2H), 5.33 (m, 1H), 6.70 (s, 1H), 7.17 (dd, *J* = 2.0, 8.0 Hz, 1H), 7.36 (t, *J* = 8.0 Hz, 1H), 7.41 (t, *J* = 8.0 Hz, 2H), 7.60 (d, *J* = 16.0 Hz, 1H), 7.67 (s, 1H), 7.87 (d, *J* = 16.0 Hz, 1H), 13.27 (s, 1H, OH). ESI-MS *m/z*: 413 $[M + H]^+$.

*2-Hydroxy-2′,4,4′-tri(methoxymethoxy)-5-(3,3-dimethylallyl)-chalcone **6e***

Reagent: compound **5c** (502 mg, 1.90 mmol), 2,4-di(methoxymethoxy)-benzaldehyde (451 mg, 2.00 mmol); purification: silica gel column chromatography (petroleum ether / ethyl acetate = 10 : 1, v/v). A yellow oil (582 mg, 62%); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.74 (s, 3H), 1.76 (s, 3H), 3.29 (d, *J* = 6.8 Hz, 2H), 3.48 (s, 3H), 3.50 (s, 3H), 3.52 (s, 3H), 5.21 (s, 2H), 5.25 (s, 2H), 5.28 (s, 2H), 5.29 (m, 1H), 6.64 (s, 1H), 6.76 (dd, *J* = 2.0, 8.0 Hz, 1H), 6.87 (d, *J* = 2.0 Hz, 1H), 7.58 (d, *J* = 16.0 Hz, 1H), 7.61 (d, *J* = 8.0 Hz, 1H), 7.62 (s, 1H), 8.17 (d, *J* = 16.0 Hz, 1H), 13.41 (s, 1H, OH). ESI-MS *m/z*: 473 $[M + H]^+$.

*General method for synthesis of compounds **4a – e***

To a solution of **6a – e** in 5 mL methanol / THF (1 : 1, v/v), 0.5 mL 3 N HCl was added, and the mixture was stirred at 40°C for 6 h. After cooling to room temperature, the reaction mixture was poured into cold water and extracted with ethyl acetate. The organic phase was washed with brine, dried over anhydrous Na_2SO_4. After removal of the solvent, the residue was purified to give **4a – e**.

*2,4,4′,6-Tetrahydroxy-3-(3,3-dimethylallyl)chalcone **4a***

Reagent: compound **6a** (300 mg, 0.64 mmol); purification: silica gel column chromatography using petroleum ether / ethyl acetate (1 : 1, v/v). Yellow amorphous powder (119 mg, 55%), m.p.: 154 – 155°C. ^{1}H-NMR (Acetone-d_6, 400 MHz) δ: 1.61 (s, 3H), 1.69 (s, 3H), 3.13 (d, *J* = 7.2 Hz, 2H), 5.14 (m, 1H), 6.03 (s, 1H), 6.85 (d, *J* = 8.0 Hz, 2H), 7.52 (d, *J* = 8.0 Hz, 2H), 7.72 (d, *J* = 16.0 Hz, 1H), 8.07 (d, *J* = 16.0 Hz, 1H), 8.81 (s, 1H, OH), 9.01 (s, 1H, OH), 9.62 (s, 1H, OH), 14.41 (s, 1H, OH). ESI-MS *m/z*: 341 $[M + H]^+$.

*2,4,6-Trihydroxy- 4′-methoxy-3-(3,3-dimethylallyl)chalcone **4b***

Reagent: compound **6b** (300 mg, 0.68 mmol); purification: silica gel column chromatography using petroleum ether / ethyl ace-

tate (4 : 1, v/v). Yellow amorphous powder (162 mg, 67%), m.p.: 151–152°C. ^{1}H-NMR (Acetone-d_6, 400MHz) δ: 1.58 (s, 3H), 1.70 (s, 3H), 3.21 (d, *J* = 7.2 Hz, 2H), 3.81 (s, 3H), 5.19 (m, 1H), 6.05 (s, 1H), 6.95 (d, *J* = 8.4 Hz, 2H), 7.59 (d, *J* = 8.4 Hz, 2H), 7.71 (d, *J* = 16.0 Hz, 1H), 8.10 (d, *J* = 16.0 Hz, 1H), 9.12 (s, 1H, OH), 9.56 (s, 1H, OH), 14.30 (s, 1H, OH). ESI-MS *m/z*: 355 $[M + H]^+$.

2,4,4′-Trihydroxy-3-(3,3-dimethylallyl)chalcone ***4c***

Reagent: compound **6c** (300 mg, 0.73 mmol); purification: silica gel column chromatography using petroleum ether / ethyl acetate (2 : 1, v/v). Yellow amorphous powder (165 mg, 70%), m.p.: 157–159°C; ^{1}H-NMR (Acetone-d_6, 400MHz) δ: 1.64 (s, 3H), 1.77 (s, 3H), 3.36 (d, *J* = 7.2 Hz, 2H), 5.27 (m ,1H), 6.52 (d, *J* = 8.4 Hz, 1H), 6.92 (d, *J* = 8.4 Hz, 2H), 7.72 (d, *J* = 8.4 Hz, 2H), 7.74 (d, *J* = 16.0 Hz, 1H), 7.82 (d, *J* = 16.0 Hz, 1H), 7.96 (d, *J* = 8.4 Hz, 1H), 8.97(s, 1H, OH), 9.31 (s, 1H, OH), 13.97 (s, 1H, OH). ESI-MS *m/z*: 325 $[M + H]^+$.

2,3′,4-Trihydroxy-5-(3,3-dimethylallyl)chalcone ***4d***

Reagent: compound **6d** (300 mg, 0.73 mmol); purification: silica gel column chromatography using petroleum ether / ethyl acetate (2 : 1, v/v). Yellow amorphous powder (147 mg, 62%), m.p.: 179–180°C. ^{1}H-NMR (acetone-d_6, 400MHz) δ: 1.66 (s, 3H), 1.68 (s, 3H), 3.25 (d, *J* = 7.2 Hz, 2H), 5.29 (m, 1H), 6.36 (s, 1H), 6.90 (dd, *J* = 2.0, 8.0 Hz, 1H), 7.20 (d, *J* = 2.0 Hz, 1H), 7.24 (m, 2H), 7.72 (d, *J* = 16.0 Hz, 1H), 7.80 (d, *J* = 16.0 Hz, 1H), 7.93 (s, 1H), 8.53 (s, 1H, OH), 9.51 (s, 1H, OH), 13.30 (s, 1H, OH). ESI-MS *m/z*: 325 $[M + H]^+$.

2,2′,4,4′-Tetrahydroxy-5-(3,3-dimethylallyl)chalcone ***4e***

Reagent: compound **6e** (300 mg, 0.63 mmol); purification: silica gel column chromatography using petroleum ether / ethyl acetate (1 : 1, v/v). Yellow amorphous powder (54 mg, 25%), m.p.: >135°C (des.). ^{1}H-NMR (Acetone-d_6, 400MHz) δ: 1.68 (s, 6H), 3.24 (d, *J* = 7.2 Hz, 2H), 5.29 (m, 1H), 6.34 (s, 1H), 6.41 (dd, *J* = 2.4, 8.4 Hz, 1H), 6.47 (d, *J* = 2.4 Hz, 1H), 7.57 (d, *J* = 8.0 Hz, 1H), 7.75 (d, *J* = 16.0 Hz, 1H), 7.80 (s, 1H), 8.12 (d, *J* = 16.0 Hz, 1H), 8.85 (s, 1H, OH), 9.18 (s, 1H, OH), 9.39 (s, 1H, OH), 13.59 (s, 1H). ESI-MS *m/z*: 341 $[M + H]^+$.

Vasorelaxant activity assay

Vascular rings were prepared from the aorta of male Sprague-Dawley rats (four to six months old and weighing on average 250 g) and contraction studies were performed following the general procedure detailed in the literature [16]. After an equilibration period of at least 1 h, isometric contractions induced by PE (1 μM) were obtained. When contraction of the tissue in response to this vasoconstrictor agent had stabilized (after about 20 min), cumulatively increasing concentrations of the tested compounds were added to the bath at 15–20 min intervals (the time needed to obtain steady-state relaxation). Control tissues were simultaneously subjected to the same procedures, but omitting the compounds and adding the vehicle. All data were expressed as mean ± SD (n = 3∼4). The flavonoids-induced maximal relaxation (E_{max}) in aortic rings was calculated as a percentage of the contraction in response to PE (1 μM). The half-maximum effective concentration (EC_{50}) was defined as the concentration of the flavonoids that induced 50% of maximum relaxation from the contraction elicited by PE (1 μM) and was calculated from the concentration-response curve by nonlinear regression (curve fit) using GraphPad Prism.

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