Synthesis, Biological Evaluation of Prenylflavonoids as Vasorelaxant and Neuroprotective Agents

Xiaowu Dong[a], Lingling Qi[b], Chaoyi Jiang[a], Jing Chen[a], Erqing Wei[b], Yongzhou Hu[a] *

[a]*ZJU-ENS joint laboratory of medicinal chemistry, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou, 310058, China*

[b] *Department of Pharmacology and Institute of Neuroscience, School of Medicine, Zhejiang University, Zhejiang University, Hangzhou, 310058, China*

SUPPORTING INFORMATION

* Corresponding author. Tel.:086-571-88208460; Fax: 086-571-88208460; e-mail: huyz@zju.edu.cn

Chemistry

Melting points were obtained on a B-540 Büchi melting-point apparatus and are uncorrected. ^{1}H NMR spectra was recorded on a Brüker AM 400 instrument at 400 MHz (chemical shifts are expressed as δ values relative to TMS as internal standard). ESI (positive) was recorded on an Esquire-LC-00075 spectrometer. Elemental analyses were performed on a Flash EA 1112 elemental analyzer.

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 3N HCl in MeOH/THF (1/1, v/v) using corresponding flavanones **7b-d** as shown in Scheme1.

Leachianone G **1b**: Mp 147-149 ^{o}C, ^{1}H-NMR (Acetone-d_6, 400M, δ): 1.55 (s, 3H), 1.57 (s, 3H),2.70 (dd, J =3.2, 16.8 Hz, 1H), 3.05 (dd, J = 13.6, 16.8 Hz, 1H), 3.18 (d, 2H, J = 7.2 Hz), 5.16 (m, 1H), 5.63 (dd, 1H, J = 13.6 Hz, 3.2 Hz), 5.98 (s, 1H), 6.38 (dd, 1H, J = 2.0, 8.4 Hz,), 6.42 (d, J = 2.0 Hz), 7.30 (d, J = 8.4 Hz, 1H), 8.33 (s, 1H, OH), 8.59 (s, 1H, OH), 9.51 (s, 1H, OH), 12.11 (s, 1H, OH). ESI-MS: m/z $[M+H]^+$ 357. Anal. calcd. for $C_{20}H_{20}O_6$: C, 67.41; H, 5.66; Found C, 67.55; H, 5.51.

2',3',4',5,7-pentahydoxy-8-(3,3-dimethylallyl)-flavanone **1d**: Mp > 170 ^{o}C (dec.) ^{1}H-NMR (Acetone-d_6, 400M, δ): 1.55 (s, 3H), 1.57 (s, 3H), 2.72 (dd, J = 2.8, 16.8 Hz, 1H), 3.06 (dd, J = 13.8, 16.8 Hz, 1H), 3.18 (d, 2H, J = 7.2 Hz), 5.16 (m, 1H), 5.64 (dd, 1H, J = 12.8 Hz, 2.8 Hz), 5.97 (s, 1H), 6.43 (dd, 1H, J = 8.4 Hz,), 6.84 (d, 1H, J = 8.4 Hz), 7.49 (s, 1H, OH), 7.62 (s, 1H, OH), 8.27 (s, 1H, OH) , 9.45 (s, 1H, OH), 12.12 (s, 1H, OH). ESI-MS: m/z $[M+H]^+$ 373. Anal. calcd. for $C_{20}H_{20}O_7$: C, 64.51; H, 5.41; Found C, 64.40; H, 5.55.

4',5,7-trihydroxy-8-(3,3-dimethylallyl)-aurone **2a**: Mp: 158-161 ^{o}C. ^{1}H-NMR (Acetone-d_6, 400M, δ):

1.64 (s, 3H), 1.81 (s, 3H), 3.39 (d, 2H, *J* = 7.2 Hz), 5.31 (m, 1H), 6.16 (s, 1H), 6.56 (s, 1H), 6.91 (d, 2H, *J* = 8.4 Hz)), 7.83 (d, 2H, *J* = 8.4 Hz), 8.66 (s, 1H, OH), 8.87 (s,1H, OH), 9.68 (s, 1H, OH). ESI-MS: m/z $[M+H]^+$ 339. Anal. calcd. for $C_{20}H_{18}O_5$: C, 70.99; H, 5.36; Found C, 70.78; H, 5.49.

2',4',5,7-tetrahydroxy-8-(3,3-dimethylallyl)-aurone **2b**: Mp > 250 ^oC (des.) ^1H-NMR (Acetone-d_6, 400M, δ): 1.69 (s, 3H), 1.86 (s, 3H), 3.44 (d, 2H, *J* = 7.2 Hz), 5.36 (m, 1H), 6.19 (s, 1H), 6.51 (dd, 1H, *J* = 8.4 Hz), 6.53 (s, 1H), 7.17 (s, 1H), 8.17 (d, 1H, *J* = 8.4 Hz), 8.65 (s, 1H, OH), 8.87 (s,1H, OH), 9.14 (s, 1H, OH), 9.72 (s, 1H, OH). ESI-MS: m/z $[M+H]^+$ 355. Anal. calcd. for $C_{20}H_{18}O_6$: C, 67.79; H, 5.12; Found C, 67.91; H, 5.34.

Demethylxanthohumol **3a**: Mp: 154-155 ^oC, ^1H-NMR (Acetone-d_6, 400M, δ): 1.61 (s, 3H), 1.69 (s, 3H), 3.13 (d, 2H, *J* = 7.2 Hz), 5.14 (m,1H), 6.03 (s, 1H), 6.85 (d, 2H, *J* = 8.0 Hz), 7.52 (d, 2H *J* = 8.0 Hz), 7.72 (d, 1H, *J* = 16.0 Hz), 8.07 (d, 1H, *J* = 16.0 Hz), 8.81 (s, 1H, OH), 9.01 (s, 1H, OH), 9.62 (s, 1H, OH), 14.41 (s, 1H, OH). ESI-MS: m/z $[M+H]^+$ 341. Anal. calcd. for $C_{20}H_{20}O_5$: C, 70.57; H, 5.92; Found C, 70.41; H, 5.99.

2',4',5,7-tetrahydroxy-8-(3,3-dimethylallyl)-flavone **4b:** Mp > 250 ^oC (dec.) ^1H-NMR (Acetone-d6, 400 MHz, δ): 1.60 (s, 3H), 1.74 (s, 3H), 3.48 (d, 2H, *J* = 6.8 Hz), 5.22 (m, 1H), 6.27 (s, 1H), 6.52 (dd, 1H, *J* = 2.0, 8.4 Hz), 6.56 (d, 1H, *J* = 2.0 Hz), 7.01 (s, 1H), 7.82 (d, 1H, *J* = 8.4 Hz), 9.22 (s, 1H, OH), 9.64 (s, 2H, OH), 13.02 (s, 1H, OH). ESI-MS: m/z $[M+H]^+$ 355. Anal. calcd. for $C_{20}H_{18}O_6$: C, 67.79; H, 5.12; Found C, 67.65; H, 5.22.

3',4',5,7-tetrahydroxy-8-(3,3-dimethylallyl)-flavone **4c** Mp > 250 C^o(dec.) 1H-NMR (Acetone-d6, 400 MHz, δ): ^1H-NMR (Acetone-d6, 400 MHz, d): 1.61 (s, 3H), 1.77 (s, 3H), 3.51 (d, 2H, *J* = 6.8 Hz), 5.25 (m, 1H), 6.28 (s, 1H), 6.53 (s, 1H), 6.97 (d, 1H, *J* = 8.4 Hz), 7.45 (dd, 1H, *J* =2.0, 8.4 Hz), 7.50 (d, 1H, *J* = 2.0 Hz), 8.41 (s, 1H, OH), 8.78 (s, 1H, OH), 9.53 (s, 1H, OH), 12.92 (s, 1H, OH). ESI-MS: m/z

$[M+H]^+$ 355. Anal. calcd. for $C_{20}H_{18}O_6$: C, 67.79; H, 5.12; Found C, 67.61; H, 5.28.

2',3',4',5,7-pentahydroxy-8-(3,3-dimethylallyl)-flavone **4d**: Mp >210 C°(dec.) H^1-NMR (Acetone-d6, 400 MHz, δ): 1.62 (s, 3H), 1.75 (s, 3H), 3.50 (d, 2H, *J* = 6.8 Hz), 5.24 (m, 1H), 6.27 (s, 1H), 6.78 (s, 1H), 6.86 (d, 1H, *J* = 8.4 Hz), 7.53 (d, 1H, *J* = 8.4 Hz), 8.39 (s, 1H, OH), 8.75 (s, 1H, OH), 9.18 (s, 1H, OH), 9.62 (s, 1H, OH), 13.01 (s, 1H, OH). ESI-MS: m/z $[M+H]^+$ 371. Anal. calcd. for $C_{20}H_{18}O_6$: C, 64.86; H, 4.90; Found C, 64.69; H, 5.13.

Vasodilatory effect assay

Vascular rings were prepared from the aorta of male 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.[24] 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. The flavonoids-induced maximal relaxation (Emax) in aortic rings was calculated as a percentage of the contraction in response to PE (1 μM). The half maximum effective concentration (EC50) 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.

Neuroprotective effect assay

PC12 Cells were rinsed twice and incubated in Earle's solution without glucose. Then, the cells were

introduced into an anaerobic chamber containing a mixture of 95% N_2 and 5% CO_2 at 37 ^oC for 2 h. This procedure decreased pO2 in the solution from 152.0 ± 5.0 to 29.0 ± 1.0 mmHg (mean ± SD, n = 5). At the end of OGD, the medium was replaced, and cells were cultured in normal condition for 24 h of recovery. Prenylflavonoids and edaravone at designated concentration were continuously applied from 30 min before OGD to the end of recovery.

Cell viability was determined by the colorimetric MTT reduction assay. Briefly, cells were cultured on 96-well plates. At the end of the experiments, the cells were incubated with 0.5 mg/ml MTT for 2 h at 37 ^oC. Then, the supernatant layer was carefully removed and 100 μl DMSO was added into each well, and the absorbance at 490 nm of the MTT product formazan was determined on a microplate recorder (ELX 800; Bio-Tek Instruments Inc.). Results are expressed as percentage of control.