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Synthesis and promotion angiogenesis effect of chrysin derivatives coupled to NO donors

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ABSTRACT

Two types of new chrysin derivatives were prepared by coupling NO donors of alkyl nitrate and furazan derivatives and were fully characterized by ^{1}H NMR and other techniques. These compounds were tested in human umbilical vein endothelial cells (HUVECs-12) and all the compounds exhibited cell proliferation. Notable effects of promoting angiogenesis were observed for all the modified compounds using chick chorioallantoic membrane (CAM) assay.

Angiogenesis, the formation of new blood vessels from pre-existing vasculature, is a crucial step in the management of ischemic disease such as coronary heart disease, ischemic cerebral embolism and fracture healing, due to its ability of supply oxygen and nutrients to the ischemia target. Brownlee proposed that the diabetic patients with vascular injuries in diabetic vascular complications is one of the main reasons.[1] One of the key mediators of blood vessel formation during development is vascular endothelial growth factor (VEGF),[2] which can stimulate the proliferation and migration in endothelial cells. Endothelial cells proliferation plays an essential role in the regulation of various vascular biological function and diseases.

Recently, considerable fundamental researches and clinical researches indicate that oxidative stress plays a key role in the pathogenesis of diabetic vascular complications. An early step of such damage is considered to be the development of an endothelial dysfunction.[3] Oxidative stress and diabetic vascular complications are closely related to the development of diabetes, suggesting that anti-oxidant treatment is expected to become the prevention and treatment of vascular complications of diabetes new ways.[4]

Chrysin(5,7-dihydroxyflavone) is a naturally wide distributed flavonoid, which has been reported to have many different biological activities such as anti-viral,[5] anti-cancer,[6] anti-bactericidal,[7] anti-inflammatory,[8] anti-allergic,[9] DNA cleavage,[10] vasodilator,[11] anti-mutagenic,[12] anti-anxiolytic[13] and anti-oxidant effects.[14] However, research on the role of chrysin derivatives in cardiovascular is rare.[15] In order to investigate the effect of chrysin derivatives in promoting angiogenesis, in this paper, we made attempts to report that chrysin derivatives were coupled to different NO donors and their effect on promoting angiogenesis was evaluated by HUVEC-12 and CAM assay.

To begin our study, chrysin derivatives were coupled to nitrate following the synthetic route illustrated in Scheme 1. An alkyl chain with bromide was introduced to the 7-position of chrysin (**1**) selectively by reacting with the corresponding dibromo alkane and yielded the 7-bromo alkane derivatives (**2a–2c**), which were subsequently converted to the final nitro ester (**3a–3c**) by treatment with $AgNO_3$ in anhydrous acetonitrile. The synthesis of furazan derivatives is shown in Scheme 2.[16] Saturated aqueous sodium nitrite was added to a solution of cinnamyl alcohol (**4**) in glacial acetic acid. The resulting reaction mixture was diluted with water and extracted with diethyl ether. The combined organic layer was washed with brine and dried over $MgSO_4$. The volatiles were removed in vacuo and the residue was recrystallized from dichloromethane–petroleum ether to give **5**. Chrysin derivatives were successfully coupled to furazan as shown in Scheme 3. To a stirring solution of chrysin **1** in acetone, K_2CO_3 was added and then a mixture of ethyl bromoacetate and acetone was added dropwise to give compound **6**. Compound **6** was treated in KOH solution (methanol) and after that most of the volatiles were evaporated. The residue was dissolved in water and the solution was adjusted to pH 2 by using HCl solution to give **7**. Chrysin derivatives **7** was suspended in anhydrous dichloromethane with stirring at room

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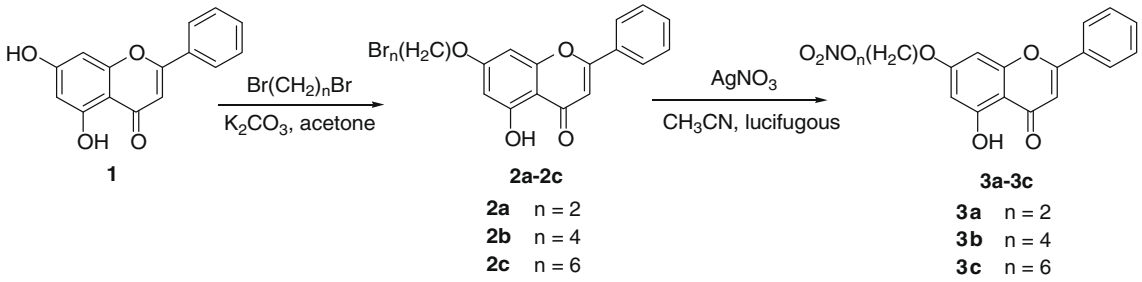
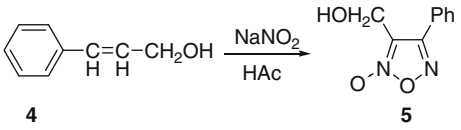


Scheme 1. Preparation methods for **3a**–**3c**.



Scheme 2. Preparation methods for **6**.

temperature, then furazan derivatives **5**, dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) were added. The reaction solution was stirred for 12 h at room temperature and dicyclohexylurea (DCU) was formed, which was removed through filtering. The residue solution was evaporated to get a pale yellow solid which was recrystallized from dichloromethane–petroleum ether and washed with K_2CO_3 solution and water to give **8**. All of the compounds were characterized by means of ^{1}H NMR and MS (EI).[17]

MTT assay was performed to evaluate the effect of these compounds on HUVECs-12 proliferation. Incubation with the compounds at concentration of 10 μmol/L for 24 h showed promoted proliferation of HUVECs-12. Generally, HUVECs were split at a density of 2.5×10^5 cells/mL in 96-well plates and 200 μL suspension per well cultured in DMEM containing 10% FCS for 24 h, then starved by serum deprivation for 24 h, treated with the products (10 μM) and vehicle (0.1% DMSO) for 24 h.[18] Twenty microliter of MTT (5 mg/mL) was added and incubated for 4 h. Culture medium was removed and 150 μL DMSO was added to each well. The absorbance (OD) was measured at 490 nm using a microplate spectrophotometer (Bio-Rad) (see Fig. 1). It was observed that all the compounds exhibited cell proliferation. However, with a degree of variation, cell proliferation of compound **3b** is the most obvious.

CAM assay was used to examine whether the compounds exhibit an angiogenic activity. General CAM assay: neutral filter paper was loaded with products (**1, 3a, 3b, 3c, 8, ECGF, vehicle** or PBS), respectively, which, after air-drying, were then applied to the CAM surface of 7-day-old chick embryos. Two days later, an appropriate volume of 10% formaldehyde solution was injected into the 9-day-old embryo chorioallantois and angiogenesis was observed under a microscope (see Fig. 2). Whereas the control vehicle (0.3% DMSO) and PBS both showed no angiogenic activity in the

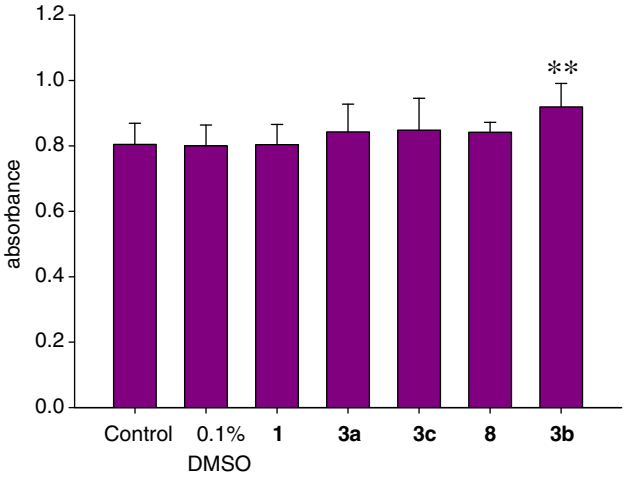
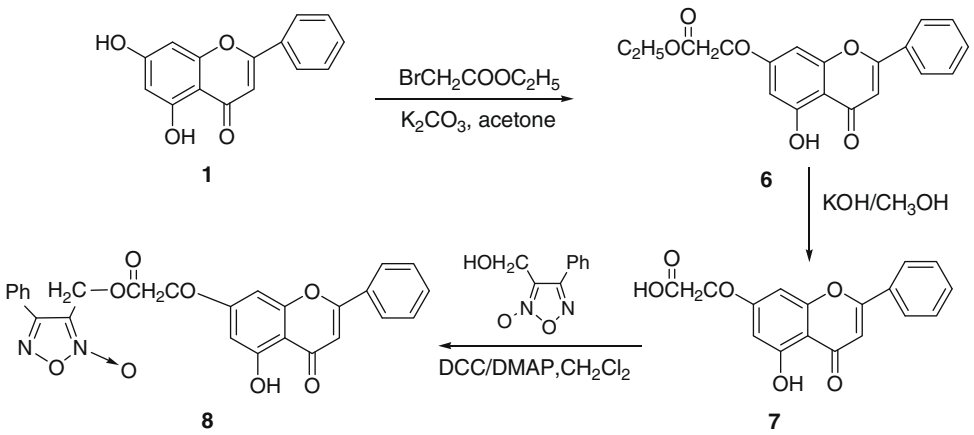


Figure 1. Effects of products (10 μM) on the proliferation of HUVECs-12. The proliferation of HUVECs-12 was determined by MTT assay. Each value is the mean ± SEM of six determinations. $^{**}p < 0.05$ versus 0.1% DMSO.



Scheme 3. Preparation methods for **8**.

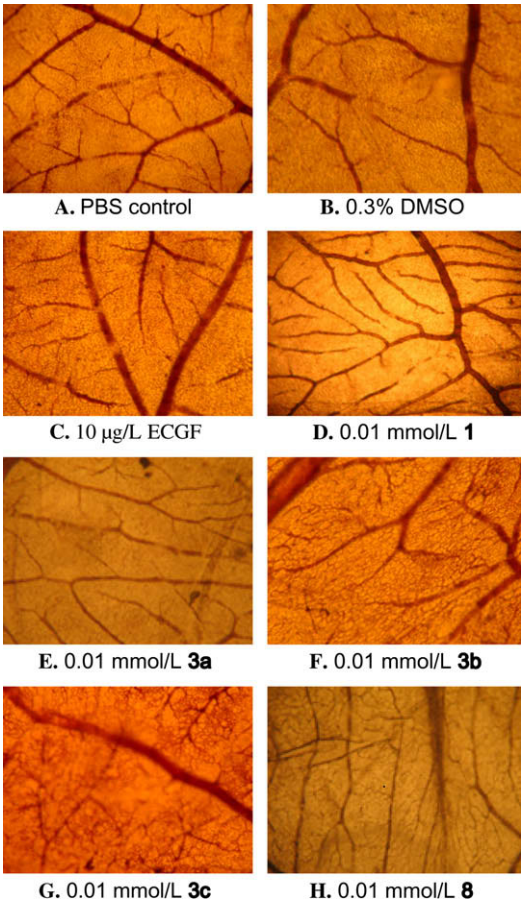


Figure 2. Effect of products (10 μL/egg) on angiogenesis in the CAM assay.

eggs, the products (**3a**, **3b**, **3c**, **8**, 10 μL/egg) and the positive control ECGF showed strong angiogenic activities (D, E, F, G, H). These results indicated that these compounds showed excellent angiogenic effects in vivo.

From the cell proliferation and CAM assay experimental results, it can be seen that angiogenesis was caused partly due to a vascular endothelial cell proliferation.

Angiogenesis is impaired in conditions where NO activity is attenuated. Exogenously applied NO donors have been shown to stimulate endothelial cells (ECs) growth and migration in vitro and promote angiogenesis. The fact indicates that these compounds merit further investigation as potential pharmaceutical agents in processes in which angiogenesis plays an important role, for example, wound healing, fracture repair, peptic ulcers and the production of sufficient collateral flow to rescue ischemic organ.

In conclusion, we have prepared two types of chrysin derivatives coupled with NO donors of alkyl nitrate and furazan derivatives, and have tested in HUVEC-12 and CAM assay. The results indicated that all of the compounds exhibited cell proliferation in vitro and angiogenic activity in vivo. Our study on the hypoglycemic effect of these compounds is ongoing. Further results will be released in subsequent papers.

Acknowledgment

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17. *Compound **3a**:* Mp 135–136 °C; ^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 12.79 (s, 1H), 8.09–8.08 (d, J = 8.4 Hz, 2H), 7.60–7.56 (m, 3H), 7.03 (s, 1H), 6.81–6.80 (d, J = 2.0 Hz, 1H), 6.39–6.38 (d, J = 2.0 Hz, 1H), 4.59 (s, 2H), 4.14 (s, 2H); MS (EI) m/z: 343.*Compound **3b**:* Mp 119–120 °C; $_1$H NMR (400 MHz,$CDCl_3$, ppm) δ 12.73 (s, 1H), 7.90–7.88 (dd, J = 1.2 Hz, 2H), 7.54–7.52 (m, 3H), 6.67 (s, 1H), 6.50–6.49 (d, J = 2.0 Hz, 1H), 6.36–6.35 (d, J = 2.0 Hz, 1H), 4.56 (s, 2H), 4.09 (s, 2H), 1.96 (s, 4H); MS (EI) m/z: 371.*Compound **3c**:* Mp 105–106 °C; ^{1}H NMR (400 MHz,$CDCl_3$, ppm) δ 12.71 (s, 1H), 7.90–7.88 (m, 2H), 7.57–7.50 (m, 3H), 6.67 (s, 1H), 6.50–6.49 (d, J = 2.0 Hz, 1H), 6.37–6.36 (d, J = 2.0, 1H), 4.49–4.46 (t, J = 7.0 Hz, 2H), 4.06–4.03 (t, J = 7.0 Hz, 2H), 1.88–1.75 (m, 4H), 1.53–1.50 (m, 4H); MS (EI) m/z: 399.*Compound **8**:* Mp 174–175 °C; ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 12.77 (s, 1H), 7.89–7.87 (m, 2H), 7.66–7.64 (m, 2H), 7.57–7.49 (m, 6H), 6.69 (s, 1H), 6.48–6.47 (d, J = 2.0 Hz, 1H), 6.34–6.33 (d, J = 2.0 Hz, 1H), 5.32 (s, 2H), 4.77 (s, 2H); MS (EI) m/z: 486.
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