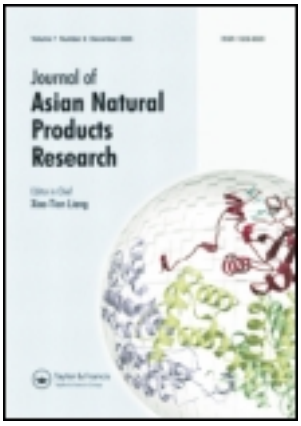

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Design, synthesis, and vasorelaxation activity of novel imperatorin derivatives

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Design, synthesis, and vasorelaxation activity of novel imperatorin derivatives

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In this study, a series of novel imperatorin derivatives **7a**–**7e** were designed and synthesized. Their vasorelaxation activities were evaluated by the pharmacological experiments *in vitro*. Most of the tested compounds exhibited better water solubility and vasorelaxation activity in different degrees, especially **7b** and **7c** with EC_{50} values of 2.29 and 2.63 μM, respectively on mesenteric artery, **7d** and **7e** with EC_{50} values of 1.04 and 2.65 μM, respectively on brain artery. The results indicated that these novel compounds have a potential interest for the development of novel and potent vasorelaxant agents for different kinds of arteries.

Keywords: hypertension; imperatorin; xanthotoxol; vasorelaxation activity; synthesis

1. Introduction

Hypertension is one of the most important public health problems worldwide and has been recognized as an important cardiovascular disorder since the dawn of the twentieth century [1]. It is also a major risk for accelerated atherogenesis and cardiovascular morbidity [2]. Currently, several types of agents to lower blood pressure are either new or investigational. The most effective treatment for hypertension can be taking vasorelaxant agents, which can cause arterial vasodilation in selected arterial beds [3–6]. Therefore, the development of novel vasorelaxant agents causes wide interest of researchers.

Imperatorin, which is a naturally occurring furocoumarin compound, can be isolated from the roots of *Angelica dahurica* and fruits of *Angelica archangelica* [7–9]. It exhibits a wide range of biological properties, such as antioxidative activity [10], central nervous system activity [11], hepatoprotective activity, and antibacterial activity [12]. In the previous work, we have found that imperatorin may exert a vasodilatory effect via the method of vascular smooth muscle/cell membrane chromatography [13,14]. However, due to the poor solubility, the druggability of imperatorin is subject to a certain restriction.

In this study, in an attempt to develop novel imperatorin derivatives with improved solubility, we retained the furanocoumarin, saturated the double bond, and then introduced a nitrogen atom in the side chain (Figure 1). Thus, five new imperatorin derivatives have been designed, synthesized, and evaluated for the first time as vasorelaxant agents in different isolated arteries of rat.

2. Results and discussion

2.1 Structure analysis

The main structural feature of imperatorin from other vasodilator agents is the special furocoumarin. However, the large conjugated system of imperatorin leads to the poor water solubility. Through the struc-

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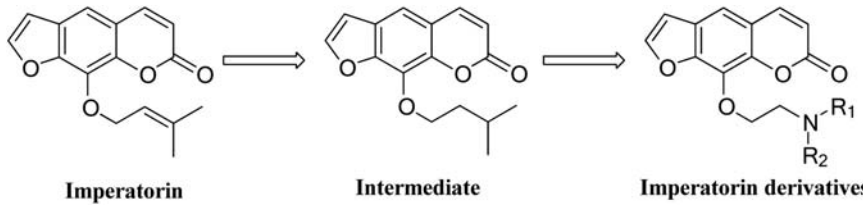
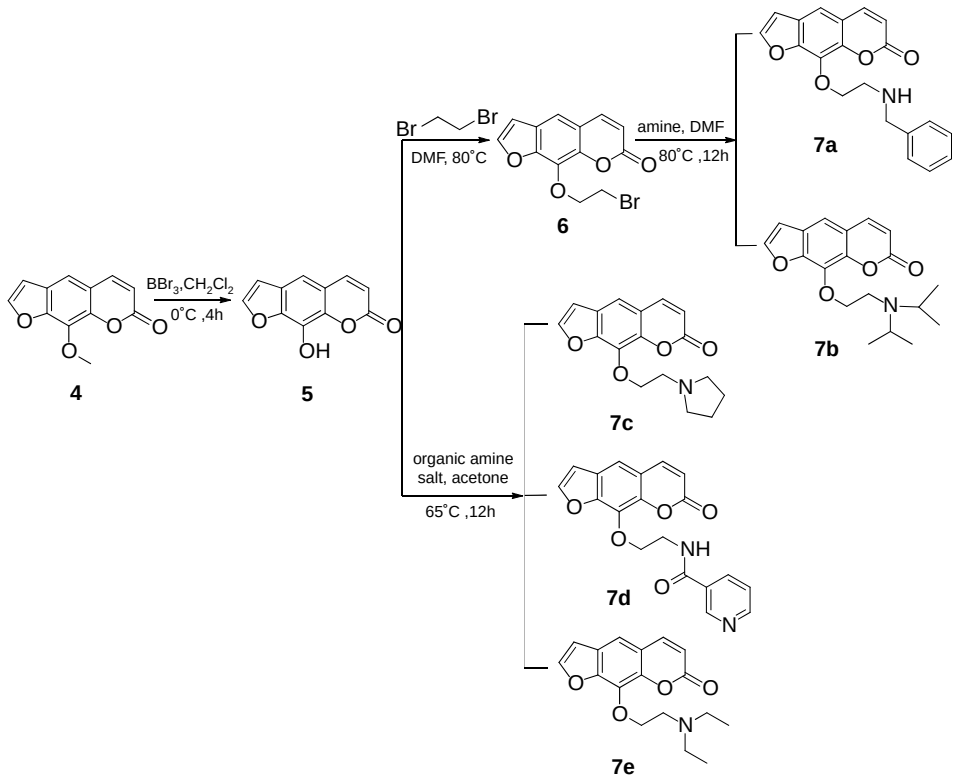
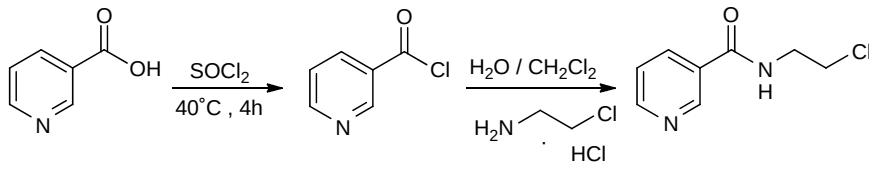


Figure 1. Design of the novel imperatorin derivatives.

ture analysis of traditional vasodilators such as dihydropyridine and phenylalkylamine [15], there is always a nitrogen atom in the molecule. In fact, it is easy to form a salt with the existence of nitrogen atom in the molecular structure. Therefore, the incorporation of nitrogen atom in the side chain will not affect the bone structure of furocoumarin but can increase the possibility of forming salt. In these imperatorin derivatives, the water solubility order is **7d** (1.83 g) > **7c** (0.22 g) > **7e** (1.18×10^{-3} g) > **7b** (7.00×10^{-4} g) > **7a** (1.50×10^{-4} g) > imperatorin (8.00×10^{-5} g). All the data were measurements obtained in 10 ml of water. The new compounds **7a**–**7e** were synthesized according to Scheme 1. *N*-(2-chloroethyl) nicotinamide, a key intermediate for the synthesis of **7d**, was prepared following Scheme 2.



Scheme 1. General routes for the synthesis of target compounds (**7a**–**7e**).



Scheme 2. Synthesis of *N*-(2-chloroethyl) nicotinamide.

2.2 Vasorelaxation activity

The vasorelaxation activity of imperatorin derivatives was evaluated on isolated rat mesenteric artery, basilar artery and renal artery. Imperatorin was used as the positive control. The results were expressed by their potency (EC_{50}, $p\,EC_{50}$) and efficacy (E_{max}). Therein, the data of EC_{50} was defined as the concentration of the tested compounds that induced 50% of maximum relaxation from the contraction elicited by a K^+-rich Krebs or U46619 solution. The $p\,EC_{50}$ was the logarithm of half maximum effective concentration. The E_{max}% was the maximal relaxation.

Through analysis of the data shown in Tables 1–3 and Figure 2, we found that all the tested compounds promoted relaxation in a dose-dependent manner and their maximal effects were observed at 100 μM. Therein, **7b** and **7c** showed excellent half maximum effective concentration ($EC_{50} = 2.29$, 2.63) compared with that of imperatorin ($EC_{50} = 6.31$) and could induce maximal relaxation (E_{max}%) more than 95% on mesenteric artery. Compounds **7c**, **7d**, and **7e** showed excellent EC_{50} (3.16, 1.04, and 2.45) compared with that of imperatorin (7.41) and could induce better E_{max}% on basilar artery. However, all the tested compounds exhibited similar relaxation activity with imperatorin on renal artery. The analysis results suggested that the incorporation of nitrogen atom not only improved the water solubility but also enhanced the pharmacological activity in different degrees. Therefore, these new compounds could be considered to develop good vasorelaxant agents for different kinds of arteries.

3. Experimental

3.1 General experimental procedures

The melting points were measured on an XT-4 instrument without correction (Henan, China). The infrared (IR) spectra were recorded on a Shimadzu Fourier transform (FT)-IR 440 spectrometer in the 4000–500 cm^{-1} range (Shimadzu, Kyoto, Japan). ^1H NMR spectra were recorded on a Bruker AVANCF 400 MHZ instrument in $CDCl_3$ (Bruker, Zurich, Switzerland). The chemicals shifts (δ values) are given in parts per million downfield from tetramethylsilane as the internal reference. The molecular weights were performed on a Shimadzu GC-MS-QP2010 instrument (Shimadzu). All the solvents and chemicals were obtained from commercial sources and were used without further

Table 1. Vasorelaxation activities of imperatorin derivatives on mesenteric artery rings.

Compounds	*n*	$p\,EC_{50}$	EC_{50} (μM)	E_{max}%
Imperatorin	7	5.20 ± 0.09	6.31	96.00 ± 1.00
7a	7	5.30 ± 0.06	5.01	87.33 ± 0.42
7b	7	5.64 ± 0.10	2.29	98.44 ± 0.98
7c	7	5.58 ± 0.03	2.63	98.44 ± 0.98
7d	7	4.39 ± 0.06	40.74	34.18 ± 6.96
7e	7	5.36 ± 0.08	4.37	97.64 ± 2.09

Table 2. Vasorelaxation activities of imperatorin derivatives on basilar artery rings.

Compounds	*n*	pEC_{50}	EC_{50} (μM)	E_{max}%
Imperatorin	6	5.13 ± 0.25	7.41	53.12 ± 2.986
7a	6	5.07 ± 0.14	8.51	64.70 ± 4.35
7b	6	4.81 ± 0.02	15.49	108.5 ± 8.55
7c	6	5.50 ± 0.21	3.16	62.47 ± 4.81
7d	6	5.98 ± 0.23	1.04	84.76 ± 8.15
7e	6	5.61 ± 0.43	2.45	63.79 ± 6.13

purification unless otherwise stated. The synthetic procedure was controlled by the method of thin-layer chromatography on 0.25 mm silica gel plates (60 GF-254) and visualized by UV light. The products were purified by recrystallization or flash chromatograph.

3.2 Synthesis of xanthotoxol (5)

Boron tribromide (3.50 ml) in 15 ml CH_2Cl_2 was added dropwise to a solution of xanthotoxin (2.16 g, 10.00 mmol) in 30 ml anhydrous CH_2Cl_2 under nitrogen at 0°C. The reaction mixture was stirred at 0°C for 4 h. Then, the reaction mixture was poured slowly into a stirred solution of saturated aqueous sodium bicarbonate (100 ml) and stirred for another 1 h. The precipitate was removed by filtration, and the filtrate was treated with 2 M HCl for further product. The product was purified by column chromatography (EtOAc:petroleum ether = 1:1) to afford 1.62 g (81%) isolated yield light yellow solid with mp 251–253°C (lit. [16] 247°C). IR (KBr) υ_{max} (cm^{-1}): 3294 (OH), 1697 (C═O). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.35 (1H, d, J = 9.6 Hz, CH═*H*C—C═O), 7.01 (1H, d, J = 2.4 Hz, C*H*═HC—O), 7.42 (1H, s, Ar—*H*), 8.04 (1H, d, J = 2.4 Hz, CH═*H*C—O), 8.07 (1H, d, J = 9.6 Hz, C*H*═HC—C═O). EI-MS: *m/z* 201.9 $[M]^+$.

3.3 9-(2-Bromoethoxy)-7H-furo[3,2-g]chromen-7-one (6)

Xanthotoxol (2.02 g, 10.00 mmol) was dissolved in anhydrous *N*,*N*-dimethylformamide (10 ml). Anhydrous K_2CO_3 (1.66 g, 12.00 mmol) was added and the reaction mixture was stirred under nitrogen atmosphere for 30 min at room temperature. Then, 1,2-dibromoethane (5 ml) was added and the reaction mixture was stirred at 80°C for another 12 h under nitrogen atmosphere. After the reaction, the mixture was cooled and poured into cold water (100 ml). Then, the mixture was extracted with EtOAc (3 × 50 ml) and the dried (Na_2SO_4) organic layer was evaporated *in vacuo*. The oil residue was purified by column chromatography (EtOAc:petroleum ether = 1:2) to give the product as a white solid (2.63 g, 85%), mp 148–149°C. IR (KBr) υ_{max} (cm^{-1}): 1697 (C═O). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.75 (2H, t, J = 6.4 Hz, CH_2—C*H*$_2$—Br),

Table 3. Vasorelaxation activities of imperatorin derivatives on renal artery rings.

Compounds	*n*	pEC_{50}	EC_{50} (μM)	E_{max}%
Imperatorin	6	4.76 ± 0.02	17.38	99.16 ± 2.479
7a	6	4.76 ± 0.04	17.38	97.93 ± 6.25
7b	6	4.86 ± 0.21	13.80	67.21 ± 5.99
7c	6	4.34 ± 0.05	45.70	50.92 ± 9.22
7d	6	4.61 ± 0.16	24.54	32.66 ± 8.47
7e	6	4.53 ± 0.02	29.51	83.70 ± 3.20

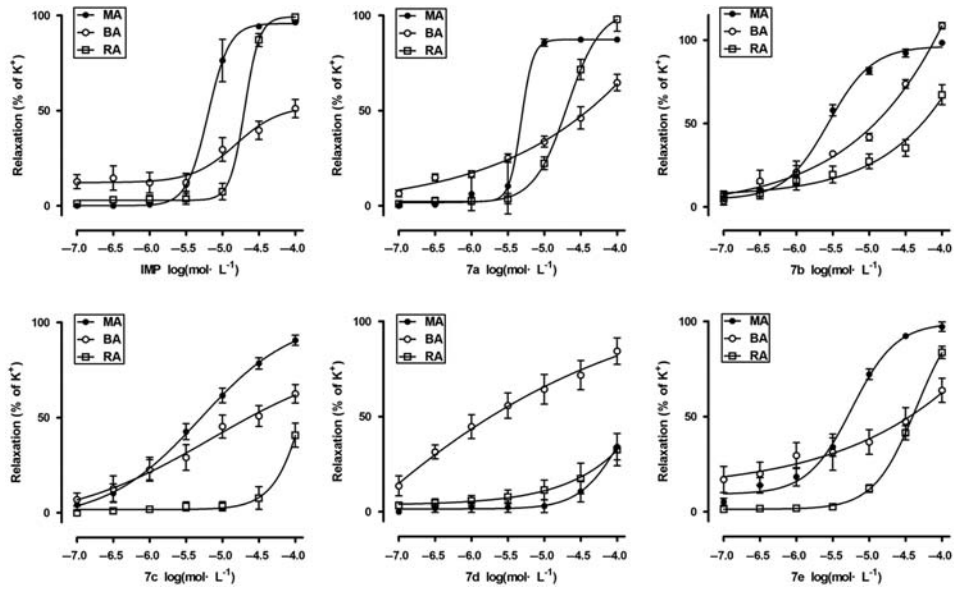


Figure 2. Effects of imperatorin derivatives **7a**, **7b**, **7c**, **7d**, and **7e** on relaxation in mesenteric artery (MA), basilar artery (BA), and renal artery (RA), compared with imperatorin (IMP).

4.76 (2H, t, $J = 6.4$ Hz, CH_2—CH_2—Br), 6.39 (1H, d, $J = 9.6$ Hz, CH=HC—C=O), 6.84 (1H, s, CH=HC—O), 7.41 (1H, s, Ar—H), 7.72 (1H, s, CH=HC—O), 7.78 (1H, d, $J = 9.6$ Hz, CH=HC—C=O). EI-MS *m/z*: 307.9 $[M]^+$.

3.4 Synthesis of nicotinoyl chloride

Nicotinic acid (1.23 g, 10.00 mmol) was dissolved in $SOCl_2$ (10 ml). Three drops of triethylamine were added to the mixture by stirring. Then, the reaction mixture was stirred at 40°C for 4 h. After the reaction, the excess $SOCl_2$ was removed by evaporating *in vacuo*. The crude white product was used directly without further purification.

3.5 Synthesis of N-(2-chloroethyl) nicotinamide

2-Chloroethylamine hydrochloride (1.38 g, 12.00 mmol) was dissolved in 10 ml H_2O and 20 ml CH_2Cl_2. Then, the mixture was treated with 7 ml 2 M NaOH to pH 8 and kept at 0°C for 20 min. After that, crude nicotinoyl chloride was dissolved in 40 ml anhydrous CH_2Cl_2 and added dropwise. The reaction mixture was stirred overnight at room temperature. After the reaction, the mixture was neutralized with saturated aqueous sodium bicarbonate and extracted with CH_2Cl_2 (4×50 ml). The dried (Na_2SO_4) organic layer was evaporated *in vacuo* and the residue was purified by column chromatography (EtOAc:methanol = 10:1) to give the product as a white solid (1.17 g, 64%), mp 87–88°C (lit. [17] 89–91°C). IR (KBr) υ_{max} (cm^{-1}): 3425, 3263 (N—H), 1666 (C=O). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.77 (2H, t, $J = 5.2$ Hz, CH_2—CH_2—Cl), 3.84 (2H, t, $J = 5.2$ Hz, CH_2—CH_2—Cl), 6.64 (1H, s, pyridine-CH), 7.42 (1H, s, pyridine-CH), 8.13 (1H, s, pyridine-CH), 8.76 (1H, s, pyridine-CH), 9.02 (1H, s, HN—C=O). EI-MS: *m/z* 184.0 $[M]^+$.

3.6 General procedure for imperatorin derivatives 7a and 7b

Compound **6** (3.08 g, 10.00 mmol), anhydrous K_2CO_3 (1.66 g, 12.00 mmol) and amine (12.00 mmol) were dissolved in

anhydrous *N*,*N*-dimethylformamide (10 ml). Then, the reaction mixture was stirred at 80°C for 12 h under nitrogen atmosphere. After the reaction, the mixture was cooled and poured into cold water (100 ml) and the aqueous layer was extracted with EtOAc (3 × 50 ml). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by column chromatography. Both **7a** and **7b** were chromatographed on silica gel (EtOAc:petroleum ether = 1:1).

*3.6.1 9-(2-(Benzylamino)ethoxy)-7H-furo[3,2-g]chromen-7-one (**7a**)*

2.40 g (72% yield) isolated as a pale white solid, mp 225–226°C. IR (KBr) υ_{max} (cm^{-1}): 3425 (N—H), 1620 (C═O). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.42 (4H, s, CH_2—CH_2—NH, NH—CH_2—Ar), 4.61 (2H, t, J = 4.4 Hz, CH_2—CH_2—NH), 6.54 (1H, s, CH═*H*C—C═O), 6.60 (1H, s, C*H*═HC—O), 6.68 (2H, s, Ar—*H*), 7.36 (2H, s, Ar—*H*), 7.57 (2H, s, Ar—*H*), 7.88 (1H, s, CH═*H*C—O), 7.93 (1H, s, C*H*═HC—C═O). EI-MS: *m/z* 335.1 $[M]^+$.

*3.6.2 9-(2-(Diisopropylamino)ethoxy)-7H-furo[3,2-g]chromen-7-one (**7b**)*

1.77 g (54% yield) isolated as a white solid, mp 129–130°C. IR (KBr) υ_{max} (cm^{-1}): 1716 (C═O). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.60 (12H, s, CH_3), 3.96 (2H, s, C*H*—$(CH_3)_2$), 4.60 (2H, s, CH_2—CH_2—*CH), 5.00 (2H, s, CH_2*—O), 6.40 (1H, d, J = 9.0 Hz, CH═*H*C—C═O), 6.85 (1H, s, C*H*═HC—O), 7.42 (1H, s, Ar—*H*), 7.70 (1H, s, CH═*H*C—O), 7.80 (1H, d, J = 9.0 Hz, C*H*═HC—C═O). EI-MS: *m/z* 329.1 $[M]^+$.

3.7 General procedure for other imperatorin derivatives (7c–7e)

Xanthotoxol (2.02 g, 10.00 mmol) was dissolved in anhydrous acetone (50 ml). Anhydrous K_2CO_3 (1.66 g, 12.00 mmol) was added and the reaction mixture was stirred under nitrogen atmosphere for 30 min at room temperature. Then, different organic amine salt (12.00 mmol) was added respectively and the reaction mixture was stirred at 65°C for another 8 h under nitrogen atmosphere. After the reaction, the mixture was cooled and concentrated *in vacuo*. The residue was dissolved in water and extracted with EtOAc or $CHCl_3$ (3 × 50 ml). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The product was purified by column chromatography. Therein, **7c** was chromatographed on silica gel ($CHCl_3$:MeOH = 5:1), **7d** was chromatographed on silica gel (EtOAc:MeOH = 30:1) and **7e** was chromatographed on silica gel (EtOAc:MeOH = 5:1).

*3.7.1 9-(2-(Pyrrolidin-1-yl)ethoxy)-7H-furo[3,2-g]chromen-7-one (**7c**)*

1.72 g (58% yield) isolated as a white solid, mp 225–226°C. IR (KBr) υ_{max} (cm^{-1}): 1635 (C═O). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.30 (4H, s, CH_2—CH_2—N), 3.64 (6H, m, CH_2—CH_2—O, CH_2—CH_2—N), 5.03 (2H, t, J = 3.8 Hz, CH_2—O), 6.40 (1H, d, J = 9.6 Hz, CH═*H*C—C═O), 6.85 (1H, s, C*H*═HC—O), 7.45 (1H, s, Ar—*H*), 7.73 (1H, s, CH═*H*C—O), 7.81 (1H, d, J = 9.6 Hz, C*H*═HC—C═O). EI-MS: *m/z* 299.1 $[M]^+$.

*3.7.2 N-(2-((7-oxo-7H-furo[3,2-g]chromen-9-yl)oxy)ethyl)nicotinamide (**7d**)*

2.18 g (62% yield) isolated as a pale white solid, mp 157–158°C. IR (KBr) υ_{max} (cm^{-1}): 3440 (N—H), 1720, 1599 (C═O). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.90 (2H, t, J = 4.6 Hz, O—CH_2—CH_2—N—C═O), 4.64 (2H, t, J = 4.6 Hz, O—CH_2—CH_2—N—C═O), 6.40 (1H, d, J = 10.4 Hz, C*H*═HC—O), 6.85 (1H, s, CH═*H*C—C═O), 7.40 (1H, s, Ar—*H*), 7.44 (1H, d, J = 10.4 Hz, CH═*H*C—O),

7.38 (1H, dd, $J_1 = 4.8$ Hz, $J_2 = 7.9$ Hz, pyridine-C*H*), 7.81 (1H, s, C*H*═HC—C═O), 8.25 (1H, d, $J = 7.9$ Hz, pyridine-C*H*), 8.73 (1H, d, $J = 4.8$ Hz, pyridine-C*H*), 9.17 (1H, s, pyridine-C*H*). EI-MS: *m/z* 350.0 $[M]^+$.

*3.7.3 9-(2-(Diethylamino)ethoxy)-7H-furo[3,2-g]chromen-7-one (**7e**)*

2.48 g (83% yield) isolated as a white solid, mp 79–80°C. IR (KBr) υ_{max} (cm^{-1}): 1720 (C═O). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.11 (6H, t, $J = 7.1$ Hz, CH_3), 2.77 (4H, q, $J = 7.1$ Hz, N—CH_2—CH_3), 3.02 (2H, t, $J = 6.4$ Hz, O—CH_2—CH_2), 4.63 (2H, t, $J = 6.4$ Hz, O—CH_2—CH_2), 6.38 (1H, d, $J = 9.6$ Hz, CH═*H*C—C═O), 6.79 (1H, s, C*H*═HC—O), 7.40 (1H, s, Ar—*H*), 7.70 (1H, s, CH═*H*C—O), 7.76 (1H, d, $J = 9.6$ Hz, C*H*═HC—C═O). EI-MS: *m/z* 301.1 $[M]^+$.

3.8 Vasodilatory effect assay

The vasodilatory activity was evaluated on rat mesenteric artery, basilar artery, and renal artery according to the literatures [18,19]. Sprague-Dawley rats weighing 250–300 g were anesthetized and sacrificed by decapitation. For instance, small mesenteric arteries (2 mm segments of second order branch of the superior mesenteric artery) were dissected free of fat and connective tissue and mounted in Multi-wire myograph system (Danish Myo Technology A/S, Inc., Skejbyparken, Denmark). Vessels were maintained at 37°C in physiological Krebs buffer that was bubbled with a carbogen (95% O_2, 5% CO_2) to maintain the buffer at pH 7.4. After a 30 min equilibration period, vessel tension was increased to 3 mN on mesenteric artery and renal artery, 1 mN on basilar artery. That was equilibrated for another 1.5 h before the experiments were started. After equilibration, segments were pre-contracted. The contractile capacity of each vessel segment (mesenteric artery and renal artery) was tested by exposure to a K^+-rich Krebs solution (with 60 mM KCl), in which NaCl was exchanged for an equimolar concentration of KCl. While, U46619 (1 μM) was used for basilar artery segments. When two reproducible contractions have been achieved, the vessels could be used for further experiments. After equilibration, segments were pre-contracted. Once the sustained tension was obtained, the new imperatorin derivatives (0.1 μM to 0.1 mM) were added cumulatively to the baths, and the concentration–response curves to the new imperatorin derivatives were constructed. Control tissues were subjected to the same procedures simultaneously, but omitting the compounds and adding the vehicle. The data of logarithm of half maximum effective concentration (pEC_{50}) and the maximal relaxation (E_{max}) in artery rings were expressed as mean ± SEM.

4. Conclusions

In conclusion, we have synthesized a series of novel imperatorin derivatives via introducing a nitrogen atom in the side chain of the furocoumarin and evaluated the vasorelaxation activity of the new compounds on different arteries. Generally, most of the new compounds displayed better vasorelaxation activity and water solubility than imperatorin itself on mesenteric artery and basilar artery, but the similar vasorelaxation activity as imperatorin on renal artery. The structure analysis was also discussed in this study. In particular, according to the different vasorelaxation activity, **7b** and **7c** can be used to develop a new kind of vasodilator agent for mesenteric artery, while **7d** and **7e** can be used to develop a new kind of vasodilator agent for basilar artery. In addition, since all the tested compounds are the imperatorin derivatives, we hypothesized that the novel derivatives have the similar mechanism of actions with the imperatorin [20]. The further

research on the mechanism of actions of the new compounds needed more in-depth research and the result will be reported in the near future.

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