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5-Phenyl Coumarin Derivatives: Design, Synthesis and vasodilatory activity

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In continuation of our previous efforts towards the development of coumarin derivatives with potential vasodilatory activity, 5-phenyl coumarin derivatives were designed and synthesized. Target compounds and their precursors exhibited moderately vasodilatory ability with EC_{50} at 2.5-49.0 μM. And docking study also revealed the well binding mode of compound 8c with the target protein. Moreover, Intermediates and final product also exhibited different fluorescent properties due to the substituent effect. These results may provide new idea for synthesis and application of 5-substituted coumarins.

Keywords: 5-Phenyl coumarin • Vasodilatory activity • Fluorescence • Molecular docking

Introduction

Coumarins, structurally composing of fused benzene and a-pyrone rings, formed a great class of naturally occurring compounds that possess considerable therapeutic prospect due to their diverse pharmacological activities. Some natural coumarin compounds were confirmed to exhibit inhibitory effects on LPS-induced NO production, vascular smooth muscle cell proliferation and six human CA isozymes (CAs I, II, VII, IX, XII and XIII).[1-3] Besides, a series of synthetic coumarin sulfonamides,

triazole-coumarins, thiazole-coumarins, indolyl-coumarins, imidazole-coumarin and 4-substituted coumarins derivatives were found to have anti-HIV, antibacterial, antioxidant, inhibiting ACE, antifungal, scavenging of reactive oxygen species (ROS), anti-inflammatory, and anticancer activities.[4-12] More importantly, some of them have made their way to clinics including warfarin (anticoagulant), hymecromone (choleretic and antispasmodic), armillarisin A (antibiotic), carbochromen (coronary disease), and cloricromene (antithrombotic and antiplatelet), as shown in Fig 1.

Moreover, coumarin derivatives, both natural and synthetic compounds, also exhibited cardiovascular activity, such as lipid lowering effects, antihyperglycemic, in vitro vasorelaxant activity and in vivo antihypertensive effect.[13-19] Najmanová et al summarized the effects of selected coumarins on experimental major cardiovascular diseases, including cardiac hypertrophy caused by hypertension, experimental dysrhythmias, myocardial ischaemia/reperfusion, and experimental myocardial injury and heamorrhagic shock, which all indicating the tremendous potentiality of coumarins on cardiovascular diseases. [20]

Imperatorin (**Fig 1**), a furanocoumarin compound, was found to induce vasodilatation possibly via inhibiting voltage dependent calcium channel and receptor-mediated Ca^{2+} influx and release, and it also was identified as a L-type calcium channel blocker in our previous works.[21][22] After that, we have spent great efforts on study of imperatorin, concentrating on the synthesis and screening for vasorelaxant activity of its derivatives. As a result, a series of furanocoumarin and 5-phenyl furanocoumarin compounds were obtained, and they all exhibited considerable vasodilatory, antihypertensive, vascular remodeling effects and optical property.[23-27]

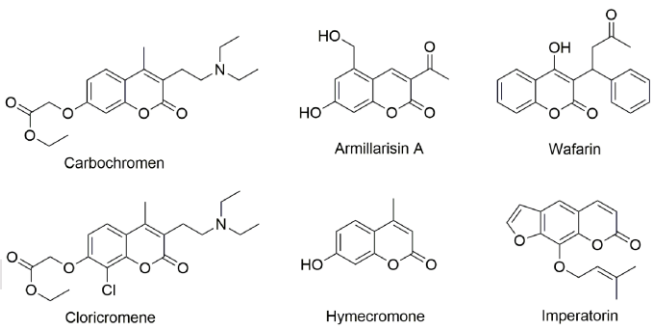


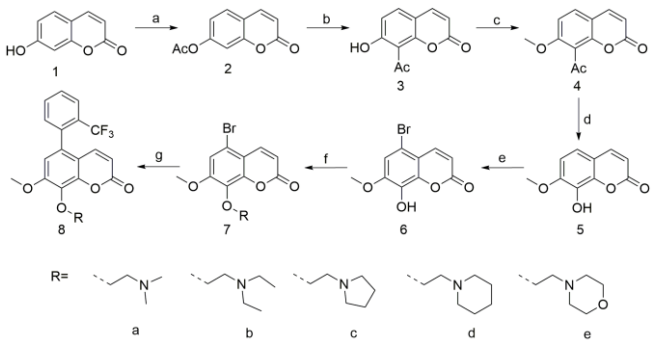
Figure 1. Structures of clinically used coumarin derivatives and imperatorin.

In view of our interest in developing coumarin derivatives with vasodilatory activity to expand its structural diversity, coumarin substructure in skeleton of imperatorin was kept. Following this idea, a new series of 5-phenyl coumarin compounds were designed, synthesized, explored for their bioactivity and fluorescence in present study. The docking study was also performed to investigate the structural influence on the bioactivity and binding mode.

Results and Discussion

Chemistry

Target compounds were prepared *via* seven facile reactions, esterification, Fries rearrangement, methylation, Baeyer-Villiger oxidation, bromination, etherification and Suzuki-coupling reaction, as depicted in **scheme 1**. Initially, with triethylamine as alkaline reagent, 7-OH coumarin (**1**) was esterified by Ac_2O in anhydrous THF within 0.5 h to give 7-acetyloxy coumarin (**2**) with >90% yield. Fries reaction of **2** could afford two rearrangement products at 6-substitute and 8-substituted, respectively. Since 8-substitute was thermodynamic product rather than the dynamic compound (6-substitute), enhanced temperature could result in improved yield of 8-acetyl-7-hydroxycoumarin (3), and 3 can obtained with 67.5% yield at 160℃. Dimethyl sulphate can exhibit the strongest reaction activity only at 50℃, hence, 8-acetyl-7-methoxycoumarin (**4**) was obtained with remarkable yield under this moderate condition. Baeyer-Villiger oxidation was the limited step of targets due to its low yield. Concentrated sulphuric acid served as solvent and reagent, inducing carbonization during the process, which may contribute to the low yield. Moreover, as oxidant, hydrogen peroxide must be dropwise added, in case of overheating even explosion. Finally, 8-hydroxy-7-methoxy coumarin (**5**) was achieved. 5-Bromo-8-Hydroxy-7-methoxycoumarin (**6**) was then obtained by electrophilic substitution reaction at C-5 of compound **5** within acetic acid in presence of Br_2, which attributed to the stronger orientation effect of 8-hydroxy than 7-methoxyl. As the reaction progressed, product precipitated from the solution, which made it convenient to purify. Compounds **7** were given by a similar procedure with **4**, and etherification of 8-hydroxy ensured the success of last step. Finally, target compounds **8** were constructed by the classical Suzuki–Miyaura cross-coupling reaction, while it could not be accomplished on **6** with hydroxy group. Overall, we realized our goals *via* the operable designed path.



Scheme 1. Design route of target compounds. Reagents and conditions: (a) Et_3N, $(CH_3CO)_2O$; (b) $AlCl_3$, 160℃; (c) acetone, K_2CO_3, reflux; (d) H_2SO_4, H_2O_2, 0℃; (e) CH_3COOH, Br_2, 0℃, -r.t.; (f) acetone, K_2CO_3, reflux; (g) 1, 4-dioxane, H_2O, Na_2CO_3, 2-(trifluoromethyl)-phenylboronic acid, 90℃.

Vasodilatory activity

To evaluate the potential vasodilatory activity of compounds **8**, a K^+-induced contractions model of rat mesenteric artery rings was introduced. Compounds **7** were performed to investigate the substituent effect between them. As leading compound, imperatorin was also tested. And the

results were shown in **table 1**. **8b** with *N*, *N*-diethyl ethylamine substituent was more effective than **8a** with *N*, *N*-dimethyl ethylamine substituent, and change from *N*, *N*-diethyl to pyrrole group afforded **8c**, the rigid structure may be favor to vasodilatory effect, comparing the EC_{50} and E_{max}% between **8c** and **8b**. However, when the larger group, piperidyl, replaced pyrrole group, a lower vasodilatory **8d** was obtained, indicating the smaller rigid substituent may be beneficial for bioactivity. Morpholinyl, a more hydrophilic group, induced slightly decrease of activity, compared **8d** with **8e**. On account of above rules, **8c** exhibited most potent vasodilator activity. And the same phenomenon in compounds **7** further supported our speculation. Similarly, **table 1** revealed **7** generally possessed higher bioactivity than corresponding **8**, may suggested that CF_3-substituted phenyl in 5-coumarin was not suitable for their vasodilator activity. Furthermore, compounds **8** even showed poorer vasodilator activity compared with imperatorin, the lead compound. Which suggests that further study still need to improve.

Table 1. *In vitro* vasodilator activity of compounds **7-8**.

Compds	EC_{50}(μM) (n=6)	E_{max}%	Compds	EC_{50}(μM) (n=6)	E_{max}%
7a	5.3±0.7	93.9±5.0	**8a**	49.0±1.5	102.9±2.5
7b	3.0±0.5	101.7±6.2	**8b**	8.3±1.4	95.8±3.4
7c	2.5±0.2	102.4±0.9	**8c**	4.2±0.1	113.4±6.4
7d	3.4±0.3	103.8±3.1	**8d**	13.2±1.2	104.0±5.9
7e	3.6±0.3	102.7±4.2	**8e**	20.0±1.2	100.2±2.0
imperatorin	12.6±0.6	83.1±0.65%	**---**	---	---

Docking study

Chains E and F of transmembrane segments (Fig. 4B) of L-type calcium channels (PDB code: 3G43) was demonstrate participate in binding with L-type calcium channel blocker, as well as imperatorin.[28] Molecule docking study was further performed to investigate the binding mode of these compounds to the target protein, 3G43. The best vasodilatory compound, **8c** was chosen for docking study, and the result was presented in **Fig. 4a**. Generally, four critical hydrogen bonds formed between compound **8c** and protein, contributing to its potent bioactivity. Two oxygen atoms of pyrone formed two hydrogen bonds with HIS107 and THR110 with bond lengths of 2.50 Å and 2.14 Å, respectively. Two hydrogen bonds were found between two fluorine atoms in trifluoromethyl bond with GLU82 with distance of 2.24 Å and 2.45 Å, respectively. Docking mode in this study was quite different from that of 5-phenyl furanocoumarins, which revealed our target compounds may work in a diverse way to function vasodilatory effect. **Fig. 4b** clearly depicted that coumarin kept a certain angle with 5-phenyl in **8c** while bonding with protein.

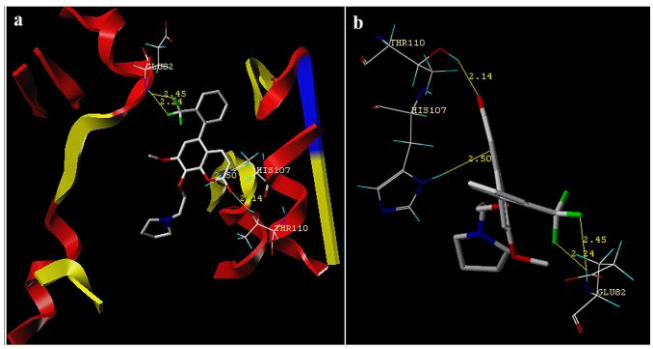


Figure 4. Binding mode of compounds **8c** to L-calcium channel (PDB code: 3G43); (a) binding mode; (b) stereochemistry of compound **8c**.

Fluorescent study

Dual functional biomaterials or compounds, especially the bioactive fluorescence probes have drawn much attention because they opened up the possibility of visualized imaging in diagnosis and treatment of tumor and other disease.[29-31] And we wished that our target compounds could also served as fluorescence probes. Thus, the fluorescence of the compounds was also discussed. The substituent effect on coumarin was explored by comparing the fluorescence of compounds **1-8**. As an electrophilic group, appearance of acetyl reduced electron density in **2** and **3**, consequently, generating obviously decreased fluorescence intensity. After 7-hydroxy was methylated, fluorescence intensity of **4** was clearly stronger than that of **3**, as shown in **Fig. 2a**. When acetyl was lost to afford **5**, apparent red shift can be detected. And bromination of **5** obtained **6**, fluorescence intensity and λ_{em} both increased, which may attribute to the lone pairs in bromine, forming conjugated system with coumarin. Substituted amine contributed to the electron density, resulting the increasing fluorescence intensity in **7**. 5-(2-CF_3)-phenyl in **8** offered less electron than Br in **7**, resulting reduced fluorescence intensity.

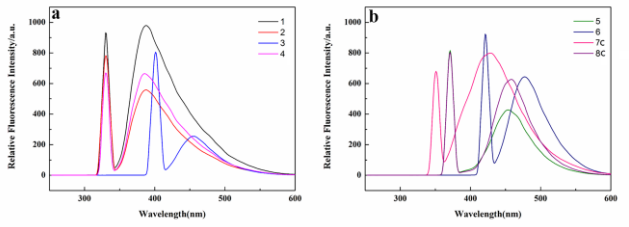


Figure 2. Fluorescent property of compounds **1-8**; (a) fluorescent property of **1-4**; (b) fluorescent property for **5**, **6**, **7c** and **8c**.

Fig. 3 depicted the effect of solvents and pH on **8c**. The increasing polarity of the solvents resulted in parabola trend in fluorescence intensity and red-shift. **8c** exhibited highest and lowest fluorescence intensity in DMF and MeCN, respectively (**Fig. 3a**). As shown in **Fig. 3b**, fluorescence intensity obviously decreased along with reducing pH, probably because of tertiary amine substructure in **8c**. In acidic condition, **8c** bond proton, as a result, electron density decreased, resulting in reducing fluorescence intensity.

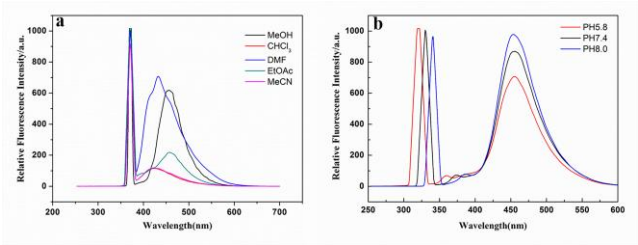


Figure 3. Solvents (a) and pH (b) effects on Fluorescence of **8c**.

Moreover, the ability of **8c** to emit the absorbed light energy was characterized quantitatively by the fluorescence quantum yield (Φ_F). 7-Hydroxy-4-methylcoumarin (Φ_F=0.63, λ_{ex}=450 nm) was served as reference to calculate the fluorescence quantum yield. And the fluorescence quantum yield of 8c was found to be 0.15, which implied that out compound may serve as fluorescence probe as well as vasodilatory entity.

Conclusions

In summary, we designed, synthesized novel 5-phenyl coumarin derivatives and determined their vasodilator activity. Fluorescent investigation revealed the substituent influence on optical property of coumarin, leading us the direction for further modification. Study on structure-activity relationship among compounds **7 (a-e)** and **8 (a-e)**, and molecular docking suggested that the prepared compounds in this paper may act *via* different mechanism from that of previously reported 5-phenyl furocoumarin. These results may point out a different idea for further structural optimization aiming at more potential coumarin-based entities, and further research is well underway.

Experimental Section

Chemistry

Reagents and Materials

All reagents and solvents were purified and dried by standard techniques. Melting points were determined on an X-4 apparatus without correction. Thin layer chromatography (TLC) on silica gel plates was routinely employed to follow the course of reactions and to check the purity of the products. Mass spectra were performed on a Shimadzu GC-MS QP 2010 instrument (Shimadzu, Japan). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCF 400 MHz instrument in $CDCl_3$ solution with TMS as internal standard. Chemical shift multiplicities were reported as follows: s, singlet; d, doublet; t, triplet; m, multiplet.

General procedure

*Synthesis of 7-Acetoxycoumarin (**2**)*

7-Hydroxycoumarin (**1**, 6.48 g, 40.0 mmol) was dissolved in tetrahydrofuran, followed adding triethylamine (7.50 mL). Acetic anhydride was dropwise added in. The mixture was stirred at room temperature for 0.5 h. Product was obtained after evaporated solvent *in vacuum* and washed with alcohol. Yield: 93.4%. M.p.142-144 ℃. ^{1}H NMR (400 MHz, DMSO) δ 8.08 (d, *J* = 9.6 Hz, 1H), 7.77 (d, *J* = 8.4 Hz, 1H), 7.28 (d, *J* = 2.1 Hz, 1H), 7.17 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.48 (d, *J* = 9.6 Hz, 1H), 2.31 (s, 3H).

*Synthesis of 8-Acetyl-7-hydroxycoumarin (**3**)*

Mixture of 7-Acetoxycoumarin (**2**, 7.10 g, 34.8 mmol) and aluminium trichloride (19.9 g, 149.2 mmol) powder was heated at 160°C for 2.5 h. After cooled, water was added and stirred for 0.5 h. The aqueous phase was extracted with ethyl acetate (100×3 mL), the combined organic phase was washed with saturated brines, dried with Na_2SO_4 and evaporated *in vacuo*. The residue was purified on a silica gel column eluting with $CHCl_3$/EtOAc (20:3) to give yellow-green needle crystal (4.79 g). Yield: 67.5%. M.p.166-168 ℃. ^{1}H NMR (400 MHz, DMSO) δ 11.81 (s, 1H), 7.99 (d, *J* = 9.6 Hz, 1H), 7.67 (d, *J* = 8.6 Hz, 1H), 6.91 (d, *J* = 8.6 Hz, 1H), 6.29 (d, *J* = 9.5 Hz, 1H), 2.62 (s, 3H).

*Preparation for 8-Acetyl-7-methoxycoumarin (**4**)*

8-Acetyl-7-hydroxycoumarin (**3**, 4.79 g, 23.5 mmol) was dissolved in anhydrous acetone, followed adding anhydrous potassium carbonate (12.98 g, 94.0 mmol) and stirring at room for 0.5 h. Dimethyl sulphate (3.31 mL) was added and heated at 50°C for 2 h. After that, the reaction mixture was filtered and the filtrate was concentrated *in vacuum*. The crude product was washed with petroleum ether for white product (4.99 g). Yield: 97.3%. M.p.117-118 ℃. ^{1}H NMR (400 MHz, DMSO) δ 8.03 (d, *J* = 9.6 Hz, 1H), 7.78 (d, *J* = 8.7 Hz, 1H), 7.18 (d, *J* = 8.6 Hz, 1H), 6.34 (d, *J* = 9.5 Hz, 1H), 3.91 (s, 3H), 2.51 (s, 3H).

*Preparation for 8-Hydroxy-7-methoxycoumarin (**5**)*

8-Acetyl-7-methoxycoumarin (**4**, 4.99 g, 22.9 mmol) was dissolved in sulphuric acid under ice bath, and hydrogen peroxide was slowly dropwise added in. After reacting for 10 min, the result mixture was poured slowly into a stirred solution of ice water and adjusted to neutral with sodium bicarbonate. The aqueous phase was extracted with ethyl acetate (100×3 mL), the combined organic phase was washed with saturated brines, dried with Na_2SO_4 and evaporated *in vacuo*. The residue was purified by flash chromatography on a silica gel column eluting with $CHCl_3$/EtOAc (20:3) to give yellow-green needle crystal (0.77 g). Yield: 17.5%. M.p.175-177 ℃. ^{1}H NMR (400 MHz, DMSO) δ 9.48 (s, 1H), 7.95 (d, *J* = 9.5 Hz, 1H), 7.16 (d, *J* = 8.6 Hz, 1H), 7.04 (d, *J* = 8.6 Hz, 1H), 6.27 (d, *J* = 9.5 Hz, 1H), 3.89 (s, 3H).

*Preparation for 5-Bromo-8-Hydroxy-7-methoxycoumarin (**6**)*

8-Hydroxy-7-methoxycoumarin (**5**, 0.77 g, 4.0 mmol) was dissolved in cooled acetic acid, followed dropwise adding bromine (22.0 mmol). After reacting completed, the reaction mixture was filtered and filter cake was washed with ice water. The filtrate was diluted by water and extracted with ethyl acetate (30×3 mL), the combined organic phase was washed with saturated brines, dried with

Na_2SO_4 and evaporated *in vacuo*. Total product was 0.98 g. Yield: 89.8%. M.p.172~173 ℃. MS: EI-MS (m/z) 272.0 [M^+]. ^{1}H NMR (400 MHz, DMSO) δ 8.28 (s, 1H), 7.93 (d, *J* = 9.6 Hz, 1H), 7.37 (s, 1H), 6.22 (d, *J* = 9.5 Hz, 1H), 3.92 (s, 3H).

*General procedure for compounds (**7**)*

5-Bromo-8-hydroxy-7-methoxycoumarin (**6**, 0.50 g, 1.8 mmol) was dissolved in anhydrous acetone, followed adding anhydrous potassium carbonate (1.02 g, 7.4 mmol) and stirring at room for 0.5 h. Substituted amine hydrochlorides (2.16 mmol) was added and reflux. After reaction completed, the mixture was filtered and evaporated *in vacuo*. The residue was purified by flash chromatography on a silica gel column eluting with EtOAc/ MeOH (1:1), EtOAc/ MeOH (5:1) or EtOAc/MeOH (10:1).

7a M.p. 162-166 ℃. MS: EI-MS (m/z): 342.80 [M^+]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, *J* = 9.8 Hz, 1H), 7.12 (s, 1 H), 6.34 (d, *J* = 9.8 Hz, 1H), 4.59 (s, 2H), 3.97 (s, 3H), 3.41 (s, 2H), 1.62 (s, 6H).

7b M.p. 193-195 ℃. MS: EI-MS (m/z): 370.85 [M^+]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, *J* = 9.8 Hz, 1H), 7.12 (s, 1H), 6.34 (d, *J* = 9.8 Hz, 1H), 4.26 (t, *J* = 6.1 Hz, 2H), 3.95 (s, 3H), 3.06 (t, *J* = 6.0 Hz, 2H), 2.82 (s, 4H), 1.14 (t, *J* = 7.1 Hz, 6H).

7c M.p. 166-169 ℃. MS: EI-MS (m/z): 368.85 [M^+]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (d, *J* = 9.8 Hz, 1H), 7.11 (s, 1H), 6.32 (d, *J* = 9.8 Hz, 1H), 4.57-4.40 (m, 2H), 3.99 (s, 3H), 3.43-3.38 (m, 6H), 2.16-2.10 (m, 4H).

7d M.p. 173-178 ℃. MS: EI-MS (m/z): 380.85 [M^+]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, *J* = 9.8 Hz, 1H), 7.11 (s, 1H), 6.32 (d, *J* = 9.8 Hz, 1H), 4.38 (s, 2H), 3.91 (s, 3H), 3.00 (s, 2H), 2.74 (s, 4H), 1.69 (s, 4H), 1.50 (s, 2H).

7e M.p. 130-135 ℃. MS: EI-MS (m/z): 382.80 [M^+]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (d, *J* = 9.8 Hz, 1H), 7.10 (s, 1H), 6.33 (d, *J* = 9.8 Hz, 1H), 4.27 (s, 2H), 3.94 (s, 3H), 3.79 (s, 4H), 2.90 (s, 2H), 2.70 (s, 4H).

*General procedure for target compounds (**8**)*

A mixture of compound **7**, Na_2CO_3, 2-(trifluoromethyl) phenylboronic acid, tetrakis (triphenylphosphine) palladium, dioxane and H_2O was stirred at 90°C for 2.5 h. After cooling, H_2O was added in. The aqueous phase was extracted with ethyl acetate (50×4 mL), the combined organic phase was dried with Na_2SO_4 and evaporated *in vacuo*. The residue was purified by flash chromatography on a silica gel column eluting with EtOAc/Ether (2:1) and EtOAc/MeOH (10:1).

8a Yeild: 43.3%. White solid. M.p.145-147 ℃. MS: EI-MS (m/z): 407.1 [M^+]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, *J* = 7.4 Hz, 1H), 7.66-7.58 (m, 2H), 7.45 (d, *J* = 7.3 Hz, 1H), 7.13 (d, *J* = 9.7 Hz, 1H), 6.68 (s, 1H), 6.15 (d, *J* = 9.7 Hz, 1H), 4.57 (s, 2H), 3.92 (s, 3H), 3.45 (s, 2H), 2.62 (s, 6H).

8b Yeild: 45.6%. White solid. M.p.136-138 ℃. MS: EI-MS (m/z): 435.1[M^+]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (d, *J* = 7.4 Hz, 1H), 7.71-7.59 (m, 2H), 7.42 (d, *J* = 7.2 Hz, 1H), 7.15 (d, *J* = 9.7 Hz, 1H), 6.79 (s, 1H), 6.16 (d, *J* = 9.7 Hz, 1H), 4.65 (s, 2H), 4.00 (s, 3H), 3.94 (s, 4H), 3.55 (s, 2H), 1.53-1.50 (m, 6H).

8c Yeild: 33.30%. White solid. M.p. 90-92 ℃. MS: EI-MS (m/z): 433.1 [M^+].

8d Yeild: 53.4%. White solid. M.p. 133-135 ℃. MS: EI-MS (m/z): 447.1 (M^+). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (d, *J* = 7.4 Hz, 1H), 7.64-7.60 (m, 2H), 7.29 (s, 1H), 7.13 (d, *J* = 9.7 Hz, 1H), 6.76 (s, 1H), 6.14 (d, *J* = 9.7 Hz, 1H), 4.38 (s, 2H), 3.91 (s, 3H), 3.00 (s, 2H), 2.74 (s, 4H), 1.69 (s, 4H), 1.50 (s, 2H).

8e Yeild: 49.2%. White solid. M.p. 114-117 ℃. MS: EI-MS (m/z): 449.1 [M^+]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (d, *J* = 7.5 Hz, 1H), 7.65-7.57 (m, 2H), 7.29 (d, *J* = 7.4 Hz, 1H), 7.14 (d, *J* = 9.7 Hz, 1H), 6.77 (s, 1H), 6.14 (d, *J* = 9.7 Hz, 1H), 4.33 (t, *J* = 5.3 Hz, 2H), 3.90 (s, 3H), 3.76 (s, 4H), 2.90 (s, 2H), 2.66 (s, 4H).

Vasodilatory activity

Activity evaluation was performed following our previous reports.[34] Briefly, Sprague-Dawley rats were anesthetized and sacrificed by decapitation. 2 mm segments of second order branch of mesenteric artery were obtained. The segments were mounted in Multi-wire myograph system at 37 ℃ and maintained in physiological Krebs buffer and carbogen (95% O_2, 5% CO_2). After equilibration for 30 min, vessel tension increased to 3 mN, followed by equilibrating for another 1.5 h. The sustained tension of segments was obtained by exposure to a K^+-rich Krebs solution (with 60 mM KCl), target compounds were then added cumulatively, and the concentration response curves were constructed. Control vessels were subjected to the same processes simultaneously with adding vehicle only. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health. The experimental protocols for using the mice were approved by the Animal Ethics Committee at Xi'an Jiaotong University, Xi'an, China (Permit Number: XJTU 2011-0045).

Docking study

Molecular docking was carried out following our previous study.[35] Briefly, the small molecules and the X-ray crystal structure of protein (PDB code: 3G43) were imported. Water molecules were removed and hydrogen was added. Tripos force field and Pullman charge were applied to minimize. **8c** was depicted by Sybyl/Sketch module (Tripos Inc.), optimized by Powell's method with Tripos force field with convergence criterion at 0.005 kcal / (Å mol), and assigned with Gasteiger–Hückel method.

Fluorescent study

The spectral characteristics of the compounds were also measured. Compounds 1-**6**, **7c** and **8c** were performed by dissolved them in methanol separately obtaining 4×10^{-2} mol/L stock solutions. **8c** was dissolved separately in methanol, ethyl acetate, N, N-dimethylformamide, acetonitrile and chlorform to obtain 4×10^{-5} mol/L solution. In another experiment, the solution of **8c** in methonal was freshly prepared by the buffer solution to the corresponding pH (5.7, 7.4 and 8.0). The emission spectra were obtained by using RF-5301 spectrofluorometer. Test conditions: room temperature, adjust the excitation wavelength λ_{ex}, the sample pool is 1 cm×1 cm×4 cm quartz cuvette, slit width of 5 nm. Moreover, 7-Hydroxy-4-Methylcoumarin (ΦF=0.63 in ethanol, λex=450 nm)[32] was chosen as standard dye to measure and calculate the fluorescence quantum yields of the synthesized dye (8c) follow our previous procedure.[33]

Acknowledgements

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Author Contribution Statement

H. Z. He designed and managed the project. C. Wang and Y. J. Li contributed to synthetic work activity evaluation. T. Zhang and D. Wei

conducted the optical experiments. Y. J. Hou worked on the docking study.

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Entry for the Table of Contents