Bioorganic & Medicinal Chemistry Letters 26 (2016) 640–644

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and fluorescent study of 5-phenyl furocoumarin derivatives as vasodilatory agents

Cheng Wang, Tao Wang, Limin Huang, Wen Lu, Jie Zhang, Huaizhen He *

School of Pharmacy, Xi'an Jiaotong University, Xi'an 710061, China

ARTICLE INFO

Article history:
Received 26 June 2015
Revised 11 November 2015
Accepted 17 November 2015
Available online 17 November 2015

Keywords:
Furocoumarin
Synthesis
Vasodilatory activity
Fluorescence properties
Theoretical studies

ABSTRACT

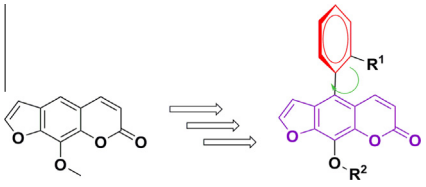
Two series of 5-phenyl furocoumarin derivatives were designed and prepared based on our previous research. All new compounds were characterized by ^{1}H NMR, ^{13}C NMR and mass spectra. Furthermore, they were screened for their vasodilatory activity on the mesenteric artery of Sprague-Dawley rats, and they all presented with moderate vasodilatory activity. Fluorescent properties of the target compounds were tested in methanol. The fluorescence variation of **4a** was investigated in different solvents, various pH and the migration time was determined. All results indicated that this type of fluorescent compound can be used as vasodilatory agents and probes simultaneously after further structural modifications.

Hypertension is increasing in prevalence worldwide due to aging of the population and rising rates of obesity.[1] Although novel agents and channelopathy for the treatment of hypertension have made substantial progress,[2–5] hypertension remains an incurable disease. Because vasodilatory capacity damnification is a significant hallmark of hypertension, vascular studies have become an interesting field for vasodilatory agents.

Furocoumarins form a large class of naturally occurring compounds, which possess surprising pharmacological activity, optical properties and promising therapeutic prospects.[6–14] During the search for novel furocoumarin-based potent antihypertensive agents, we developed a number of furocoumarin derivatives and screened them for potent vasodilatory activity, vascular remodeling effects and fluorescent activity.[15–19] Theoretical studies reveal that geometric structures of diphenyl–furocoumarin derivatives are altered following the change of the ortho-substituent of phenyl. The dihedral angle between furocoumarin and phenyl gradually increases with the change of F, Cl, Br, CF_3, which is consistent with their vasodilatory activity. The dihedral angle is almost 90° with the CF_3 substituent. This suggests that the dihedral angle between furocoumarin (marked in purple) and phenyl (marked in red) may effect the vasodilatory activity. Further theoretical studies show that the substituents of methoxycarbonyl and ethoxycarbonyl could afford a 90° dihedral angle. Moreover, introduction of an ester group to the phenyl could change the electron distribution, which may enhance the conjugation, contributing to the fluorescence of the molecule.[20,21]

Based on these observations, we developed two series of compounds (Scheme 1) to enhance the structural diversity and fluorescence potency. The docking results confirm our hypothesis of the geometric structure of the target compounds. Activity evaluation indicates all the target compounds possess favorable vasodilatory activity and optical properties.

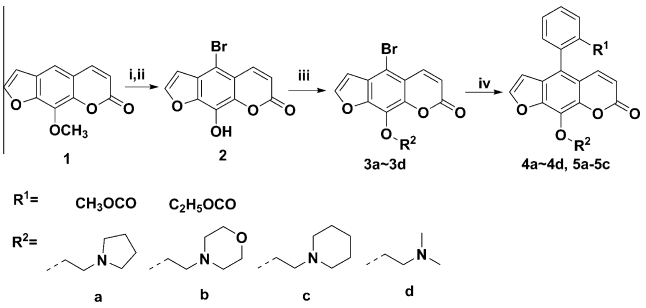
The general methods for synthesis of compounds **4a**–**5c** are summarized in Scheme 2. The option of methoxycarbonyl and ethoxycarbonyl for R^1 was based on theoretical study. The R^2 were selected based on our previous studies in consideration of bioactivity and fluorescence. Compounds substituted with pyrrolidyl, morpholinyl and piperidyl possessed high bioactivity and fluorescence compared with other substituents, as previously reported. Compound **4d** was synthesized to investigate the substitution effect on bioactivity. The reaction were monitored by TLC under



Scheme 1. Design strategy of target compounds.

* Corresponding author. Tel./fax: +86 29 82655451.
E-mail address: hehuaizhen@mail.xjtu.edu.cn (H. He).

http://dx.doi.org/10.1016/j.bmcl.2015.11.056
0960-894X/

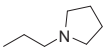
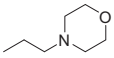
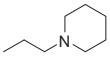
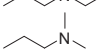
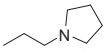
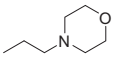
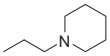


Scheme 2. Synthetic route of target compounds. Reagents and conditions: (i) Br_2, AcOH, 0 °C–rt; (ii) BBr_3, CH_2Cl_2, −5 °C to rt; (iii) K_2CO_3, acetone, reflux; (iv) Na_2CO_3, $Pd(PPh_3)_4$, dioxane/H_2O (v/v = 3:1).

365 nm because compounds **4a**–**5c** showed favorable primary fluorescence.

The vasodilator activities of the synthesized derivatives were evaluated on the in vitro rat mesenteric artery rings against a K^+-induced contractions model, and the results were summarized in Figure 1 and Table 1. Generally, all novel designed compounds were more effective than the parent scaffold. Compounds with an ethoxycarbonyl substituent had higher vasodilator activity than that of methoxycarbonyl, as shown in compound **4a** compared with **5a**, **4b** compared with **5b**, **4c** compared with **5c**, separately. This may be because the ethoxycarbonyl group contributes more electrons to the molecule, and the larger substituent favors the forming of a vertical dihedral angle. The result also supports our hypothesis. The pentacyclic pyrryl in **4a** was replace with a larger moiety piperidyl to yield **4c**, and the vasodilator activities were simultaneously improved. The same trend is observed in **5a** and **5c**. However, **4d** exhibited higher activity than **4a** and **5a**, which suggests that a flexible substituent is beneficial for activity. Altering the piperidyl of **4c** to an oxygen-contained isostere morpholinyl yielded the more effective compound **4b**, which was consistent with **5b**. This result may be because an oxygen atom in the morpholinyl is beneficial for forming a hydrogen bond with the target protein. In conclusion, the lipophilic substituent ethoxycarbonyl in the R^1 position enhances the bioactivity as well as the morpholinyl moiety in R^2 position. Compound **5b** with a pEC_{50} = 5.81 ± 0.13 was the most efficient agent.

Table 1
Structures and in vitro vasodilator activity of title compounds

Compd	R^1	R^2	pEC_{50} (μM) (n = 6)	E_{max} (%)
4a	CH_3OCO		4.71 ± 0.03	102.2 ± 2.22
4b	CH_3OCO		5.59 ± 0.06	100.1 ± 2.44
4c	CH_3OCO		5.35 ± 0.07	115.9 ± 3.04
4d	CH_3OCO		5.27 ± 0.01	101.5 ± 0.05
5a	C_2H_5OCO		5.15 ± 0.01	100.1 ± 0.10
5b	C_2H_5OCO		5.81 ± 0.13	113.3 ± 1.17
5c	C_2H_5OCO		5.38 ± 0.03	113.4 ± 6.45
Imperatorin	—	—	4.95 ± 0.14	85.6 ± 0.40
Verapamil	—	—	7.51 ± 0.09	95.8 ± 1.92

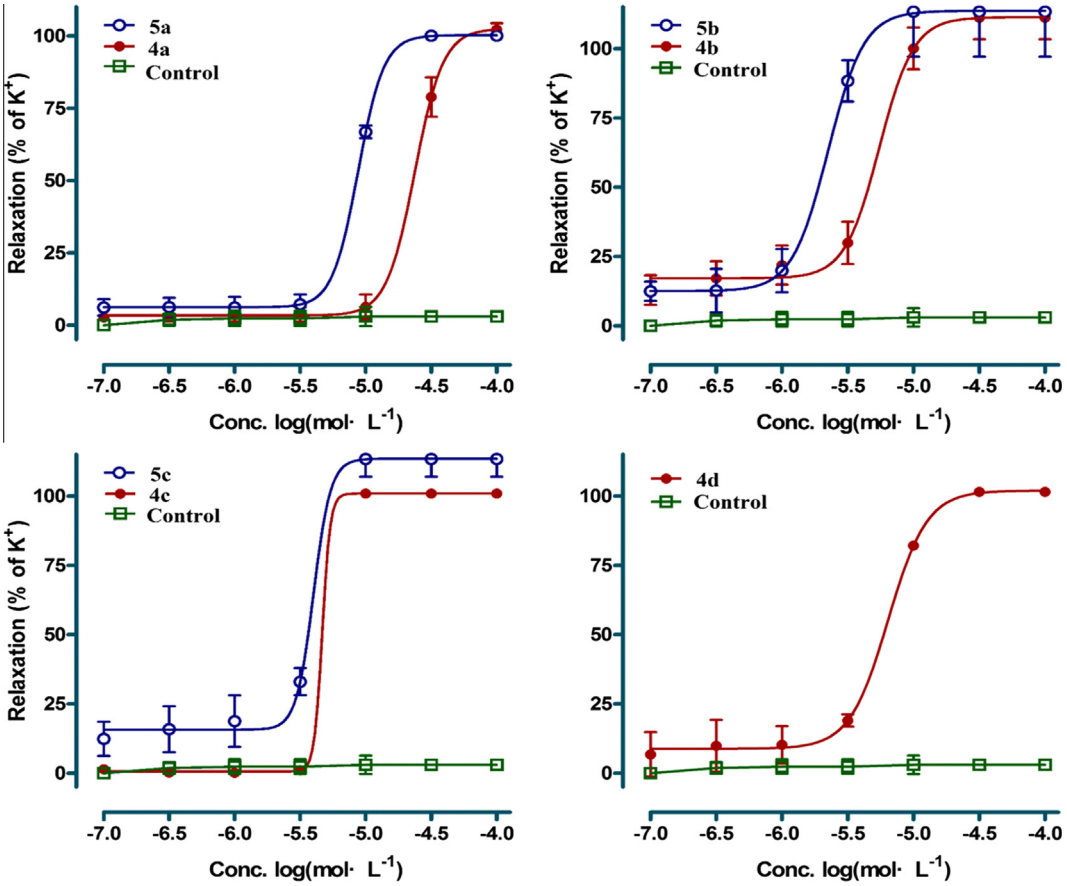


Figure 1. In vitro vasodilator activity induced by title compounds.

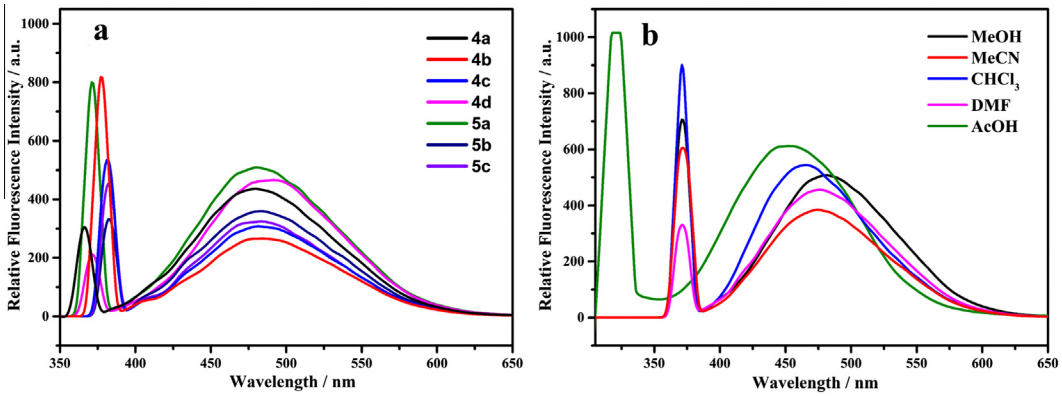


Figure 2. (a) The emission spectra of **4a**–**5c** in $CHCl_3$; (b) solvent effect on fluorescence emission spectra of **5a**.

The optical properties of the target compounds were also explored. As shown in Figure 2a, **4a**–**5c** all showed blue emission with λ_{em} at 480 ± 1 nm, except **4d** showed emission with λ_{em} at 493 nm. Series **4** had a stronger relative fluorescence intensity than series **5**, possibly due to the ethoxycarbonyl devoting more to the conjugation. Furthermore, with the same R^1, the relative fluorescence intensity generally decreased with a larger substitution at R^2 in **4a** compared with **4b**, **4c** compared with **4d**, and **5a** compared with **5c**. Larger substitution may influence the conjugation, and relative fluorescence intensity consequently decreased. The effect of solvents on the most highly fluorescent agent, **5a**, was also evaluated in methanol, *N*,*N*-dimethyl formamide, acetonitrile, acetic acid and chloroform in Figure 2b. It distinctly revealed that **5a** is sensitive to different solvents. The decreasing polarity of the solvents resulted in a blue-shift of the maximum emission peaks (λ_{em}) from 482 nm (in methanol) to 463 nm (in chloroform), except for acetic acid. It may be because acetic acid could react with **5a** and greatly changes its conjugation.

Figure 3 shows the influence of pH on **5a**. As depicted in Figure 3a, **5a** maintained its fluorescence property at both pH 7.4 and 9.2, whereas the relative fluorescence intensity was slightly decreased in acidic solution. Figure 3b–d and Table 2 describe

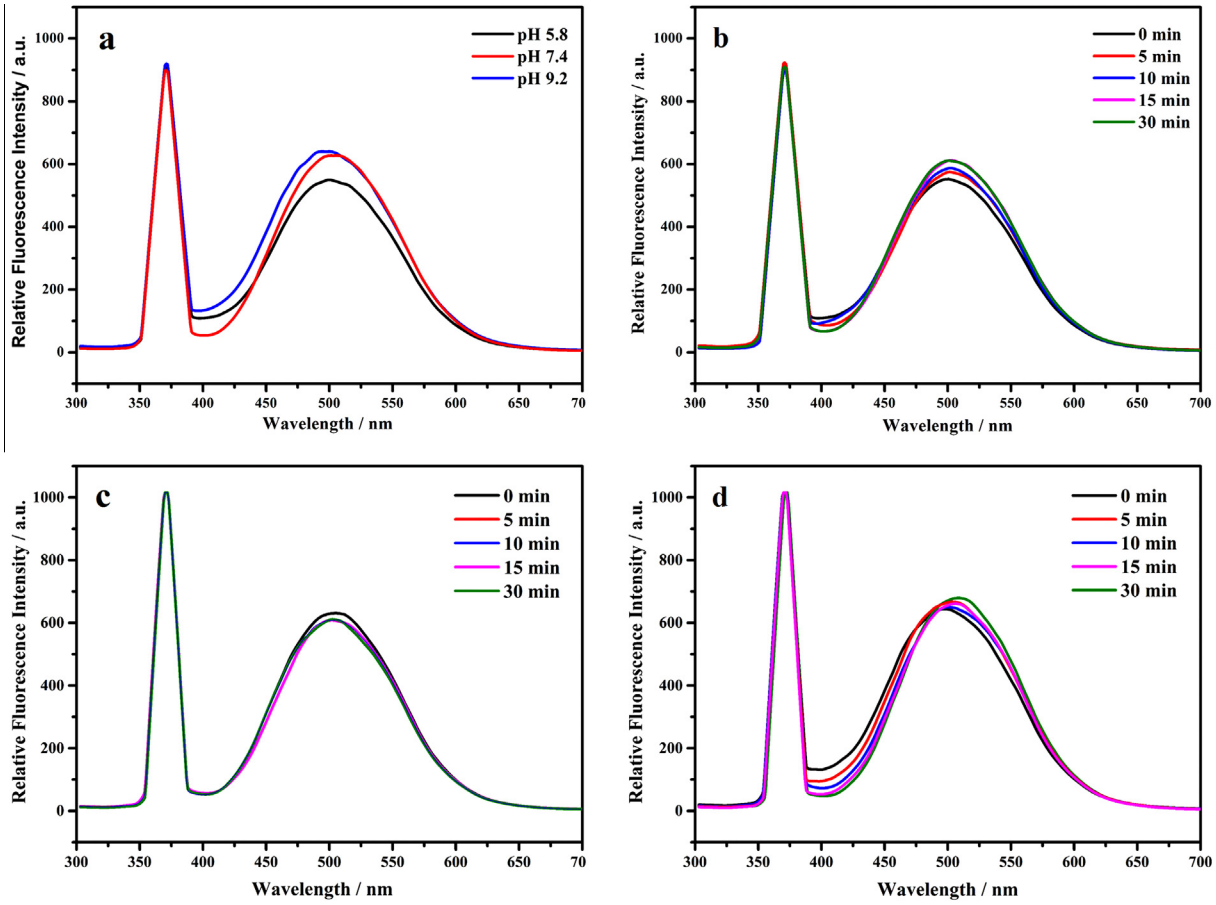


Figure 3. (a) Fluorescence spectra of **5a** at various pH; (b) fluorescence spectra of **5a** at different time at pH 5.8; (c) fluorescence spectra of **5a** at different time at pH 7.4; (d) fluorescence spectra of **5a** at different time at pH 9.2.

Table 2
Change of λ_{em} and relative fluorescence intensity of **5a** in various pH and migration time

	pH 5.8		pH 7.4		pH 9.2	
	λ_{em} (nm)	RFI*	λ_{em} (nm)	RFI*	λ_{em} (nm)	RFI*
0 min	507	555	500	632	496	654
5 min	510	565	498	615	497	682
10 min	511	594	501	614	509	665
15 min	507	616	498	615	505	665
30 min	506	618	501	631	504	709

* Relative fluorescence intensity.

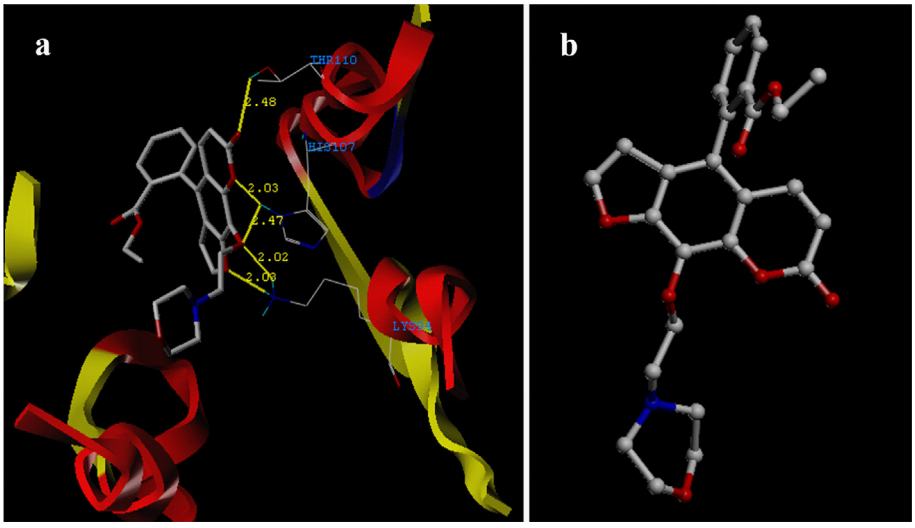


Figure 4. Binding mode of compounds **5b** to L-calcium channel (PDB code: 3G43).

the change of λ_{em} and relative fluorescence intensity with time in the above three buffer solutions. The λ_{em} ranged successively from 506–511 nm, 498–501 nm and 496–509 nm. The relative fluorescence intensity ranged from 555–618 nm, 614–632 nm and 654–709 nm. Although the relative fluorescence intensity in the alkaline solution (pH 9.2) was better than in the neutral solution (pH 7.4), there was a larger change of λ_{em} and relative fluorescence intensity in both the acidic solution (pH 5.8) and alkaline solution (pH 9.2) than in the neutral solution (pH 7.4) indicating that these compounds may be stable for labels in vivo.

Our previous studies confirmed that imperatorin, the scaffold core of 5-phenyl furocoumarin derivatives, possessed calcium antagonism and affinity to L-type calcium channel (PDB code: 3G43).[22] A molecule docking study was performed to investigate the binding mode of these compounds to the L-calcium channel. The result is presented in Figure 4a. Generally, the target compound **5b** formed five critical hydrogen bonds with the protein, thus contributing to its potent bioactivity. The oxygen atom of furan and the oxygen atom connected to the benzene ring formed two hydrogen bonds with LYS94. The bond lengths were 2.03 Å and 2.02 Å, respectively. The oxygen atom in lactone and the oxygen atom connected to the benzene ring formed two hydrogen bonds with HIS107. The bond lengths were 2.03 Å and 2.47 Å, respectively. The oxygen atom in the carbonyl formed a hydrogen bond with THR110 with distance of 2.48 Å.

The minimized energy optimization of **5b** revealed that furocoumarin and phenyl were almost mutually perpendicular, as shown in Figure 4b. Figure 4a also shows that **5b** maintained the geometric structure while bonded with the protein, which confirmed our initial conjecture.

In this study, we report the synthesis, bioactivity, fluorescence and docking study of seven 5-phenyl furocoumarin derivatives. The biological and fluorescent evaluations revealed they all exhibit moderate vasodilator activity and photoluminescent properties. The optical study on **5a** indicates that this series of compounds possesses favorable stability in different solvents and in various pH solutions. This study offers new ideas for the structural optimization for potent agents, and provides bioactive labeled entity for further efforts.

Acknowledgment

Financial support from the National Natural Science Foundation of China (Nos. 81202494, 81302737) is greatly appreciated.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.11.056.

References and notes

1. Bhatt, D. L.; Kandzari, D. E.; O'Neill, W. W.; D'Agostino, R.; Flack, J. M.; Katzen, B. T.; Leon, M. B.; Liu, M.; Mauri, L.; Negoita, M.; Cohen, S. A.; Oparil, S.; Rocha-Singh, K.; Townsend, R. R.; Bakris, G. L. N. *Engl. J. Med.* **2014**, *370*, 1393.
2. Grimminger, F.; Weimann, G.; Frey, R.; Voswinckel, R.; Thamm, M.; Bölkow, D.; Weissmann, N.; Mück, W.; Unger, S.; Wensing, G.; Schermuly, R. T.; Ghofrani, H. A. *Eur. Respir. J.* **2009**, *33*, 785.
3. Ma, L.; Roman-Campos, D.; Austin, E. D.; Eyries, M.; Sampson, K. S.; Soubrier, F.; Germain, M.; Trégouët, D. A.; Borczuk, A.; Rosenzweig, E. B.; Girerd, B.; Montani, D.; Humbert, M.; Loyd, J. E.; Kass, R. S.; Chung, W. K. N. *Engl. J. Med.* **2013**, *369*, 351.
4. Miyagawa, K.; Emoto, N. *Ther. Adv. Cardiovasc. Dis.* **2014**, *8*, 202.
5. Ito, T.; Ozaki, Y.; Son, Y.; Nishizawa, T.; Amuro, H.; Tanaka, A.; Tamaki, T.; Nomura, S. J. *Med. Case Rep.* **2014**, *8*, 250.
6. Santana, L.; Uriarte, E.; Roleira, F.; Milhazes, N.; Borges, F. *Curr. Med. Chem.* **2004**, *11*, 3239.
7. Girennavar, B.; Poulose, S. M.; Jayaprakasha, G. K.; Bhat, N. G.; Patil, B. S. *Bioorg. Med. Chem.* **2006**, *14*, 2606.

8. Messer, A.; Raquet, N.; Lohr, C.; Schrenk, D. *Food Chem. Toxicol.* **2012**, *50*, 756.
9. Baumgart, A.; Schmidt, M.; Schmitz, H. J.; Schrenk, D. *Biochem. Pharmacol.* **2005**, *69*, 657.
10. Wu, S. B.; Pang, F.; Wen, Y.; Zhang, H. F.; Zhao, Z.; Hu, J. F. *Planta Med.* **2010**, *76*, 82.
11. Curini, M.; Cravotto, G.; Epifano, F.; Giannone, G. *Curr. Med. Chem.* **2006**, *13*, 199.
12. Fylaktakidou, K. C.; Hadjipavlou-Litina, D. J.; Litinas, K. E.; Nicolaides, D. N. *Curr. Pharm. Des.* **2004**, *10*, 3813.
13. Marques, A. D. S.; Takahata, Y.; Junior, J. R. L.; Souza, M. C.; Simoes, S. S.; Azevedo, W. W.; de Sa, G. F. *J. Lumin.* **2002**, *97*, 237.
14. Kitamura, N.; Kohtani, S.; Nakagaki, R. J. *Photochem. Photobio. C.* **2005**, *6*, 168.
15. Li, N.; He, J. Y.; Zhan, Y.; Zhou, N.; Zhang *J. Med. Chem.* **2011**, *7*, 18.
16. Wang, C.; Wang, T.; Zhou, N.; Pan, X. Y.; He, H. Z. *J. Asian. Nat. Prod. Res.* **2014**, *16*, 304.
17. Zhou, N.; Wang, T.; Song, J.; He, H. Z.; He, J. Y.; He, L. C. *Clin. Exp. Pharmacol. Physiol.* **2014**, *41*, 571.
18. Wang, T.; Wang, C.; Zhou, N.; Pan, X. Y.; He, H. Z. *Med. Chem. Res.* **2015**, *24*, 2417.
19. He, H. Z.; Wang, C.; Wang, T.; Zhou, N.; Wen, Z. Y.; Wang, S. C.; He, L. C. *Dyes Pigments* **2015**, *113*, 174.
20. Valeur, B.; Leray, I. *Coord. Chem. Rev.* **2000**, *205*, 3.
21. Chen, K. Y.; Tsai, H. Y.; Lin, W. C.; Chu, H. H.; Weng, Y. C.; Chan, C. C. *J. Lumin.* **2014**, *154*, 168.
22. Zhang, Y.; Cao, Y. J.; Wang, Q. L.; Zheng, L.; Zhang, J.; He, L. C. *Fitoterapia* **2010**, *82*, 988.