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Synthesis and vasodilative activity of tanshinone IIA derivatives ☆

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ABSTRACT

A series of 2,2′-(substituted methylene)bis-(1,6,6-trimethyl-6,7,8,9-tetrahydrophenanthro[1,2-*b*]furan-10,11-dione) derivatives were synthesized by the reaction of tanshinone IIA (**D₁**) and aromatic aldehyde in the presence of *p*-TsOH. Bromination derivative of **D₁** and hydrolysis product of cryptotanshinone (**D₂**) were also prepared in this work. Vasodilation activity in vitro of them was valuated on the contractile response of vascular thoracic aorta smooth muscle from Wistar rats for the first time. Most of them exhibited a concentration-dependent inhibition on the contractile response of norepinephrine.

Salvia miltiorrhiza (**SM**) has been widely used in the treatment of cardiovascular and coronary artery disease in traditional Chinese medicine practice.[1–4] It has been reported that the relaxation by **SM** in rat aorta ring is endothelium dependent and is possibly mediated by the nitric oxide–guanylyl cyclase pathway.[5–7] Tanshinone IIA (**D₁**), which is the major bioactive lipophilic ingredient of SM, shows anti-cancer, anti-bacterial, cardiovascular system activities and could reduce inflammatory reaction of cerebral ischemia tissue.[8–11] Cryptotanshinone (**D₂**), another bioactive natural ingredient in the **SM**, has been fully proved anti-inflammatory and anti-oxidative (Fig. 1). But there were few reports about the vasodilation activity of vascular thoracic aorta smooth muscle of tanshinones and their derivatives.

In the study, tanshinone derivatives were synthesized by the reactions of **D₁** with some aromatic aldehydes.[12] Bromination derivative of **D₁** and hydrolysis product of cryptotanshinone (**D₂**) were also prepared. Vasodilation activity of them was investigated in vitro and compounds **1**, **2**, **10** and **11** reveal remarkable vasodilation activity.

Compound **1** was synthesized by the hydrolysis of **D₂** in the presence of sodium hydroxide (Scheme 1). In the ^{1}H NMR spectra,[13] the shift of two hydrogen protons in the $C_{1'}$ of the dihydrofuran ring of compound **1** were upfield at δ_H 3.95 and δ_H 3.84 in comparison to that of **D₂** at δ_H 4.98 and δ_H 4.43. In the ^{13}C NMR spectra, the shift of $C_{1'}$ in compound **1** at δ 65.4, was upfield in comparison to that of **D₂** at δ 81. It was concluded that dihydrofuran ring was opened in the reaction. However, hydrolysis reaction of **D₁** was failed.

2,2′-(Substituted methylene)bis-(1,6,6-trimethyl-6,7,8,9-tetrahydrophenanthro[1,2-*b*]furan-10,11-dione) derivatives (**2–13**) were synthesized by the reactions of **D₁** and aldehydes at the pres-

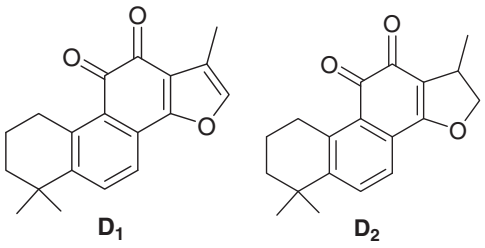
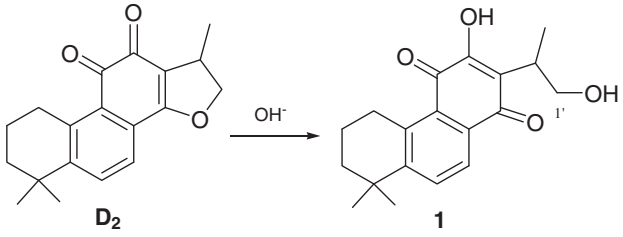


Figure 1. Structures of tanshinone IIA (**D₁**) and cryptotanshinone (**D₂**).



Scheme 1. Reagents and conditions: 2 mol/l NaOH, anhydrous CH_3CH_2OH, rt 12 h.

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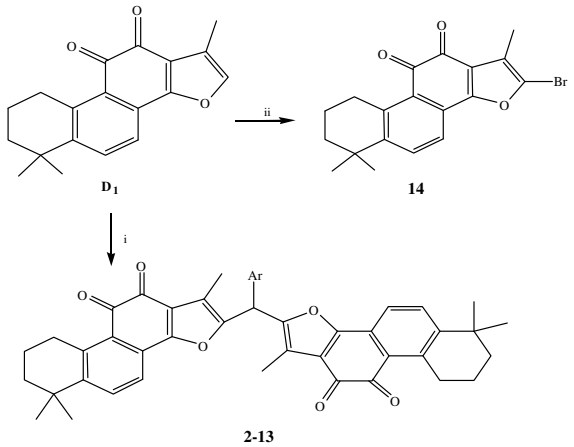
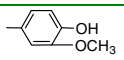
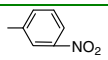
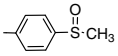
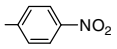
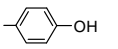
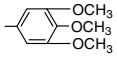
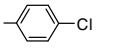
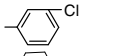
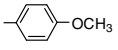
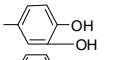
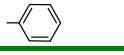
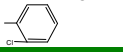
ence of *p*-TsOH (Scheme 2). In order to avoid oxidation of protocatechualdehyde, the reaction carried out under the nitrogen protection. In the reaction, the solvent, chloroform was purified by the process of washing with water to remove EtOH, followed by drying, refluxing and distilling, which could improved the reaction yield. In addition, 2-bromo-1,6,6-trimethyl-6,7,8,9-tetrahydrophenanthro[1,2-*b*]furan-10,11-dione (**14**) was prepared by the reaction of $\mathbf{D_1}$ and bromine liquid in the carbon tetrachloride (Scheme 2).

Vasodilation activity for the synthesized tanshinones derivatives was investigated in vitro using thoracic aortic rings of male



Comp.	Ar	Comp.	Ar
2		**8**	
3		**9**	
4		**10**	
5		**11**	
6[12]		**12**	
7[12]		**13**	

Scheme 2. Reagents and conditions: (i) 1 equiv aromatic aldehyde, *p*-TsOH, anhydrous $CHCl_3$, 50–60 °C, 12 h; (ii) 1 equiv Br_2, CCl_4, rt 20 min.

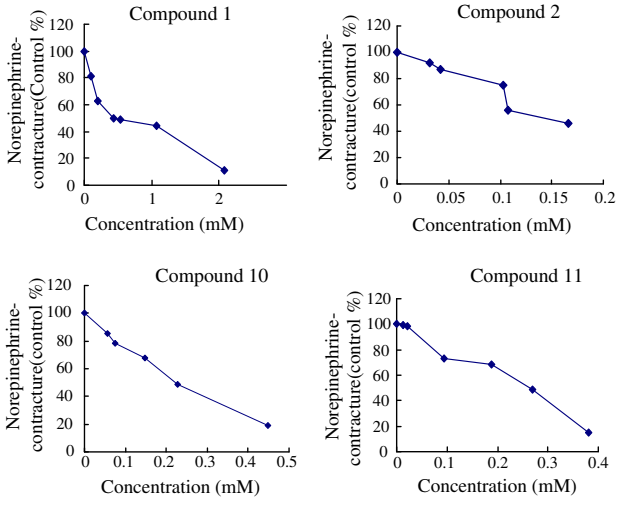


Figure 2. Influence of synthesized compounds on contracture induced by norepinephrine bitartrate in thoracic aortic rings of rats.

Table 1
The relaxant effects of norepinephrine (10^{-6} mol/l) induced contraction in rat thoracic aorta ($X \pm$ SEM) (*n*, the number of cases used)

Compd	% Inhibition of norepinephrine contraction in rat thoracic aorta	
	10^{-4} M	*n*
1	59.18 ± 9.09	5
2	42.78 ± 6.14	6
3	10.31 ± 3.73	5
4	15.22 ± 4.35	3
5	1.00 ± 1.00	3
6	10.98 ± 6.21	6
7	2.95 ± 2.22	3
8	18.84 ± 5.71	6
9	11.37 ± 0.78	3
10	54.98 ± 14.20	3
11	56.04 ± 15.53	3
12	−0.86 ± 7.63	6
13	−29.17 ± 7.9	4
14	4.24 ± 2.18	9
$\mathbf{D_1}$	2.92 ± 1.61	6

Wistar rats pre-contracted with norepinephrine bitartrate (1×10^{-6} mM) according to the known standard procedure.[14,15] The result was presented in terms of percentage of the maximal control norepinephrine-induced responses. Most of the compounds showed a concentration-dependent inhibition on the contractile response of norepinephrine (Fig. 2, Table 1). Especially, **1**, **2**, **10** and **11** (IC_{50}, 0.48, 0.13, 0.2 and 0.26 mM, respectively) were exhibited better activity than that of $\mathbf{D_1}$.[16]

The structure–activity relationship showed that *meta*-substituted derivatives greatly affect the activity than *para*-substituted derivatives. The methoxyl-substituting group in the *meta* position of aromatic aldehydes, presented better pharmacological vasodilation properties than that of nitro substitution derivative (Table 1). The bioactivity of two methoxyl-substituting derivatives in the *meta* position of **10** was better than that of monomethoxyl substitution derivative. In addition, chloro-substituting group in the *meta* position of aromatic aldehydes **11** produced far better activity than that of the *ortho* position and *para* position. It was interesting that chloro-substituting derivative in the *ortho* position of **13** presented obvious vasoconstriction responses. The basic hydrolysis product **1** of $\mathbf{D_2}$ exhibited good bioactivity with IC_{50} of 0.48 mM.

In summary, a series of tanshinone IIA derivatives have been synthesized and vasodilation activity in vitro of them was valuated on the contractile response of vascular thoracic aorta smooth muscle from Wistar rats. The results revealed that the synthetic products exhibited good activity. It is valuable to develop potent vasodilation activity tanshinone IIA derivatives. Interactions of different substituent groups of aromatic aldehydes for pharmacological vasodilation bioactivity are currently underway, which will be reported in due course.

Acknowledgment

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References and notes

1. Fang, Z. Y.; Lin, R.; Yuan, B. X.; Liu, Y.; Zhang, H. *Life Sci.* **2007**, *81*, 1339.
2. Wu, G. B.; Zhou, E. X.; Qing, D. X. *Eur. J. Pharmacol.* **2009**, *617*, 102.
3. Honda, G.; Koezuka, Y.; Tabata, M. *Chem. Pharm. Bull.* **1988**, *36*, 408.
4. Brovkovych, V.; Stolarczyk, E.; Oman, J.; Tomboulian, P.; Milinski, T. *J. Pharm. Biomed. Anal.* **1999**, *19*, 135.
5. Anonymous, *Chin. Med. J.* **1977**, *3*, 224.
6. Zhao, B. L.; Jiang, W.; Hou, J. W., et al *Biochem. Mol. Biol. Int.* **1996**, *38*, 1171.
7. Zhang, F.; Ma, X. D.; Jin, H. F. *Mod. J. Integr. Trad. Chin. West. Med.* **2007**, *16*, 1318.
8. Wang, H.; Gao, X. M.; Zhang, B. L. *J. Ethnopharmacol.* **2005**, *99*, 93.
9. Huang, K. J.; Wang, H.; Xie, W. Z.; Zhang, H. S. *Spectrochim. Acta, Part A* **2007**, *68*, 1180.

10. Houlihan, C. M.; Ho, C. T.; Chang, S. S. *J. Am. Oil Chem. Soc.* **1985**, *62*, 96.
11. Wu, W. L.; Chang, W. L.; Lee, A. R.; Lin, H. C.; King, M. L. *J. Med. Sci.* **1985**, *6*, 159.
12. Qin, J. K.; Bu, X. Z.; Wang, Y. F.; Du, Z. Y.; Wang, X. Y.; Huang, Z. S.; Ma, L.; An, L. K.; Gu, L. Q. *Chin. Chem. Lett.* **2005**, *16*, 1451.
13. All newly synthesized compounds have adequate spectral data, for example, compound **11**, IR (KBr, cm^{-1}): 3439, 2927, 2856, 1673, 1581, 1467, 1381, 1274, 1199. ^{1}H NMR (400 MHz, CDC_{13}, TMS): δ 7.62 (d, 2H, J = 8.2 Hz, H-5,5′), 7.47 (d, 2H, J = 8.1 Hz, H-4,4′), 7.16–7.32 (m, 4 H, H-2″,4″,5″,6″), 5.61 (s, 1H, –CH), 3.18 (t, 4H, J = 6.2 Hz, H-9,9′), 2.22 (s, 6 H, $-CH_3 \times 2$), 1.77–1.80 (m, 4 H, H-8,8′), 1.64–1.66 (m, 4H, H-7,7′), 1.30 (s, 12 H, $-CH_3 \times 4$). ^{13}C NMR (400 MHz, CDC_{13}, TMS): δ 183.27 (C-10, C-10′), 175.67 (C-11, C-11′), 160.70 (C-3a, C-3′a′), 150.40 (C-5a, C-5′a′), 149.45 (C-2, C-2′), 144.68 (C-9a, C-9′a′), 139.03 (C-3″), 134.88 (C-1″), 133.54 (C-5, C-5′), 130.18 (C-2″), 128.08 (C-4″, C-5″), 127.00 (C-3b, C-3′b′), 126.48 (C-9b, C-9′b′), 126.12 (C-6″), 120.48 (C-4, C-4′), 120.23 (C-11a, C-11′a′), 117.98 (C-1, C-1′), 40.13 (–CH), 37.74 (C-7, C-7′), 34.67 (C-6, C-6′), 31.83 ($-CH_3 \times 4$), 29.70 (C-9, C-9′), 19.06 (C-8, C-8′), 8.86 ($-CH_3 \times 2$). EIMS: 711.38 $[M+H]^+$.
14. Silva, A. G.; Zapata-Sudo, G.; Kummerle, A. E.; Fraga, C. A. M.; Barreiro, E. J.; Sudo, R. T. *Bioorg. Med. Chem.* **2005**, *13*, 3431.
15. Raimundo, J. M.; Sudo, R. T.; Pontes, L. B.; Antunes, F.; Trachez, M. M.; Zapata-Sudo, G. *Eur. J. Pharmacol.* **2006**, *530*, 117.
16. Screening of vasodilatiory activity synthesized compounds was carried out on the thoracic aortic rings of male Wistar rats (200–300 g), which were stunned and dislocated in the cervical. Then thoracic aortas were immediately dislodged from Wistar rats and placed in a Krebs–Henseleit solution which was composed of (in mM): NaCl, 118.0; KCl, 4.7; $NaHCO_3$, 25.0; $CaCl_2$, 2.5; NaH_2PO_4, 1.2; $MgSO_4$, 1.2; EDTA, 0.016; glucose, **12** and was saturated in a gas of 95% O_2/5% CO_2 at 4 °C. After surface tissues were removed, the aortas were cut in 2–3 mm ring and fastened in 10 mL organ baths, which maintained at 37 °C in a gas of 95% O_2/5% CO_2. The preparations were allowed to balance for 2 h under 1.5 g basal tensions and the Krebs–Henseleit solution in the organ baths was changed every 20 min.
The integrality of aorta's endothelium was checked before synthesized compounds were added to the organ baths. If the percentage of relaxation of pre-contracted aorta by norepinephrine bitartrate (1×10^{-6} mmol) by acetylcholine (1×10^{-5} mmol) was greater than 80%, endothelium was deemed to be intact. Removal of functional endothelium was confirmed by the lack of relaxation (<10%) in the presence of acetylcholine. The tested compounds were dissolved in dimethylsulfoxide (DMSO) as stock solution (50 mg/mL). Contrast tests were performed in the presence of DMSO alone, at the same concentrations as those used with the synthesized compounds, in order to check contractile response of aorta rings was not affected. All the results were performed as mean ± the standard error of the mean (SEM). Dose–response curves were used to exhibit different effects of compounds. Differences between different concentrations were considered statistically significant when paired Student's t-test was under the condition of P <0.05.