Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 16 (2006) 6155–6160
Bioorganic & Medicinal Chemistry Letters

Total synthesis and biological evaluation of viscolin, a 1,3-diphenylpropane as a novel potent anti-inflammatory agent

Chung-Ren Su,[a,†] Yuh-Chiang Shen,[b,†] Ping-Chung Kuo,[a] Yann-Lii Leu,[c] Amooru G. Damu,[a] Yea-Hwey Wang[d] and Tian-Shung Wu[a,b,*]

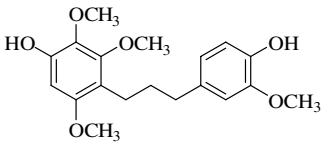
[a]*Department of Chemistry, National Cheng Kung University, Tainan 701, Taiwan*
[b]*National Research Institute of Chinese Medicine, Taipei 112, Taiwan*
[c]*Graduate Institute of Natural Products, College of Medicine, Chang Gung University, Tao-Yuan 333, Taiwan*
[d]*Institute of Pharmacology, National Yang-Ming University, Taipei, Taiwan*

Received 25 July 2006; revised 13 September 2006; accepted 15 September 2006
Available online 12 October 2006

Abstract—Total synthesis of viscolin, an anti-inflammatory 1,3-diphenylpropane isolated from *Viscum coloratum*, employing the Wittig reaction is reported. Key steps in the synthesis of viscolin depend on the selection of protecting groups to maintain the *para* hydroxyl group that is the most critical chemical structure influencing the biological activity of viscolin and the utilization of microwave-assisted Wittig olefination reaction. Anti-inflammatory potency of the synthetic viscolin, its precursor product **16**, and its analogue **17**, through their effects on reactive oxygen species (ROS), nitric oxide (NO), and pro-inflammatory cytokine production in leukocytes and microglial cells were evaluated. Excellent inhibition of ROS and NO production in inflammatory cells could confer the synthetic viscolin to be a potent anti-inflammatory agent for the treatment of oxidative stress-induced diseases.

Inflammation contributes to play an important pathogenetic role in many inflammatory disorders including asthma, gout, rheumatoid arthritis, multiple sclerosis, ischemia-reperfusion injury, etc.[1] During inflammation, production of free radicals [e.g., hydrogen peroxide (H_2O_2), superoxide anion (O_2^-)] and nitric oxide (NO) plays a pivotal role for microorganism killing and also signals the activation of leukocytes and macrophage. Conversely, over-production of these toxic reactive oxygen and nitrogen metabolites/species (ROS/RNS) may cause more damage of the surrounding tissues than the microbe per se.[1,2] Besides, activated leukocytes are preferentially to secrete cytokines [e.g., interleukin-2 (IL-2), interferon-γ (IFN-γ), and tissue necrosis factor-α (TNF-α)] for recruitment of inflammatory cells (e.g., neutrophils, macrophages, and microglial cells) to vascular endothelium and subsequently transmigrate/infiltrate to injured tissue where they release hydrolytic enzymes and enormous quantities of free radicals (ROS/RNS) that result in remarkable tissue damage and immunopathogenesis.[3] Thus, anti-inflammatory and anti-oxidant therapies are also comprehensive pharmacological approaches in the treatment of these inflammation-related disorders.[4,5] One of these important strategies is to look for natural compounds and/or their synthetic derivatives that are capable of inhibiting oxidative stress for the prevention of free radicals- and cytokine-induced cellular damage by inflammatory cells infiltrating to the injured tissue.

Viscolin **1** (Fig. 1) is a naturally occurring 1,3-diphenylpropane that was isolated from the *Viscum coloratum*,[6] a hemiparasite herb used in Chinese medicine as a curative for a number of ailments such as hemorrhage, pleurisy, gout, heart disease, epilepsy, arthritis, and



1

Figure 1. Viscolin.

Keywords: Viscolin; Cytokine; Free radicals; Leukocyte; Microglial cells; Nitric oxide.
* Corresponding author. Tel.: +886 6 2747538; fax: +886 2 2740552; e-mail: tswu@mail.ncku.edu.tw
† These authors contributed equally to this work.

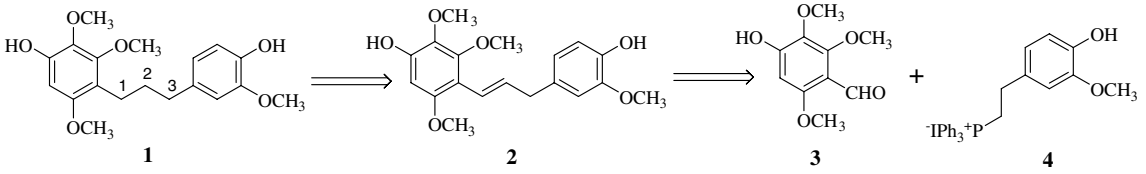
0960-894X/$ - see front matter © 2006 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2006.09.046

hypertension.[7,8] This compound displayed potent and selective activity in the inhibition of superoxide anion generation in human neutrophils activated with *N*-formyl-methionyl-leucyl-phenylalanine combined with cytochalasin B, and it also exhibited DPPH radical-scavenging property. Both these actions could mediate its anti-inflammatory activity. We began a program to explore the therapeutic potential of viscolin and its analogues. The initial goal was to design a total synthesis that is capable of producing gram quantities of **1** suitable for more indepth in vitro and in vivo studies with the flexibility to produce analogues for exploration of the structure-activity relationships. Herein, we disclose our approach toward the first total synthesis and biological evaluation of viscolin.

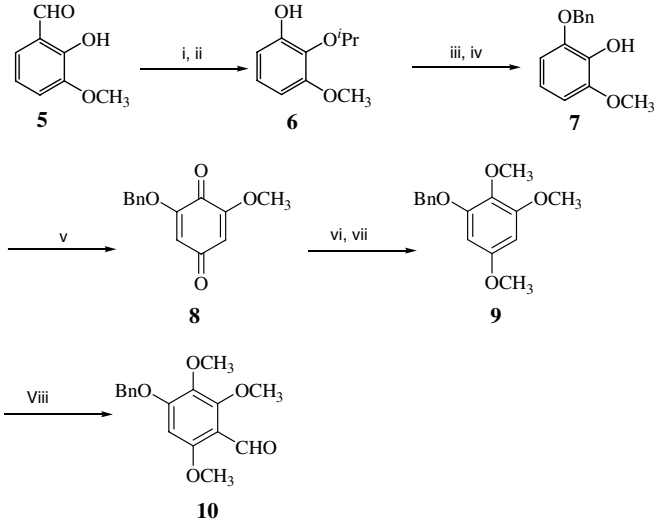
In the past, the most widely employed strategy for the synthesis of 1,3-diphenylpropanes involves the catalytic hydrogenation of the appropriate chalcones followed by subsequent Clemmensen reduction which often requires harsh conditions and long reaction time and suffers from poor yields.[9,10] The retrosynthetic analysis of viscolin **1** is shown in Scheme 1. The synthetic work began with preparation of component molecules **3** and **4** required for the convergent approach toward the projected key intermediate **2** en route to the target compound **1** involving Wittig olefination reaction and hydrogenation.[11] The most critical aspect for the synthesis is in construction of **3** without altering the *para* hydroxyl group essential for bioactivity. Compound **4** was expected to be accessible using slight modification of known methods.

With confidence that the total synthesis of viscolin **1** could be achieved in the above-mentioned manner, the preparation of a specific embodiment of differentially protected aldehyde **10** was commenced according to the reaction sequence shown in Scheme 2.

In anticipation of the Baeyer–Villiger oxidation, it was necessary to protect the hydroxyl group of *o*-vanillin **5**. For this purpose, we examined several protective reagents such as trimethylsilyl bromide, *tert*-butyldimethylsilyl bromide, methoxymethyl bromide, and isopropyl bromide, and only isopropyl bromide gave the desired product. The other protective groups would induce peroxidation or unsuccessful deprotection in the sequence. Thus, treatment of **5** with isopropyl bromide gave the protected vanillin in 94% yield, which was subjected to Baeyer–Villiger oxidation using *m*-CPBA, and the resulting formate ester was hydrolyzed in alkaline condition to yield the desired phenol **6** in 82% yield over two steps.[12] After protecting the phenolic hydroxyl group of **6** by benzyl chloride followed by selective deprotection of the isopropyl group by BCl_3, **7** was obtained.[13] Gratifyingly, H_5IO_6 and CrO_3 in aqueous acetonitrile oxidized phenol **7** to the corresponding benzoquinone **8** in high yield.[14] Compound **8** was reduced with $Na_2S_2O_4$



Scheme 1. Retrosynthetic analysis.



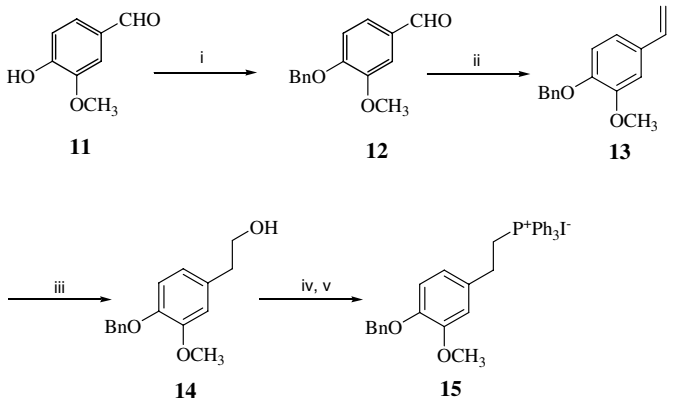
Scheme 2. Reagents and conditions: (i) $(CH_3)_2CHBr$, K_2CO_3, DMF, 94%; (ii) 1—*m*-CPBA, CH_2Cl_2; 2—NaOH, CH_3OH, 82%; (iii) BnCl, KI, K_2CO_3, acetone, 85%; (iv) BCl_3, CH_2Cl_2, 91%; (v) H_5IO_6, CrO_3, CH_3CN, 91%; (vi) $Na_2S_2O_4$, H_2O, 83%; (vii) $(CH_3)_2SO_4$, NaOH, H_2O, 86%; (viii) $POCl_3$, DMF, 58%.

and the unstable diol thus obtained was immediately treated with $(CH_3)_2SO_4$ in the alkaline solution to afford fully methylated product **9**.[15] Subsequently formylation of **9** with $POCl_3$ and DMF finally furnished the desired aldehyde **10**.

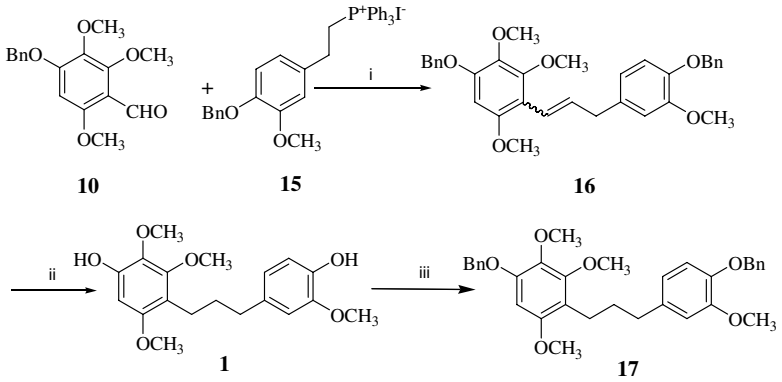
For the construction of the other component molecule **4**, vanillin **11** was treated with benzyl chloride and the ensuing protected product was subjected to Wittig olefination to provide **13**. Unfortunately, all of the conditions applied for the Wittig reaction gave either a mixture of products or the undesired polymers. After considerable experimentation, however, it was realized that, to our delight, the required olefination could be achieved with high degree efficiency under microwave-assisted conditions.[16] Hydroboration of **13** followed by the treatment with H_2O_2 and NaOH produced the primary alcohol **14**,[17] which was converted to phosphonium salt **15** by a successive treatment with I_2 in the presence of PPh_3 and then with PPh_3 in toluene. (Scheme 3).

The final stage of total synthesis is shown in Scheme 4. Wittig reaction of triphenylphosphonium salt **15** proceeded well in the presence of *n*-BuLi to afford the product **16** having the basic skeleton of viscolin. Finally, reduction of the olefin moiety of **16**, accompanied by removal of the benzyl protecting groups, upon Pd/C catalyzed hydrogenation, furnished viscolin **1** in 73% yield.[18] Importantly, analytical and spectral data obtained for synthetic **1** are in excellent agreement with those reported for the desired natural material. In order to evaluate the impact of double bond or hydroxyl groups on biological activity, benzylated viscolin **17** is prepared by treating viscolin with benzyl chloride. (Scheme 4).

To examine the efficacy of the synthetic viscolin **1**, its pro-drug product **16**, and its analogue **17** on the suppression of reactive oxygen species (ROS), nitric oxide (NO), and cytokine production in activated leukocytes or microglial cells for the use as an anti-inflammatory agent, we set up in vitro models by exposing peripheral human neutrophils (PMN), mononuclear cells (MNC), and murine microglial cells (BV2) to phorbol-12-myristate-13-acetate (PMA, a protein kinase *c*(PKC) activator), *N*-formyl-methionyl-leucyl-phenylalanine (fMLP, a G-protein-coupled activator), or lipopolysaccharide (LPS) for the induction of ROS, NO, and cytokines. Their effects on ROS, NO, and cytokines production in human leukocytes and murine microglial cells were elucidated.



Scheme 3. Reagents and conditions: (i) BnCl, KI, K_2CO_3, acetone, 87%; (ii) $CH_3P^+Ph_3I^-$, NaH, THF, microwave, 200 W, 10 min, 77%; (iii) 1—I_2, $NaBH_4$, THF; 2—H_2O_2, NaOH, 75%; (iv) PPh_3, imidazole, I_2, CH_2Cl_2, 85%; (v) PPh_3/toluene, quantitative.



Scheme 4. Reagents and conditions: (i) *n*-BuLi, THF, 71%; (ii) H_2, Pd/C, 73%; (iii) BnCl, KI, K_2CO_3, acetone, 85%.

In human leukocytes, fMLP and PMA both induced large amounts of ROS production in PMN and MNC, predominantly through a receptor/G-protein coupling pathway or the protein kinase *c*(PKC)-dependent pathway, respectively.[3,19] Viscolin significantly suppressed fMLP- and PMA-induced ROS production both in PMN and MNC. The IC_{50} values were comparable with that of quercetin (QC), a potent anti-inflammatory compound of the plant origin (Table 1). Viscolin is 3–10 times more effective in the inhibition of ROS production induced by fMLP than that of PMA, indicating that a G-protein coupling pathway could be the major site targeted by viscolin. Although less potent than viscolin, products **16**, and **17** showed a minor to mediocre activity in the inhibition of fMLP- and PMA-induced ROS production with a maximum inhibition percentage of around 37–47% and 20–42% at 25 μM, respectively, indicating that the hydroxyl groups of 1,3-diphenylpropane derivatives are important for targeting the ROS producing enzyme systems in human leukocytes. The viscolin (a 1,3-diphenylpropane derivative) is a new class of chemical compound with anti-oxidative activity. Activation of PKC as well as the quick intracellular calcium mobilization (a down steam signal for G-protein activation) displays two important signaling pathways for the ROS production in human leukocytes induced by PMA and fMLP, respectively.[19] Viscolin concentration-dependently reduced PKC activity and interfered fMLP-induced intracellular calcium mobilization (Table 2) indicating that interference of PKC- and G-protein-coupled signaling were both responsible for viscolin's effect.

Table 1. Summary of the IC_{50} values for the inhibition of fMLP- and PMA-induced ROS production by viscolin in human peripheral leukocytes

Drugs	IC_{50} (μM) in PMN		IC_{50} (μM) in MNC	
	PMA	fMLP	PMA	fMLP
Viscolin	12.0 ± 0.9*	1.4 ± 0.1	8.3 ± 2.2*	2.5 ± 0.2
16	ND	ND	ND	ND
17	ND	ND	ND	ND
QC	5.5 ± 1.8	2.5 ± 0.3	3.1 ± 0.8	4.0 ± 1.4

Data were calculated as 50% inhibitory concentration (IC_{50}). Values represent means ± SEM of 3–5 experiments performed on different days using cells from different donors. ND, values not detectable.
* $P < 0.05$ as compared with QC (quercetin), respectively.

Table 2. Summary of the inhibitory effect of viscolin on protein kinase activity and fMLP-induced calcium mobilization in human peripheral leukocytes

Drugs	Inhibition (%)		
	PKA	PKC	fMLP-induced $[Ca^{2+}]_i$
Viscolin (5 μM)	ND	34.4 ± 2.7	36.7 ± 3.5
Viscolin (25 μM)	ND	48.8 ± 6.8	53.5 ± 4.6

The activity of cyclic AMP-dependent protein kinase (PKA) and protein kinase *c* (PKC) was determined in the presence or absence of viscolin by an ELISA kit. Intracellular calcium mobilization ($[Ca^{2+}]_i$) was determined by Fura-2 pre-loaded cells in the presence of 2 μM fMLP. Data were calculated as percentage (%) of inhibition by taking the values of protein kinase activity or calcium increment in drug-free samples as 100%. Values represent means ± SEM of 3 experiments performed on different days using cells from different donors. ND, values not detectable.

Microglial cells are also important free radical-producing cells in the central nervous system. We have observed rapid production of ROS by NADPH oxidase (NOX) and nitric oxide (NO) by NO synthase (NOS) in microglial cells.[20] We further evaluated whether viscolin, **16**, and **17** could inhibit NOX-dependent ROS and NOS-dependent NO production in microglial cells. Viscolin concentration-dependently suppressed ROS and NO production in microglial cells. The viscolin was less potent than diphenyleneiodonium (DPI), a specific NOX inhibitor, in the inhibition of ROS production, but was more potent than L-NAME, a specific NOS inhibitor, in the inhibition of NO production (Table 3). Products **16** and **17** showed effectiveness in the inhibition of NOS activity with IC_{50} value of around 19 and 20 μM, respectively, but they did not significantly target the NOX activity (Table 3), suggesting that the hydroxyl groups on the 1,3-diphenylpropane skeleton are important for targeting NOX activity but not essential for targeting NOS activity.

Pro-inflammatory cytokines produced by leukocyte and/or macrophage play pivotal roles in mediating the activation and recruitment of inflammatory cells during inflammation. Beside, drugs with anti-inflammatory activity could modulate the cytokine production.[21] Among these pro-inflammatory Th1-type cytokines, interleukin-2 (IL-2) released by $CD4^+$ Th cells, and interferon-γ (IFN-γ) and tissue necrosis factor-α (TNF-α) produced by $CD8^+$ leukocytes and microglial cells were examined in this study in the presence of viscolin, **16**, and**17**. We used PMA combination with ionomycin, or LPS to induce cytokine production in human leukocytes and microglial cells, respectively. No significant inhibition of the cytokine production was observed by viscolin or products **16** and **17**, except at relatively high concentration (25 μM) viscolin significantly inhibited the TNF-α production in microglial cells (Table 4), indicating that viscolin, **16**, and**17** are not potent chemicals for the inhibition of cytokine production.

The anti-inflammatory property of viscolin, but not products **16** and **17**, was partially due to its direct free radical-scavenging activity with IC_{50} of around 49 μM.

Table 3. Summary of the effects of viscolin on NADPH oxidase (NOX) and nitric oxide synthase (NOS) activity in murine microglial cells

Drugs	IC_{50} (μM) in NOX	IC_{50} (μM) in NOS
Viscolin	29.2 ± 6.5*	24.0 ± 1.0*
16	ND	19.0 ± 1.4*
17	ND	20.0 ± 2.2*
DPI	10.2 ± 0.6	ND
L-NAME	ND	36.0 ± 3.8

NOX and NOS activity were measured by ROS and NO production, respectively, in the presence of 1–50 μM of drugs. DPI (diphenyleneiodonium, a NOX inhibitor) and L-NAME (a NOS inhibitor) were included as positive controls. Data were calculated as 50% inhibitory concentration (IC_{50}) and expressed as means ± SEM from 5 to 6 experiments performed on different days using cells from different passages.
* $P < 0.05$ as compared with relative positive control, respectively.

Table 4. Summary of the inhibitory effects of viscolin on cytokine production in human leukocytes and murine microglial cells

Drugs (μM)	IL-2/CD4$^+$	IFN-γ/CD8$^+$	TNF-α/BV2
	MCF	MCF	MCF
Control (drug free)	47 ± 6	17 ± 2	32 ± 3
Activator	111 ± 13	82 ± 2	185 ± 7
+Viscolin (25)	112 ± 20	67 ± 1	121 ± 10*
+Viscolin (5)	99 ± 7	72 ± 1	155 ± 14
+**16** (25)	103 ± 19	65 ± 2	155 ± 17
+**16** (5)	101 ± 12	60 ± 12	164 ± 23
+**17** (25)	102 ± 11	65 ± 32	154 ± 22
+**17** (5)	100 ± 22	59 ± 09	164 ± 25

Data are expressed as means ± SEM from 3 to 5 experiments performed on different days using cells from different donors or passages. MCF, mean channel fluorescence of the intracellular cytokine staining.
* $P<0.05$ as compared with activator alone, respectively.

Table 5. Summary of the effects of viscolin on DPPH free radical-scavenging activity in cell free system and cytotoxicity on microglial cells

Drugs	DPPH scavenging (IC_{50}μM)	Cytotoxicity (% of dead cells)*		
		25 μM	10 μM	5 μM
16	ND	10.0 ± 3.9	8.1 ± 3.2	8.6 ± 3.0
17	ND	9.6 ± 2.6	8.9 ± 2.6	14.6 ± 5.6
Viscolin	49.2 ± 1.1	14.6 ± 5.6	8.9 ± 2.6	9.6 ± 2.6
Trolox	40.5 ± 3.0	ND	ND	ND

* For cytotoxicity study, all drugs were examined at the concentration as indicated in the table. The background cell death in the solvent control (0.5% DMSO) was 8.5 ± 2.7%. Trolox, an antioxidant included as a control. Data are expressed as means ± SEM from 3 to 5 experiments performed on different days using cells from different passages. ND, values not detectable.

Viscolin, **16**, and **17** did not show significant cytotoxic effect (Table 5).

In summary, the present work provides the first total synthesis of viscolin and affirmed the structure originally reported. We demonstrated in the study for the first time that viscolin, a 1,3-diphenylpropane derivative, exhibits leukocyte inhibitory activity by suppressing free radicals, possibly through modulation of PKC activity and calcium mobilization, and NO production with moderate free radical-scavenging effects that give viscolin the potential to be anti-inflammatory agent for the treatment of oxidative stress-induced diseases. On the basis of this route, further studies directed toward the synthesis of artificial congeners of viscolin as well as evaluation of anti-inflammatory properties will be reported in due course.

Acknowledgments

We acknowledge the financial supports, in part, from Grants of NSC-94-2113-M-006-008, NSC-94-2320-B-077-007, NRICM94-DBCMR-09, and 94-T21 from National Science Council and National Research Institute of Chinese Medicine to Y. C. Shen and T. S. Wu, respectively.

References and notes

1. Nathan, C. *Nature* **2002**, *420*, 846.
2. Casimir, C. M.; Teahan, C. G. In *Immunopharmacology of Neutrophils*; Hellewell, P. G., Williams, T. J., Eds.; Acedemic Press: San Diego, 1994; p 27.
3. Wardlaw, A. J.; Walsh, G. M. In *Immunopharmacology of Neutrophils*; Hellewell, P. G., Williams, T. J., Eds.; Acedemic Press: San Diego, 1994; p 134.
4. Cuzzocrea, S.; Riley, D. P.; Caputi, A. P.; Salvemini, D. *Pharmacol. Rev.* **2001**, *53*, 135.
5. Middleton, E., Jr.; Kandaswami, C.; Theoharides, T. C. *Pharmacol. Rev.* **2000**, *52*, 673.
6. Leu, Y. L.; Hwang, T. L.; Chung, Y. M.; Hong, P. Y. *Chem. Pharm. Bull.* **2006**, *54*, 1063.
7. Chiu, S. T.; *Flora of Taiwan*; 2nd ed.; Editorial Committee of the Flora of Taiwan, Taipei, Taiwan, 1996; Vol. II, pp 282–285.
8. Chiu, S. T.; Medicinal Resource of Formosan Mistletoes; In *Proceedings of International Symposium on Plant Biodiversity and Development of Bioactive Natural Products*; Nov. 18–20, Taichung.
9. Schwartz, M. A.; Rose, F. F.; Holton, R. A.; Scott, S. W.; Vishnuvajjala, B. *J. Am. Chem. Soc.* **1977**, *99*, 2571.
10. Iinuma, M.; Matoba, Y.; Tanaka, T.; Mizuno, M. *Chem. Pharm. Bull.* **1986**, *34*, 1656.
11. Chen, C. C.; Huang, Y. L.; Shiao, Y. J.; Huang, R. L.; Fu, T. T.; Shen, C. C. *Chin. Pharm. J.* **2003**, *55*, 381.
12. Heckrodt, T. J.; Mulzer, J. *J. Am. Chem. Soc.* **2003**, *125*, 4680.
13. Iinuma, M.; Iwashima, K.; Tanaka, T.; Matsuura, S. *Chem. Pharm. Bull.* **1984**, *32*, 4217.
14. Yamazaki, S. *Tetrahedron Lett.* **2001**, *42*, 3355.
15. Baker, W. *J. Chem. Soc.* **1941**, 662.
16. Synthesis of 1-Benzyloxy-2-methoxy-4-vinylbenzene (**13**). To a suspension of NaH (60% in paraffin oil, 840 mg, 21 mmol) in THF (20 mL) was added dropwise a solution of methyl triphenyl phosphonim iodide (3.9 g, 9.8 mmol) in THF (10 mL), followed by a solution of **12** (1.7 g, 7 mmol) in THF (20 mL). The mixture was stirred for 10 min at 80 °C under MW irradiation (200 W). After cooling, the solution was quenched by adding H_2O in ice-water bath. The mixture was extracted with EtOAc, washed with brine, and dried over Na_2SO_4. The organic solvent was evaporated and the residue was purified by silica gel column chromatography (*n*-hexane/EtOAc 9:1) to give **13** (1.3 g, 77.2%); mp 45–46 °C; IR (KBr) cm^{-1}: 1598, 1576, 1513; HREIMS *m/z*: 240.1149 (calcd for $C_{16}H_{16}O_2$: 240.1150); EIMS *m/z*: 240 (M^+, 90), 91 (100); ^1H NMR (300 MHz, $CDCl_3$) δ: 7.26–7.45 (5H, m, ArH), 6.99 (1H, d, J = 1.6 Hz), 6.88 (1H, dd, J = 8.3, 1.6 Hz), 6.83 (1H, d, J = 8.3 Hz), 6.64 (1H, dd, J = 17.6, 10.6 Hz), 5.61 (1H, d, J = 17.6 Hz), 5.16 (1H, d, J = 17.6 Hz), 5.16 (2H, s, OCH_2), 5.15 (1H, d, J = 10.6 Hz), 3.92 (3H, s, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 149.6, 148.0, 137.0, 136.4, 131.2, 128.4, 127.7, 127.1, 119.2, 113.8,111.9, 109.1, 70.9, 55.9.
17. Prasad, A. S. B.; Kanth, J. V. B.; Periasamy, M. *Tetrahedron* **1992**, *48*, 4623.
18. Data for viscolin **1**: white powder: mp 123–124 °C (lit. 118–121 °C)[6]; IR (KBr): 3418 (OH), 2937, 1599, 1515; ^1H NMR ($CDCl_3$, 300 MHz): δ 6.82 (1H, d, J = 8.6 Hz), 6.71 (1H, s), 6.70 (1H, d, J = 8.6 Hz), 6.30 (1 H, s), 5.63 (1H, br s, D_2O-exchangeable, OH), 5.45 (1H, br s, D_2O-exchangeable, OH), 3.87 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 3.73 (3H, s, OCH_3), 2.60 (2H, t, J = 6.5 Hz), 2.58 (2H, t, J = 6.0 Hz), 1.77 (2H, tt, J = 6.5, 6.0 Hz); ^{13}C NMR ($CDCl_3$, 300 MHz): δ 154.3, 151.2, 147.4, 146.2, 143.4, 134.8, 133.5, 120.9, 116.1, 114.0, 111.0, 94.2, 60.9, 60.6, 55.9, 55.7, 35.7, 31.9, 23.2; EI-MS

m/z (*rel. int.*%): 348 (M^+, 16), 197 (59), 137 (100); HR-EIMS calcd for $C_{19}H_{24}O_6$ (M^+) 348.1573. Found 348.1575. EA calcd for $C_{19}H_{24}O_6$: C, 65.50, H, 6.92. Found C, 65.27, H, 6.94.

19. Shen, Y. C.; Chiou, W. F.; Chou, Y. C.; Chen, C. F. *Eur. J. Pharmacol.* **2003**, *465*, 171.
20. Wang, Y. H.; Wang, W. Y.; Chang, C. C.; Liou, K. T.; Sung, Y. J.; Liao, J. F.; Chen, C. F.; Chang, S.; Hou, Y. C.; Chou, Y. C.; Shen, Y. C. *J. Biomed. Sci.* **2006**, *13*, 127.
21. Suzuki, J. I.; Ogawa, M.; Futamatsu, H.; Kosuge, H.; Tanaka, H.; Isobe, M. *J. Mol. Cell Cardiol.* **2006**, *40*, 688.