

## An Efficient Synthesis of a Potent Anti-inflammatory Agent, Viscolin, and Its Inducible Nitric Oxide Synthase Inhibitory Activity

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**A new and efficient synthetic pathway employed the aldol condensation between the acetophenone (3) and vanillin derivative (4) resulted in the precursor chalcone intermediate (14). The target compound viscolin (1) could be afforded through the hydrogenation of the chalcone and followed by deprotection. The present strategy described the development of a more efficient procedure that allowed large-scale production of viscolin for the further research of biological activity both *in vitro* and *in vivo*.**

**Key words** anti-inflammatory; viscolin; chalcone; aldol condensation

Viscolin **1** is a naturally occurring 1,3-diarylpropane and it consists of highly substituted aromatic ring with hydroxyl group at C-4 position in ring-A and 4-hydroxy-3-methoxy groups in ring-B joined through a propane linkage. Viscolin was identified from *Viscum coloratum* (Loranthaceae),<sup>1)</sup> which was usually used as folk medicines for the treatment of a number of ailments such as hemorrhage, gout, heart disease, epilepsy, arthritis and hypertension.<sup>2,3)</sup> The anti-inflammatory potentials of viscolin **1** had been reported to display significant inhibition of superoxide anion generation (O<sub>2</sub><sup>•-</sup>) and elastase release induced with *N*-formyl-methionyl-leucophenyl alanine combined with cytochalasin B (fMLP/CB) in human neutrophils with IC<sub>50</sub> values of 0.58±0.03 and 4.93±0.54 μg/mL, respectively.<sup>4)</sup> Viscolin does not show structural similarity to any known phosphodiesterase (PDE) inhibitor and thus provides a new chemical skeleton in the development of PDE inhibitors.<sup>4)</sup> In addition, viscolin exhibits leukocyte inhibitory activity by suppressing free radicals, possibly through modulation of protein kinase C (PKC) activity and calcium mobilization, and nitric oxide (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.<sup>4)</sup> Inflammation leads to the signaling proteins in affected cells and tissues. Inducible nitric oxide synthase (iNOS), catalyzes the formation of NO from L-arginine.<sup>5)</sup> Low concentration of NO produced by iNOS is likely to contribute to the antimicrobial activity of macrophages against certain bacterial pathogens. However, when overproduced in response to lipopolysaccharides (LPS) stimulation, excess NO can react with superoxide anion radicals to form peroxynitrite, which is a stronger oxidant than NO and cause a high oxidative stress state. It has shown to be associated with a number of chronic diseases, including asthma, rheumatoid arthritis, inflammatory bowel disease, atherosclerosis, Alzheimer's disease, and various human cancers.<sup>6,7)</sup> Thus, inhibition of NO synthesis stands as an important therapeutic goal. In our previous report, viscolin was prepared through the utilization of microwave-assisted Wittig olefination reac-

tion.<sup>8)</sup> However, this method exhibited several disadvantages, such as many steps and low yields. To explore the *in vitro* and *in vivo* anti-inflammatory potentials of viscolin, we have to construct the 1, 3-diarylpropane skeleton with a more concise synthetic methodology. Recently we report a method involving the aldol condensation between the benzaldehyde and acetophenone to produce the target molecules in fewer steps and moderate yield.<sup>9)</sup> But the reagents adapted in the synthetic protocols were not so suitable in the large scale production for the pharmaceutical industry. Therefore, in the present study we wish to develop a more efficient procedure that allowed large-scale production of viscolin for the further research of iNOS inhibitory activity both *in vitro* and *in vivo*.

### Results and Discussion

The retro-synthetic analysis of viscolin (**1**) was displayed in Fig. 1. The aldol condensation between the precursors acetophenone (**3**) and vanillin (**4**) could approach the chalcone (**2**). Moreover, the target molecule would be afforded through the hydrogenation of the chalcone intermediate (**2**). In our previous studies, two pathways were reported to synthesize the viscolin successfully.<sup>8,9)</sup> In the present research, we wish to discover a more efficient synthetic route to produce the lead compound in large scale. Therefore, one of the precursors, acetophenone (**3**) was further resolved into its starting material, 5-bromovanillin (**7**) as shown in Fig. 2.

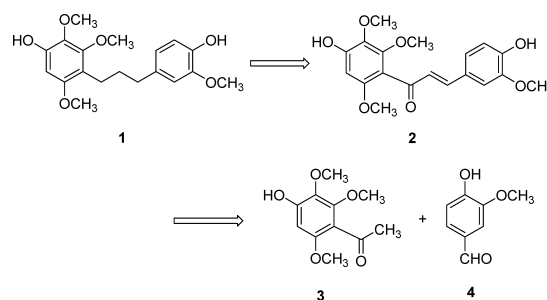


Fig. 1. Retrosynthetic Analysis of Viscolin **1**

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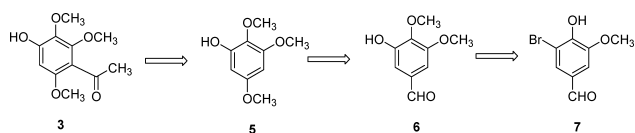


Fig. 2. Retrosynthetic Analysis of Acetophenone 3

Thus our study initiated with the preparation of the acetophenone (**3**) for the aldol condensation to the chalcone (**2**) as shown in Fig. 3. The starting material 5-bromovanillin (**7**) was hydrolyzed in the 8% sodium hydroxide aqueous solution catalyzed with copper (3%) to result in the 5-hydroxyvanillin (**8**) with the yield of 80%.<sup>10</sup> Increasing the percentages of base or metal would not improve the yields of the hydrolytic reaction. The second step is to protect the hydroxyl groups with dimethyl sulphate and isopropyl bromide successively to afford **9**. Baeyer–Villiger oxidation of **9** with *m*-chloroperbenzoic acid (*m*-CPBA) in dichloromethane ( $\text{CH}_2\text{Cl}_2$ ) gave the ester, which was further hydrolyzed with aqueous sodium hydroxide (NaOH) and methanol to furnish phenol intermediate. It was further methylated with methyl iodide in basic solution to yield colorless oil **10**. In our preliminary trials, two

hydroxyl groups of 5-hydroxyvanillin was also protected with the methyl and benzyl substituents, respectively. However, the product would be very difficult to be oxidized under Baeyer–Villiger oxidation experimental conditions. Only oxidation of **9** and followed methylation reactions could provide the desired tetra-oxygenated substituted benzene moiety with moderate yields (from **9** to **10**, 39%). Friedel–Crafts acylation of **10** with the assistance of acetyl chloride and tin(IV) chloride furnished the analogs **12** of the precursor acetophenone (**3**), accompanied by the regioisomeric products **11** with good yields of both compounds (**11**:**12**=43%:34%). Another precursor of the aldol condensation, analog **13** of vanillin (**4**), was prepared by isopropylation of the vanillin through the aid of isopropyl bromide in basic dimethylformamide solution quantitatively as shown in Fig. 3.

The yielding precursors acetophenone **12** and vanillin analog **13** were coupled by the aldol condensation reaction using sodium hydroxide methanolic solution to result in the chalcone intermediate **14** in excellent yield (97%). This could be further transformed by hydrogenation with the catalyst of 10% Pd/C in ethyl acetate–methanol (9:1) solution followed by the decarbonylation with triethylsilane in trifluoroacetic acid to

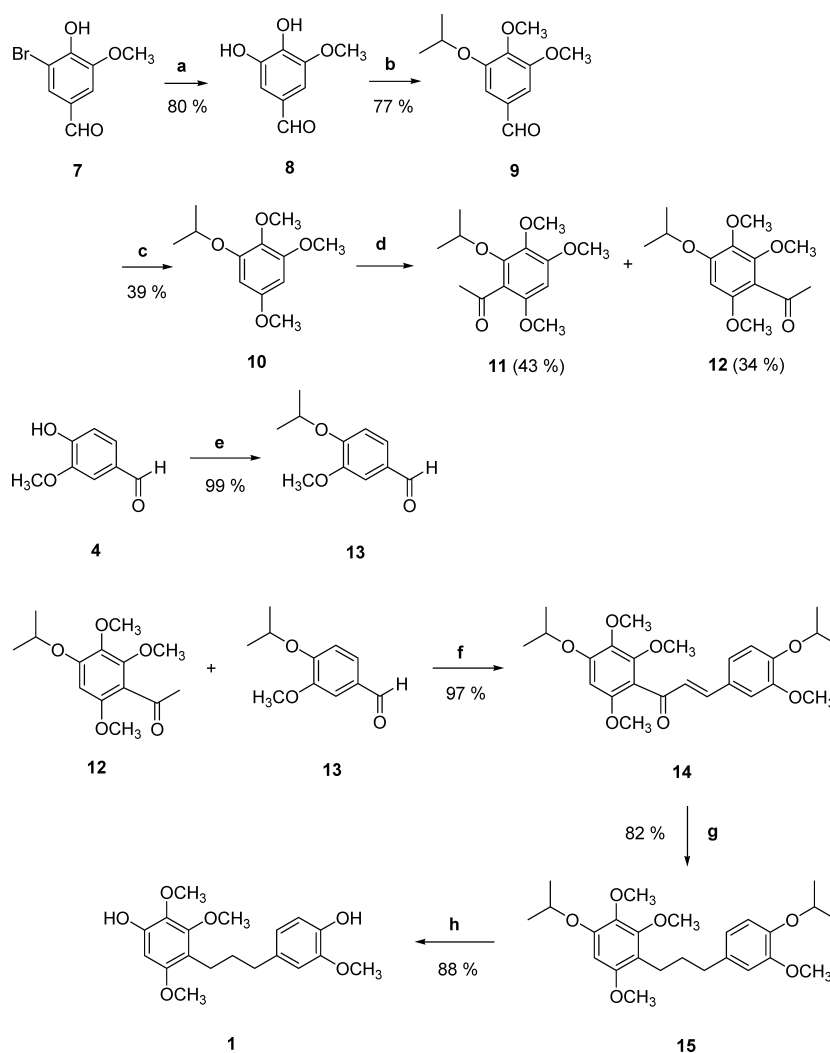


Fig. 3. Synthesis of Viscolin 1

Reagents and conditions: a) NaOH (aq), Cu, reflux; b) (i)  $(\text{CH}_3)_2\text{SO}_4$ ,  $\text{Na}_2\text{CO}_3$ , acetone, reflux; (ii)  $(\text{CH}_3)_2\text{CHBr}$ ,  $\text{K}_2\text{CO}_3$ , DMF, reflux; c) (i) *m*-CPBA,  $\text{CH}_2\text{Cl}_2$ , room temp; (ii) NaOH–MeOH, room temp; (iii)  $\text{CH}_3\text{I}$ ,  $\text{K}_2\text{CO}_3$ ,  $\text{CH}_3\text{OH}$ , room temp; d) AcCl,  $\text{SnCl}_4$ ,  $\text{CH}_2\text{Cl}_2$ , room temp; e)  $(\text{CH}_3)_2\text{CHBr}$ ,  $\text{K}_2\text{CO}_3$ , DMF, 60°C; f) NaOH, MeOH, room temp; g)  $\text{Et}_3\text{SiH}$ , TFA, room temp; h)  $\text{BCl}_3$ ,  $\text{CH}_2\text{Cl}_2$ , 0°C.

reduce the carbon-carbon double bond and carbonyl group successively. The reduction product **15** was afforded with 57% yield within two steps. In the previous studies reported by de Almeida *et al.*,<sup>11</sup> the oxygenated chalcones could be reduced to diphenylpropane accompanied with the deprotection of the benzyl groups only by hydrogenation catalyzed by Pd/C. However, in our synthetic work with the same procedures, only double bond of **14** would be reduced and no influence on the protective groups. In contrast, the oxygenated chalcones **14** could be reduced to diphenylpropane **15** in one step without any deprotection of the substituted groups by the reduction of triethylsilane in th trifluoroacetic acid with better yield (82%). Thus the triethylsilane was selected as the reducing agent and successfully reduced the chalcone **14** to the precursor of viscolin **15**. Finally the deprotection of the isopropyl groups in both phenyl rings by boron trichloride to produce viscolin **1** in good yield (88%). The present study of the synthesis of viscolin provides a more efficient synthetic pathway with fewer steps and similar yields (7 steps, 6%), compared with the previous report (15 steps, 6%).<sup>8</sup>

In the present study, the inhibitory effects of viscolin on LPS-induced NO production in mouse leukaemic monocyte macrophage cell lines (RAW 264.7) were also investigated. Nitrite accumulated in the culture medium was estimated by the Griess reaction as an index for NO release from the cells.<sup>12</sup> When RAW 264.7 macrophages were treated with different concentrations of viscolin (0, 5, 10, 20  $\mu\text{M}$ ) together with LPS (100 ng/mL) for 24 h, a significant concentration-dependent inhibition of nitrite production was detected. The IC<sub>50</sub> value for inhibition of nitrite production of viscolin was  $17.80 \pm 1.52 \mu\text{M}$ .

## Experimental

**General** Melting points were determined using Yanagimoto MP-S3 micro melting point apparatus without correction. Optical rotations were measured using a Jasco DIP-370 digital polarimeter. UV spectra were obtained on a Hitachi UV-3210 spectrophotometer, and IR spectra were recorded on a Shimadzu FT-IR DR-8011 spectrophotometer. <sup>1</sup>H-NMR (300, 400 MHz) and <sup>13</sup>C-NMR (75 MHz) spectra were recorded on Bruker AMX-400 and AVANCE-300 spectrometers using CDCl<sub>3</sub> as the solvents. Chemical shifts are shown in  $\delta$  values (ppm) with tetramethylsilane as an internal standard. Electrospray ionization (ESI) and high-resolution electrospray ionization (HR-ESI) mass spectra were measured on a Bruker APEX II mass spectrometer. Reversed-phase column chromatography was accomplished with Diaion HP-20 and Sephadex LH-20 columns. Silica gel column chromatography was carried out using Kieselgel 60 (70–230, 230–400 mesh, Merck). TLC was executed on precoated Kieselgel 60 F<sub>254</sub> plates (Merck), with compounds visualized by UV light or spraying with 10% (v/v) H<sub>2</sub>SO<sub>4</sub> followed by charring at 110°C for 10 min.

**Synthesis of 5-Hydroxyvanillin (8)** An 8% sodium hydroxide aqueous solution of 5-bromovanillin (**7**) (10.0 g, 43.3 mmol) and copper (0.09 g, 1.4 mmol) was refluxed for 24 h and the reaction progress was checked by TLC. At the last hour sodium bicarbonate was added to the reaction solution to quench the metallic copper powder. The resulting mixture was cooled down and filtered off the copper(II) bicarbonate. The filtrate was acidified by concentrated hydrochloric acid

(HCl) and extracted with ethyl acetate (EtOAc) (200 mL  $\times$  5). The combined organic phases were washed with brine and dried over anhydrous magnesium sulfate (MgSO<sub>4</sub>) and further recrystallized with toluene to afford 5-hydroxyvanillin (**8**) (5.8 g, 80%) as brown powder. **8**: UV (MeOH)  $\lambda_{\text{max}}$ : 256, 232, 208 nm; IR (neat)  $\nu_{\text{max}}$ : 3121, 1659, 1597, 1512, 1466, 1335, 1204, 1138, 1088 cm<sup>-1</sup>; <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 9.78 (1H, s, CHO-1), 7.14 (1H, s, H-5), 7.08 (1H, s, H-2), 6.03 (1H, s, OH-5), 5.56 (1H, s, OH-4), 3.96 (3H, s, OCH<sub>3</sub>-3); <sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$ : 191.0, 147.3, 144.0, 128.4, 129.0, 113.0, 102.8, 56.4; ESI-MS  $m/z$  (rel. int. %): 169 ([M+H]<sup>+</sup>, 100); HR-ESI-MS  $m/z$  169.0502 [M+H]<sup>+</sup> (Calcd for C<sub>8</sub>H<sub>9</sub>O<sub>4</sub>, 169.0501).

**Synthesis of 3-Isopropoxy-4,5-dimethoxybenzaldehyde (9)** A mixture of **8** (9.5 g, 56.7 mmol), dimethyl sulfate (2.5 mL, 26.4 mmol), and sodium bicarbonate (11.5 g, 108.5 mmol) in acetone (100 mL) was refluxed for 6 h. The reaction mixture was cooled down and filtered off the residual solids. The filtrate was concentrated and the residues were dissolved in toluene. The toluene solution was extracted with 10% aqueous NaOH (150 mL  $\times$  3). The combined water solubles were acidified by concentrated HCl and extracted with toluene (200 mL  $\times$  3). The afforded organic phases were combined and concentrated to result in oil residues (9.1 g). The residues were dissolved in dimethylformamide (DMF) (100 mL) and 2-isopropyl bromide (9.4 mL, 100.1 mmol) and potassium carbonate (13.8 g, 99.9 mmol) were also added into the mixture. The resulting solution was heated at 80°C for 12 h and then cooled down to filter off the solids. The filtrate was poured into water (200 mL) and extracted with EtOAc (200 mL  $\times$  3). The combined organic phases were washed with water and brine, and further dried over anhydrous MgSO<sub>4</sub>. The crude was further purified by column chromatography over silica gel using *n*-hexane-EtOAc (9:1) to obtain **9** (9.7 g, 77%) as yellow liquid. **9**: UV (MeOH)  $\lambda_{\text{max}}$ : 256, 232, 208 nm; IR (neat)  $\nu_{\text{max}}$ : 2978, 2939, 2835, 2731, 1694, 1585, 1496, 1323, 1234, 1119, 1003 cm<sup>-1</sup>; <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 9.85 (1H, s, CHO-1), 7.13 (1H, s, H-3), 7.11 (1H, s, H-2), 4.64 (1H, sept,  $J=6.1$  Hz, CH-1'), 3.92 (6H, s, OCH<sub>3</sub>-3, OCH<sub>3</sub>-4), 1.39 (6H, d,  $J=6.1$  Hz, CH<sub>3</sub>-2', -3'); <sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$ : 191.2, 154.0, 151.9, 145.1, 131.6, 110.7, 106.0, 71.8, 60.8, 56.2, 22.1; ESI-MS  $m/z$  (rel. int. %): 225 ([M+H]<sup>+</sup>, 100); HR-ESI-MS  $m/z$  225.1128 [M+H]<sup>+</sup> (Calcd for C<sub>12</sub>H<sub>17</sub>O<sub>4</sub>, 225.1127).

**Synthesis of 1-Isopropoxy-2,3,5-trimethoxybenzene (10)** To a solution of **9** (16.0 g, 71.4 mmol) in CH<sub>2</sub>Cl<sub>2</sub> at room temperature, the *m*-CPBA (70%, 26.5 g, 107.0 mmol) was added slowly in portions. The reaction mixture was stirred for 15 h at room temperature and then the CH<sub>2</sub>Cl<sub>2</sub> was removed under reduced pressure to obtain crude ester. This was hydrolyzed by NaOH (1 N, 30 mL) in MeOH (30 mL) at room temperature stirring for 2 h. The reaction mixture was neutralized with 5% HCl and extracted with EtOAc. The EtOAc extracts were further dissolved in methanol and stirred with potassium hydroxide (KOH) (6.1 g, 110.0 mmol) and methyl iodide (CH<sub>3</sub>I) (15.2 mL, 110.0 mmol) for 48 h at room temperature and then the methanol was removed under reduced pressure. The resulting crude was purified by column chromatography over a silica gel using *n*-hexane-EtOAc (15:1) to obtain pale yellow liquid **10** (5.9 g, 39%). **10**: UV (MeOH)  $\lambda_{\text{max}}$ : 276, 232 (sh), 208 nm; IR (neat)  $\nu_{\text{max}}$ : 2974, 2936, 2839, 1593, 1501, 1458, 1427, 1119, 1057, 1011 cm<sup>-1</sup>; <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 6.13 (1H, s, H-6), 6.13 (1H, s, H-4), 4.51 (1H, sept,  $J=6.1$  Hz,

CH-1'), 3.81 (3H, s, OCH<sub>3</sub>-5), 3.76 (3H, s, OCH<sub>3</sub>-3), 3.74 (3H, s, OCH<sub>3</sub>-2), 1.33 (6H, d,  $J=6.1$  Hz, CH<sub>3</sub>-2', -3'); <sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$ : 156.0, 153.9, 151.9, 133.9, 95.1, 92.3, 71.6, 60.7, 56.0, 55.5, 22.2; ESI-MS  $m/z$  (rel. int. %): 227 ([M+H]<sup>+</sup>, 100); HR-ESI-MS  $m/z$  227.1283 [M+H]<sup>+</sup> (Calcd for C<sub>12</sub>H<sub>10</sub>O<sub>4</sub>, 227.1283).

**Synthesis of 1-(4-Isopropoxy-2,3,6-trimethoxyphenyl)ethanone (11) and 1-(2-Isopropoxy-3,4,6-trimethoxyphenyl)ethanone (12)** To a solution of **10** (50 mg, 0.22 mmol) in CH<sub>2</sub>Cl<sub>2</sub> at 0°C, tin chloride (52  $\mu$ L, 0.44 mmol) and acetyl chloride (24  $\mu$ L, 0.34 mmol) were added slowly. The resulting mixture was stirred for 4–6 h at room temperature and then the CH<sub>2</sub>Cl<sub>2</sub> was removed under reduced pressure to obtain the crude oils. With the assistance of silica gel column chromatography eluted by *n*-hexane–EtOAc (4:1), it could afford the product **12** (26 mg, 34%) and its regioisomeric product **11** (20 mg, 43%).

**11:** Yellow liquid; UV (MeOH)  $\lambda_{\max}$ : 304 (sh), 266, 226 (sh), 208 nm; IR (neat)  $\nu_{\max}$ : 2978, 2940, 1705, 1597, 1489, 1400, 1261, 1196, 1115, 1004 cm<sup>-1</sup>; <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 6.24 (1H, s, H-5'), 4.57 (1H, sept,  $J=6.2$  Hz, CH-1''), 3.87 (3H, s, OCH<sub>3</sub>-6'), 3.77 (3H, s, OCH<sub>3</sub>-3'), 3.77 (3H, s, OCH<sub>3</sub>-2'), 2.46 (3H, s, COCH<sub>3</sub>), 1.21 (6H, d,  $J=6.2$  Hz, CH<sub>3</sub>-2''-3''); <sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$ : 201.2, 153.1, 152.4, 151.3, 137.4, 118.7, 95.6, 76.7, 61.9, 60.8, 56.0, 32.5, 22.1; ESI-MS  $m/z$  (rel. int. %): 269 ([M+H]<sup>+</sup>, 100); HR-ESI-MS  $m/z$  269.1390 [M+H]<sup>+</sup> (Calcd for C<sub>14</sub>H<sub>21</sub>O<sub>5</sub>, 269.1389).

**12:** Pale yellow powder; UV (MeOH)  $\lambda_{\max}$ : 308 (sh), 266, 224 (sh), 208 nm; IR (neat)  $\nu_{\max}$ : 2978, 2936, 1705, 1597, 1493, 1412, 1350, 1261, 1203, 1107, 1026 cm<sup>-1</sup>; <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 6.25 (1H, s, H-5'), 4.56 (1H, sept,  $J=6.3$  Hz, CH-1''), 3.87 (3H, s, OCH<sub>3</sub>-6'), 3.79 (3H, s, OCH<sub>3</sub>-4'), 3.75 (3H, s, OCH<sub>3</sub>-3'), 2.47 (3H, s, COCH<sub>3</sub>), 1.37 (6H, d,  $J=6.3$  Hz, CH<sub>3</sub>-2''-3''); <sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$ : 201.6, 154.6, 152.4, 148.8, 136.2, 119.5, 92.0, 75.9, 60.7, 56.0, 55.9, 32.6, 22.4; ESI-MS  $m/z$  (rel. int. %): 269 ([M+H]<sup>+</sup>, 100); HR-ESI-MS  $m/z$  269.1391 [M+H]<sup>+</sup> (Calcd for C<sub>14</sub>H<sub>21</sub>O<sub>5</sub>, 269.1389).

**Synthesis of 4-Isopropyl-3-methoxybenzaldehyde (13)** A mixture of **4** (5.0 g, 32.87 mmol), 2-bromopropane (6.2 mL, 66.0 mmol), and potassium carbonate (9.1 g, 66.0 mmol) in DMF (100 mL) was stirred at 60°C and the reaction mixture was checked by TLC. After reaction completed, the mixture was diluted with water (300 mL) and then extracted by EtOAc (150 mL $\times$ 3). The combined organic phases were washed with water and brine, and further dried over anhydrous MgSO<sub>4</sub>. The crude was further purified by column chromatography over silica gel using *n*-hexane–EtOAc (7:1) to obtain **13** (6.3 g, 99%). **13:** colorless liquid; UV (MeOH)  $\lambda_{\max}$ : 310, 292, 248, 206 nm; IR (neat)  $\nu_{\max}$ : 2978, 2936, 2832, 2728, 1682, 1585, 1504, 1269, 1124 cm<sup>-1</sup>; <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 9.83 (1H, s, CHO), 7.42 (1H, d,  $J=8.0$  Hz, H-6), 7.40 (1H, s, H-2), 6.97 (1H, d,  $J=8.0$  Hz, H-5), 4.68 (1H, sept,  $J=6.1$  Hz, CH-1'), 3.90 (3H, s, OCH<sub>3</sub>-3), 1.42 (6H, d,  $J=6.1$  Hz, CH<sub>3</sub>-2', -3'); <sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$ : 190.8, 153.1, 150.3, 129.7, 126.6, 112.9, 109.6, 71.3, 56.0, 21.8; ESI-MS  $m/z$  (rel. int. %): 217 ([M+Na]<sup>+</sup>, 100); HR-ESI-MS  $m/z$  217.0840 [M+Na]<sup>+</sup> (Calcd for C<sub>11</sub>H<sub>14</sub>O<sub>3</sub>Na, 217.0841).

**Synthesis of (E)-1-(4-Isopropoxy-2,3,6-trimethoxyphenyl)-3-(4-isopropoxy-3-methoxyphenyl)prop-2-en-1-one (14)** A 1 N NaOH methanol solution (100 mL) of **11** (1.8 g, 6.6 mmol) and **13** (2.5 g, 12.9 mmol) was reacted at room tem-

perature for 12 h. The methanol was removed under reduced pressure to obtain the crude oils and then extracted by EtOAc. The combined organic phases were washed with water and brine, and further dried over anhydrous MgSO<sub>4</sub>. The crude was further purified by column chromatography over silica gel using *n*-hexane–EtOAc (3:1) to obtain **14** (2.8 g, 97%). **14:** yellow syrup; UV (MeOH)  $\lambda_{\max}$ : 302 (sh), 254 (sh), 238 (sh), 208 nm; IR (neat)  $\nu_{\max}$ : 2974, 2936, 1639, 1593, 1512, 1261, 1111 cm<sup>-1</sup>; <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 7.30 (1H, d,  $J=15.8$  Hz, CH-2), 7.08 (1H, s, H-2''), 7.07 (1H, d,  $J=8.9$  Hz, H-6''), 6.87 (1H, d,  $J=15.8$  Hz, CH-3), 6.87 (1H, d,  $J=8.9$  Hz, H-5''), 6.35 (1H, s, H-5'), 4.61 (1H, sept,  $J=6.0$  Hz, CH-1'''), 4.60 (1H, sept,  $J=6.0$  Hz, CH-1''), 3.87 (3H, s, OCH<sub>3</sub>-6'), 3.86 (3H, s, OCH<sub>3</sub>-2'), 3.83 (3H, s, OCH<sub>3</sub>-3'), 3.71 (3H, s, OCH<sub>3</sub>-3'), 1.41 (6H, d,  $J=6.0$  Hz, CH<sub>3</sub>-2''''-3''''), 1.37 (6H, d,  $J=6.0$  Hz, CH<sub>3</sub>-2''''-3'''''); <sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$ : 193.9, 153.0, 152.8, 151.7, 150.2, 149.7, 145.2, 137.3, 127.6, 126.8, 123.0, 116.9, 114.5, 110.7, 95.8, 71.6, 71.2, 61.7, 60.8, 56.2, 55.9, 22.1, 21.9; ESI-MS  $m/z$  (rel. int. %): 445 ([M+H]<sup>+</sup>, 100); HR-ESI-MS  $m/z$  445.2228 [M+H]<sup>+</sup> (Calcd for C<sub>25</sub>H<sub>33</sub>O<sub>7</sub>, 445.2226).

**Synthesis of 1-Isopropoxy-4-(3-(4-isopropoxy-3-methoxyphenyl)propyl)-2,3,5-trimethoxybenzene (15)** To a solution of **14** (1.9 g, 4.3 mmol) in trifluoroacetic acid at room temperature, the triethylsilane (5.5 mL, 34.0 mmol) was added dropwise. The resulting reaction mixture was stirred at room temperature for 4 h and then poured into water (100 mL). It was extracted by EtOAc (100 mL $\times$ 3) and the combined organic phases were washed with water and brine, and further dried over anhydrous MgSO<sub>4</sub>. The crude was further purified by column chromatography over silica gel using *n*-hexane–EtOAc (5:1) to obtain **15** (1.5 g, 82%). **15:** colorless liquid; UV (MeOH)  $\lambda_{\max}$ : 280, 232 (sh), 206 nm; IR (neat)  $\nu_{\max}$ : 2955, 1593, 1485, 1246, 1118, 1030 cm<sup>-1</sup>; <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 6.82 (1H, d,  $J=8.1$  Hz, H-6''), 6.76 (1H, s, H-2''), 6.73 (1H, d,  $J=8.1$  Hz, H-5''), 6.27 (1H, s, H-6'), 4.51 (1H, sept,  $J=5.8$  Hz, CH-1'''), 4.45 (1H, sept,  $J=5.7$  Hz, CH-1'''), 3.84 (3H, s, OCH<sub>3</sub>-3''), 3.84 (3H, s, OCH<sub>3</sub>-5'), 3.81 (3H, s, OCH<sub>3</sub>-3'), 3.75 (3H, s, OCH<sub>3</sub>-2'), 2.62 (4H, br t,  $J=7.8$  Hz, CH<sub>2</sub>-1, CH<sub>2</sub>-3), 1.80 (2H, br t,  $J=7.8$  Hz, CH<sub>2</sub>-2), 1.36 (6H, d,  $J=5.8$  Hz, CH<sub>3</sub>-2''''-3''''), 1.34 (6H, d,  $J=5.7$  Hz, CH<sub>3</sub>-2''''-3'''''); <sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$ : 153.6, 152.5, 150.3, 149.6, 145.1, 140.0, 136.3, 120.3, 117.5, 116.4, 112.6, 96.6, 71.8, 71.6, 61.1, 60.7, 55.9, 55.8, 35.7, 31.7, 23.4, 22.3, 22.1; ESI-MS  $m/z$  (rel. int. %): 445 ([M+Na]<sup>+</sup>, 100); HR-ESI-MS  $m/z$  445.2410 [M+Na]<sup>+</sup> (Calcd for C<sub>25</sub>H<sub>36</sub>O<sub>6</sub>Na, 445.2409).

**Synthesis of Viscolin (1)** To a solution of **15** (100.0 mg, 0.23 mmol) in CH<sub>2</sub>Cl<sub>2</sub> at 0°C, boron trichloride (0.12 mL, 1.4 mmol) was added slowly and the reaction mixture was stirred further for 10 min at room temperature. The reaction was quenched by cold water and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The CH<sub>2</sub>Cl<sub>2</sub> extracts were combined and the organic phases were washed with water and brine, and further dried over anhydrous MgSO<sub>4</sub>. The crude was further purified by column chromatography over silica gel using *n*-hexane–EtOAc (5:1) to afford colorless powder **1** (71 mg, 88%). **1:** UV (MeOH)  $\lambda_{\max}$ : 280, 228 (sh), 206 nm; IR (neat)  $\nu_{\max}$ : 2936, 1600, 1512, 1462, 1427, 1269 cm<sup>-1</sup>; <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 6.83 (1H, d,  $J=6.7$  Hz, H-6''), 6.72 (1H, s, H-2''), 6.71 (1H, d,  $J=6.7$  Hz, H-5''), 6.31 (1H, s, H-6'), 5.72 (1H, s, OH-4'', D<sub>2</sub>O exchangeable), 5.51 (1H, s, OH-1', D<sub>2</sub>O exchangeable), 3.87

(3H, s, OCH<sub>3</sub>-5'), 3.85 (3H, s, OCH<sub>3</sub>-3''), 3.82 (3H, s, OCH<sub>3</sub>-3'), 3.74 (3H, s, OCH<sub>3</sub>-2''), 2.59 (4H, brt, *J*=7.9Hz, CH<sub>2</sub>-1, CH<sub>2</sub>-3), 1.77 (2H, brt, *J*=7.9Hz, CH<sub>2</sub>-2); <sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>) δ: 154.3, 151.3, 147.4, 146.2, 143.4, 134.9, 133.5, 120.9, 116.1, 114.1, 111.1, 94.3, 60.9, 60.6, 55.9, 55.7, 35.7, 31.9, 23.2; ESI-MS *m/z* (rel. int. %): 371 ([M+Na]<sup>+</sup>, 100); HR-ESI-MS *m/z* 371.1468 [M+Na]<sup>+</sup> (Calcd for C<sub>19</sub>H<sub>24</sub>O<sub>6</sub>Na, 371.1471).

**Measurement of NO Production** NO production was indirectly assessed by measuring the nitrite levels in the cultured media and serum determined by a colorimetric method based on the Griess reaction.<sup>11)</sup>

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