

Syntheses of Resveratrol and its Hydroxylated Derivatives as Radical Scavenger and Tyrosinase Inhibitor

Hyun Suck Lee,[†] Byung Won Lee,[‡] Mi Ran Kim,[†] and Jong-Gab Jun^{†,‡,*}

[†]Department of Chemistry and Institute of Applied Chemistry, [‡]Institute of Natural Medicine, Hallym University, Chuncheon 200-702, Korea. *E-mail: jgjun@hallym.ac.kr

Received January 12, 2010, Accepted March 3, 2010

Eight hydroxylated stilbene derivatives including resveratrol, desoxyrhapontigenin and piceatannol as potential radical scavenger and tyrosinase inhibitor are synthesized using optimized Wittig-Horner reaction for excellent trans-selectivity in good yields. Antioxidant activity was tested against ABTS radical and tyrosinase inhibitory activity was performed with L-tyrosine as the substrate based on previous procedure with some modification. In general, catecholic stilbenes showed stronger activity against ABTS radical and resorcinolic moiety showed stronger tyrosinase inhibitory activity. Synthetic piceatannol which containing both catecholic and resorcinolic moieties showed the strongest activity in both as ABTS radical scavenger and tyrosinase inhibitor with IC₅₀ values of 4.1 and 8.6 μM, respectively.

Key Words: Stilbene, Resveratrol, Piceatannol, ABTS radical, Tyrosinase inhibitor

Introduction

Among of the natural polyphenols, resveratrol **1** is one of the most famous antioxidant found in grapes and red wines (Fig. 1).¹ Resveratrol, with hydroxy substituted *trans*-stilbene structure, have multiple beneficial effects on human health such as antioxidant, anti-inflammatory and anti-cancer activities.² It has also been revealed that the compound has potent inhibitory effects on cyclooxygenase,² human F1 ATPase,³ and tyrosinase.⁴ Tyrosinase, metalloenzyme containing copper, catalyzes two distinct reactions of melanin synthesis through oxidation of copper, the hydroxylation of a monophenol and the conversion of an *o*-diphenol to the corresponding *o*-quinone.⁵ This enzyme is responsible for browning of fruits and coloring of skin in animals including human being.⁶ Inhibitors of tyrosinase is important molecular tools for food quality and skin whitening in human. The strong tyrosinase inhibitor, such as piceatannol, has potential applications as whitening agent in cosmetic preparations or anti-browning agent for food products. Since resveratrol has a simple structure, the compound is an attractive target of chemical studies in view of structure-activity relationships. In this report, we describe to synthesize of resveratrol and its hydroxylated derivatives including desoxyrhapontigenin **3** and piceatannol **4** with their antioxidant and anti-tyrosinase activities.

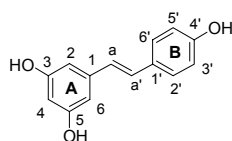


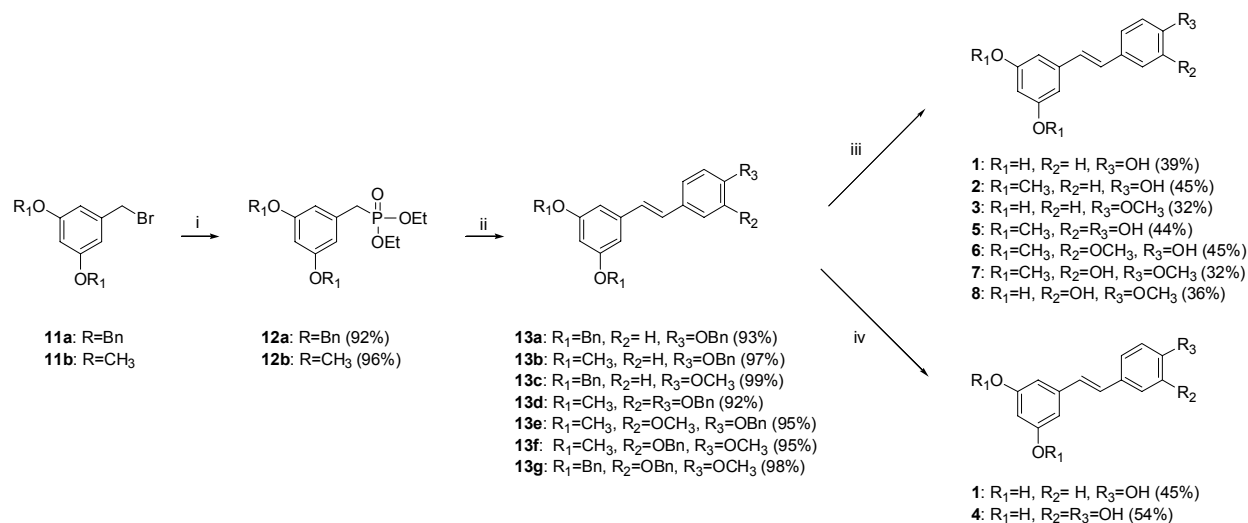
Figure 1. Structural formula of resveratrol, *trans*-3,4',5-trihydroxy-stilbene (**1**).

Results and Discussion

Among the various methods to make unsymmetrical stilbenes,⁷ we had used the standard chemical methodologies for synthesis of the hydroxylated *trans*-stilbene derivatives as shown in Scheme 1. The corresponding benzylphosphonates **12** were obtained from benzylbromides **11** via Michaelis-Arbuzov reaction with triethyl phosphite in xylene at reflux. The following Wittig-Horner reactions were carried out at reflux with the phosphonates, the corresponding benzaldehyde and sodium hydride in THF to yield the *trans*-stilbenes **13** in excellent geometrical selectivity over the *cis*-isomers as discussed in previous result.⁸ Selective deprotection of benzyl group over methyl group of phenolic ethers **13** was successfully established with 1 equivalent boron tribromide per benzyloxy group and ascorbic acid (0.2 equiv.) at -78 °C (route iii). Both deprotection of benzyl and methyl groups was performed with 3 equivalents boron tribromide per methoxy group, 1 equivalent boron tribromide per benzyloxy group and ascorbic acid (0.2 equiv.) at -20 °C (route iv). Polymerization was minimized with the use of ascorbic acid in this reaction. Melting points and NMR data of compounds **1**, **3** and **4** were in agreement with literature data.⁹

Antioxidant activity of all synthetic stilbenes **1-8** was tested against ABTS radical. The formation of ABTS radical cation takes place almost instantaneously after addition of potassium persulfate to an ABTS solution. The scavenging ability of the hydroxylated stilbenes against ABTS radicals was concentration dependent as shown in Fig. 2.

Among the hydroxylated stilbenes, compounds **4** and **5** containing catechol moiety in B-ring had high free radical scavenging activity showing ABTS IC₅₀ values of 4.1 and 10.3 μM, respectively. Remaining stilbenes which had resorcinol moiety (**1-3**) and/or destroyed catechol moiety (**6-8**) had lower ABTS radical scavenging activity than catecholic stilbenes (**4** and **5**) (Table 1). Fortunately, almost all synthetic stilbenes **1-8** showed



Scheme 1. Reagents and conditions: (i) P(OEt)₃, xylene, reflux, 10 hr; (ii) NaH, appropriate benzaldehyde derivatives, THF, reflux, 1 hr; (iii) BBr₃ (1 eq/OBn), ascorbic acid (0.2 eq), CH₂Cl₂, -78 °C, 3 hr; (iv) BBr₃ (3 eq/OMe + 1 eq/OBn), ascorbic acid (0.2 eq), CH₂Cl₂, -20 °C, 3 hr

Table 1. Radical-scavenging and tyrosinase inhibitory activities of **1-8**

Compound	ABTS		Tyrosinase	
	IC ₅₀ (μM)	% inhibition (at 100 μM)	IC ₅₀ (μM)	
<i>trans</i> -3,4',5-Trihydroxystilbene (1)	13.4 ± 0.8 (1.5) ^a	69.4 ± 1.4	61.3	
<i>trans</i> -4'-Hydroxy -3,5-dimethoxystilbene (2)	19.0 ± 1.6 (1.0)	30.2 ± 1.7	> 100	
<i>trans</i> -3,5-Dihydroxy-4'-methoxystilbene (3)	15.5 ± 0.7 (1.3)	96.8 ± 2.3	7.3	
<i>trans</i> -3, 3', 4', 5 -Tetrahydroxystilbene (4)	4.1 ± 1.1 (4.8)	95.7 ± 1.4	8.6	
<i>trans</i> -3',4'-Dihydroxy -3,5-dimethoxystilbene (5)	10.3 ± 0.7 (1.9)	40.6 ± 0.8	> 100	
<i>trans</i> -4'-Hydroxy -3, 3',5-trimethoxystilbene (6)	17.4 ± 0.9 (1.1)	14.5 ± 2.5	> 100	
<i>trans</i> -3'-Hydroxy -3, 4',5-trimethoxystilbene (7)	12.9 ± 0.7 (1.5)	12.7 ± 3.2	> 100	
<i>trans</i> -3,3',5-Trihydroxy-4'-methoxystilbene (8)	15.7 ± 1.1 (1.2)	78.5 ± 1.5	52.1	
Trolox	19.5 ± 1.2 (1.0)		NT ^b	
Kojic Acid	NT		33.5	

Each volume is the mean ± SD for *n* = 2. ^aThe relative value to that of Trolox is shown in parentheses. ^bNT; not tested.

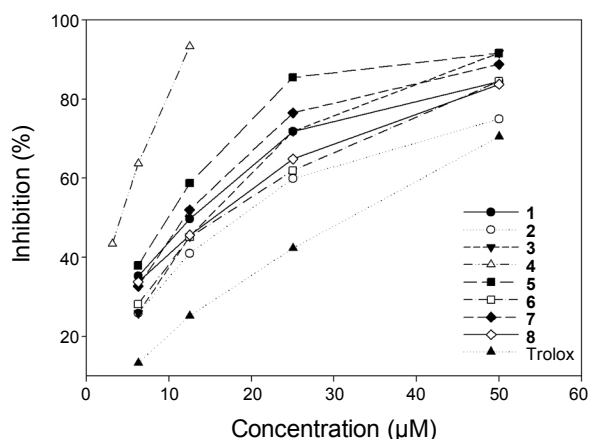


Figure 2. ABTS radical-scavenging activity of synthetic hydroxylated stilbenes **1-8** and Trolox.

stronger ABTS-radical scavenging activities than the control compound, Trolox. The relative ABTS IC₅₀ values over Trolox are shown in parentheses. It is noteworthy that the picetannol **4** showed the strongest antioxidant activity and had 4.8 times

stronger activity than Trolox as ABTS radical scavenger.

For the evaluation of tyrosinase inhibitory activity, assays were performed with L-tyrosine as the substrate based on previous procedure with some modification.¹⁰ Table 1 summarizes the percentages of inhibition and the IC₅₀ values of **1-8** compared with kojic acid as positive control.

The synthetic resveratrol **1**, *trans*-3,4',5-trihydroxystilbene, showed IC₅₀ value of 61.3 μM. As compared to this, the reference compound, kojic acid, showed IC₅₀ value of 33.5 μM. Compounds **3**, **4** and **8** having resorcinol-like structure on A-ring showed strong tyrosinase inhibitory activity with IC₅₀ values of 7.3, 8.6 and 52.1 μM, respectively. However, the *O*-dimethylated products on A ring (**2**, **5-7**) showed significantly decrease of inhibitory activity (IC₅₀ > 100 μM). The loss of activity in **2** and **5-7** was caused by the disappearance of the resorcinol-like structure on A-ring.¹¹

In conclusion, we synthesized eight hydroxylated stilbene derivatives as potential radical scavenger and tyrosinase inhibitor. All compounds having free phenol groups showed good free radical scavenging activity. Especially, catecholic stilbenes **4** and **5** had the strongest activity against ABTS radical. But,

compounds **1**, **3** and **8** with resorcinol moiety showed stronger tyrosinase inhibitory activity than catecholic stilbene **5** which has no resorcinol moiety. However, piceatannol **4**, which contains resorcinol moiety on A-ring and catechol moiety on B-ring, showed the strongest activities in both as radical scavenger and tyrosinase inhibitor with IC₅₀ values of 4.1 and 8.6 μM, respectively.

Experimental

All chemicals used were purchased from commercial sources and used as received unless otherwise stated. NMR spectra were recorded at Varian Mercury TM300 MHz FT-NMR for ¹H and 75 MHz for ¹³C, with the chemical shifts (δ) reported in parts per million (ppm) relative to TMS and the coupling constants (*J*) quoted in Hz. CDCl₃ was used as a solvent and an internal standard. Flash chromatography was carried out using silica gel Merck 60 (230 - 400 mesh). Thin-layer chromatography (TLC) was performed on DC-Plastikfolien 60, F₂₅₄ (Merck, layer thickness 0.2 mm) plastic-backed silica gel plates with visualization by UV light (254 nm) or by treatment with *p*-anisaldehyde. Melting points were measured on a MEL-TEMP II apparatus and were uncorrected.

General procedure for synthesis of diethyl benzylphosphonates **12a-b**.

Diethyl (3,5-dibenzoyloxybenzyl)phosphonate (12a): The corresponding benzylbromide (0.67 g, 1.75 mmol) was heated in dried xylene (10 mL) with triethyl phosphite (0.54 mL, 3.10 mmol) at reflux under N₂ gas. After 10 hr, the mixture was cooled to room temperature and evaporated of solvent. The residue was chromatographed on silica gel to give colorless oil (0.71 g, 92%); ¹H NMR (300 MHz, CDCl₃) δ 1.24 (6H, t, *J* = 7.2 Hz, CH₃), 3.08 (2H, d, *J* = 21.6 Hz, CH₂), 3.97 (4H, q, *J* = 7.4 Hz), 5.01 (4H, s, benzyl CH₂), 6.51 (1H, d, *J* = 2.1 Hz, C4-H), 6.55 (2H, d, *J* = 2.4 Hz, C2, C2'-H), 7.34 (10H, m, benzyl); ¹³C NMR (75 MHz, CDCl₃) δ 16.83 (CH₃), 34.38 (CH₂), 62.48 (CH₂), 70.28 (benzyl CH₂), 101.14 (C4), 109.21 (C2, C2'), 127.70 (benzyl), 128.16 (benzyl), 128.76 (benzyl), 133.90 (C1), 137.00 (benzyl), 160.01 (C3, C3').

Diethyl (3,5-dimethoxybenzyl)phosphonate (12b): (96%); ¹H NMR (300 MHz, CDCl₃) δ 3.08 (2H, d, *J* = 21.6 Hz, CH₂), 3.75 (6H, s, OCH₃), 4.02 (4H, t, *J* = 6 Hz, CH₂), 6.33 (1H, s, C4-H), 6.44 (2H, s, C2, C6-H); ¹³C NMR (75 MHz, CDCl₃) δ 16.63 (CH₃), 33.28 (CH₂), 55.44 (OCH₃), 62.28 (CH₂), 62.37 (CH₂), 99.18 (C4), 107.88 (C2), 107.97 (C6), 133.65 (C1), 160.73 (C3), 160.77 (C5).

General procedure for synthesis of compounds **13a-g**.

(E)-3,4',5-Tribenzoyloxy stilbene (13a): To a solution of NaH (0.22 g, 5.45 mmol) in THF (20 mL) was added 3,5-dibenzoyloxybenzyl diethyl phosphonate (**12a**; 0.40 g, 0.91 mmol) under N₂ gas. After 5 min, 4-henzyloxybenzaldehyde (0.25 g, 1.18 mmol) was added. The mixture was refluxed for 1 hr, and then cooled to room temperature. The solution was poured into 4 mL saturated aqueous NaHCO₃ and extracted with CH₂Cl₂ (30 mL × 3). After concentration of combined extracts, the residue was chromatographed on silica gel to give white solid (0.42 g, 93%); ¹H NMR (300 MHz, CDCl₃) δ 5.06 (6H, d, *J* = 4.8 Hz, CH₂), 6.51 (1H, t, *J* = 2.4 Hz, C4-H), 6.73 (2H, d, *J* = 2.4 Hz,

C2, 4-H), 6.87 (2H, d, *J* = 16.2 Hz, olefin H), 7.00 (1H, d, *J* = 15.9 Hz, olefin H), 7.12 (2H, m), 7.36 (16H, m); ¹³C NMR (75 MHz, CDCl₃) δ 70.26 (benzyl CH₂), 70.33 (benzyl CH₂), 101.34 (C4), 105.71 (C2, 6), 115.20 (C3', 5'), 126.37, 126.70, 127.60, 127.69, 127.94, 128.13 (olefinic C), 128.73 (olefinic C), 130.24 (C2', 6'), 132.11 (C1'), 136.98 (benzyl), 139.81 (C1), 158.64 (C4'), 160.19 (C3, C5).

(E)-4'-Benzyloxy-3,5-dimethoxystilbene (13b): Yield: (97 %); ¹H NMR (300 MHz, CDCl₃) δ 3.80 (6H, s, OCH₃), 5.05 (2H, s, CH₂), 6.35 (1H, t, *J* = 2.4 Hz, C4-H), 6.62 (2H, d, *J* = 2.1 Hz, C2, C6-H), 6.88 (1H, d, *J* = 16.2 Hz, Olefin H), 6.94 (2H, d, *J* = 9 Hz, C3', C5'-H), 7.01 (1H, d, *J* = 16.5 Hz, olefin H), 7.37 (7H, m); ¹³C NMR (75 MHz, CDCl₃) δ 55.68 (OCH₃), 70.33 (benzyl CH₂), 99.87 (C4), 104.57 (C2, C6), 115.29 (C3', C5'), 126.89, 127.69 (olefinic C), 128.02, 128.21 (olefinic C), 128.81, 128.89 (C2', C6'), 130.35 (C1'), 137.05, 139.83 (C1), 158.72 (C4), 161.09 (C3, C5).

(E)-3,5-Dibenzoyloxy-4'-methoxystilbene (13c): Yield: (99 %); ¹H NMR (300 MHz, CDCl₃) δ 3.71 (3H, s, OCH₃), 4.98 (4H, s, CH₂), 6.49 (1H, t, *J* = 2.4 Hz, C4-H), 6.17 (2H, d, *J* = 2.4 Hz, C2, C6-H), 6.83 (2H, d, *J* = 8.7, C3', C5'-H), 6.83 (1H, d, *J* = 15.9 Hz, olefin H), 6.98 (1H, d, *J* = 16.2 Hz, olefin H), 7.33 (12H, m); ¹³C NMR (75 MHz, CDCl₃) δ 55.68 (OCH₃), 70.43 (benzyl CH₂), 101.47 (C3), 105.85 (C2, C6), 114.46 (C3', C5'), 127.91, 128.16 (olefinic C), 128.32 (olefinic C), 128.92, 129.12 (C1'), 130.13 (C2', C6'), 137.19, 140.02 (C1), 159.62 (C4'), 160.37 (C3, C5).

(E)-3'4'-Dibenzoyloxy-3,5-dimethoxystilbene (13d): Yield: (92%); ¹H NMR (300 MHz, CDCl₃) δ 3.74 (6H, s, OCH₃), 5.10 (2H, s, CH₂), 5.19 (2H, s, CH₂), 6.34 (1H, d, *J* = 0.6 Hz, C4-H), 6.60 (2H, s, C2, C6-H), 6.80 (1H, d, *J* = 17.1 Hz, olefin H), 6.89 (1H, d, *J* = 15.6 Hz, olefin H), 6.97 (1H, t, *J* = 4.5 Hz, C5'-H), 7.35 (12H, m); ¹³C NMR (75 MHz, CDCl₃) δ 55.72 (OCH₃), 71.45 (benzyl CH₂), 71.67 (benzyl CH₂), 99.97 (C4), 104.67 (C2, C6), 113.02 (C2'), 115.04 (C4'), 120.83 (C6'), 127.56, 127.67, 128.11 (olefinic C), 128.14 (olefinic C), 128.79, 128.81, 129.09, 131.09 (C1'), 137.42, 137.49, 139.75 (C1), 149.13 (C4'), 149.25 (C3'), 161.15 (C3, C5).

(E)-4'-Benzyloxy-3,3',5-trimethoxystilbene (13e): Yield: (95%); ¹H NMR (300 MHz, CDCl₃) δ 3.83 (6H, s, OCH₃), 3.96 (3H, s, OCH₃), 5.18 (2H, s, CH₂), 6.39 (1H, t, *J* = 2.1 Hz, C4-H), 6.81 (2H, d, *J* = 1.8 Hz, C2, C6-H), 6.87 (1H, d, *J* = 8.1 Hz, C5'-H), 6.89 (1H, d, *J* = 15.9 Hz, olefin H), 6.97 (1H, d, *J* = 1.5 Hz, C6'-H), 7.02 (1H, d, *J* = 16.2 Hz, olefin H), 7.08 (1H, d, *J* = 1.2 Hz, C2'-H), 7.39 (5H, m); ¹³C NMR (75 MHz, CDCl₃) δ 55.67 (OCH₃), 56.35 (OCH₃), 71.33 (benzyl CH₂), 99.98 (C4), 104.62 (C2, C6), 109.73 (C2'), 114.25 (C5'), 120.08 (C6'), 127.16, 127.47, 128.06 (olefinic C), 128.74, 129.15 (olefinic C), 130.95 (C1'), 137.22, 139.72 (C1), 148.34 (C4'), 149.96 (C3'), 161.11 (C3, C5).

(E)-3'-Benzyloxy-3,4',5-trimethoxystilbene (13f): Yield: (95%); ¹H NMR (300 MHz, CDCl₃) δ 3.79 (6H, s, OCH₃), 3.87 (3H, s, OCH₃), 5.17 (2H, s, CH₂), 6.35 (1H, t, *J* = 2.4 Hz, C4-H), 6.61 (2H, d, *J* = 2.1 Hz, C2, C6-H), 6.78 (1H, d, *J* = 16.5 Hz, olefin H), 6.86 (1H, s, C5'-H), 6.96 (1H, d, *J* = 16.2 Hz, olefin H), 7.02 (1H, d, *J* = 2.1 Hz, C6'-H), 7.07 (1H, d, *J* = 1.5 Hz, C2'-H), 7.37 (5H, m); ¹³C NMR (75 MHz, CDCl₃) δ 55.69 (OCH₃), 56.37 (OCH₃), 71.38 (benzyl CH₂), 99.86 (C4),

104.56 (C2, C6), 111.94 (C2', C5'), 120.67 (C6'), 126.97, 127.62, 128.15 (olefinic C), 128.81, 129.10 (olefinic C), 130.33 (C1'), 137.24, 139.72 (C1), 148.45 (C4'), 149.83 (C3'), 161.08 (C3, C5).

(E)-3,3',5-Tribenzyloxy-4'-methoxystilbene (13g): Yield: (98%); ¹H NMR (300 MHz, CDCl₃) δ 3.89 (3H, s, OCH₃), 5.05 (4H, s, CH₂), 5.18 (2H, s, CH₂), 6.51 (1H, t, *J* = 1.8 Hz, C4-H), 6.72 (2H, d, *J* = 1.8 Hz, C2, C6-H), 6.79 (1H, d, *J* = 16.2 Hz, olefin H), 6.86 (1H, d, *J* = 8.1 Hz, C5'-H), 6.95 (1H, d, *J* = 16.2 Hz, olefin H), 7.02 (1H, s, C6'-H), 7.06 (1H, s, C2'-H), 7.37 (15H, m); ¹³C NMR (75 MHz, CDCl₃) δ 55.94 (OCH₃), 69.97 (benzyl CH₂), 70.98 (benzyl CH₂), 101.00 (C4), 105.36 (C2, C6), 111.54 (C2', C5'), 120.24 (C6'), 126.45, 127.14, 127.33, 127.68 (olefinic C), 127.79 (olefinic C), 128.37, 128.76, 129.89 (C1'), 136.60, 136.79, 139.34 (C1), 148.01 (C4'), 149.42 (C3'), 159.83 (C3, C5).

General procedure for synthesis of compounds 1-3 and 5-8 (Method iii).

(E)-3,4',5-Trihydroxystilbene (1): In a dried three-necked-flask (*E*)-3,4',5-tribenzyloxystilbene (**13a**, 0.335 g, 0.672 mmol) and ascorbic acid (0.024 g, 0.134 mmol) was solved in dried CH₂Cl₂ (20 mL) under N₂ gas and cooled to -78 °C. Then boron tribromide (1.0 M in CH₂Cl₂, 2.01 mL, 3 equivalents) was slowly added *via* syringe. Solution was stirred for 1 hr at -78 °C, and then warmed to room temperature and stirred for 2 hr. The reaction was quenched by adding 2 mL water slowly. After stirring for 20 min, the solvent was evaporated and the water-phase was extracted with EtOAc (10 mL × 3). The combined organic layer were dried over anhydrous Na₂SO₄ and concentrated. The residue was chromatographed on silica gel to give pale-yellow solid (0.059 g, 39%); ¹H NMR (300 MHz, acetone-*d*₆) δ 6.25 (1H, s, C4-H), 6.46 (2H, s, C2, C6-H), 6.74 (1H, d, *J* = 16.2 Hz, olefin H), 6.80 (2H, d, *J* = 8.1 Hz, C3', C5'-H), 6.92 (1H, d, *J* = 16.2 Hz, olefin H), 7.31 (2H, d, *J* = 8.4 Hz, C2', C6'-H), 9.05 (2H, br s, OH), 9.30 (1H, br s, OH); ¹³C NMR (75 MHz, acetone-*d*₆) δ 103.22 (C4), 105.86 (C2, C6), 116.80 (C3', C5'), 126.82 (C1'), 128.75 (C2', C6'), 129.18 (olefinic C), 129.54 (olefinic C), 140.53 (C1), 158.07 (C4'), 159.35 (C3, C5).

(E)-4'-Hydroxy-3,5-dimethoxystilbene (2): Yield: (45%); ¹H NMR (300 MHz, acetone-*d*₆) δ 3.81 (6H, s, OCH₃), 5.41 (1H, br s, OH), 6.35 (1H, d, *J* = 1.8 Hz, C4-H), 6.61 (2H, d, *J* = 2.1 Hz, C3', C5'-H), 6.67 (2H, d, *J* = 8.1 Hz, C2, C6-H), 6.86 (1H, d, *J* = 16.2 Hz, olefin H), 7.00 (1H, d, *J* = 16.2 Hz, olefin H), 7.36 (2H, d, *J* = 8.1 Hz, C2', C6'-H); ¹³C NMR (75 MHz, acetone-*d*₆) δ 55.76 (OCH₃), 55.84 (OCH₃), 99.91 (C4), 104.84 (C2, C6), 115.95 (C3', C5'), 126.62 (olefinic C), 128.33 (C1'), 129.07 (olefinic C), 130.13 (C2', C6'), 139.99 (C1), 155.69 (C4'), 160.97 (C3, C5).

(E)-3,5-Dihydroxy-4'-methoxystilbene (3): Yield: (32%); ¹H NMR (300 MHz, acetone-*d*₆) δ 3.80 (3H, s, OCH₃), 6.31 (1H, t, *J* = 2.4 Hz, C4-H), 6.54 (2H, d, *J* = 3 Hz, C2, C6-H), 6.82 (1H, d, *J* = 15.9 Hz, olefin H), 6.88 (2H, s, C3', C5'-H), 6.96 (1H, d, *J* = 16.2 Hz, olefin H), 7.41 (2H, d, *J* = 8.4 Hz, C2', C6'-H), 7.94 (2H, br s, OH); ¹³C NMR (75 MHz, acetone-*d*₆): δ 55.83 (OCH₃), 102.87 (C4), 105.84 (C2, C6), 114.85 (C3', C5'), 127.37 (olefinic C), 128.50, 128.77 (olefinic C), 130.75 (C1'), 140.54 (C1), 159.22 (C3, C5), 160.08 (C4').

(E)-3'4'-Dihydroxy-3,5-dimethoxystilbene (5): Yield: (44 %); ¹H NMR (300 MHz, acetone-*d*₆) δ 3.82 (6H, s, OCH₃), 5.39 (2H, br s, OH), 6.37 (1H, d, *J* = 2.4 Hz, C4-H), 6.62 (2H, d, *J* = 1.8 Hz, C2, C6-H), 6.83 (1H, d, *J* = 8.1 Hz, C5'-H), 6.84 (1H, d, *J* = 15.9 Hz, olefin H), 6.94 (1H, d, *J* = 8.1 Hz, C6'-H), 6.95 (1H, d, *J* = 15.3 Hz, olefin H), 7.05 (1H, s, C2'-H); ¹³C NMR (75 MHz, acetone-*d*₆) δ 55.69 (OCH₃), 99.89 (C4), 104.62 (C2, C6), 113.183 (C2'), 115.70 (C5'), 120.42 (C6'), 127.08 (olefinic C), 128.89 (olefinic C), 130.92 (C1'), 139.69 (C1), 143.75 (C3'), 143.88 (C4'), 161.04 (C3, C5).

(E)-4'-Hydroxy-3,3',5-trimethoxystilbene (6): Yield: (45%); ¹H NMR (300 MHz, acetone-*d*₆) δ 3.80 (6H, s, OCH₃), 3.90 (3H, s, OCH₃), 5.78 (1H, br s, OH), 6.36 (1H, t, *J* = 2.4 Hz, C4-H), 6.63 (2H, d, *J* = 2.1 Hz, C2, C6-H), 6.86 (1H, d, *J* = 15.3 Hz, olefin H), 6.87 (1H, d, *J* = 1.5 Hz, C5'-H), 6.90 (1H, s, C6'-H), 6.99 (1H, d, *J* = 7.2 Hz, C2'-H), 7.00 (1H, d, *J* = 15.6 Hz, olefin H); ¹³C NMR (75MHz, Acetone-*d*₆) δ 56.04 (OCH₃), 56.22 (OCH₃), 99.85 (C4), 104.50 (C2, C6), 108.44 (C2'), 114.81 (C5'), 120.83 (C6'), 128.41 (olefinic C), 128.48 (olefinic C), 129.93 (C1'), 139.76 (C1), 145.84 (C4'), 146.88 (C3'), 161.06 (C3, C5).

(E)-3'-Hydroxy-3,4',5-trimethoxystilbene (7): Yield: (32%); ¹H NMR (300 MHz, acetone-*d*₆) δ 3.79 (16H, s, OCH₃), 3.83 (3H, s, OCH₃), 5.76 (1H, br s, OH), 6.36 (1H, t, *J* = 2.4 Hz, C4-H), 6.62 (2H, d, *J* = 2.4 Hz, C2, C6-H), 6.77 (1H, d, *J* = 8.4 Hz, C5'-H), 6.85 (1H, d, *J* = 15.2 Hz, olefin H), 6.92 (1H, d, *J* = 2.1 Hz, C6'-H), 6.97 (1H, d, *J* = 15.9 Hz, olefin H), 7.12 (1H, d, *J* = 1.8 Hz, C2'-H); ¹³C NMR (75 MHz, acetone-*d*₆) δ 55.71 (OCH₃), 99.91 (C4), 104.59 (C2, C6), 110.88 (C5'), 112.12 (C2'), 119.71 (C6'), 128.40 (olefinic C), 128.50 (olefinic C), 130.99 (C1'), 139.76 (C1), 145.92 (C3'), 146.74 (C4'), 11.06 (C3, C5).

(E)-3,3',5-Trihydroxy-4'-methoxystilbene (8): Yield: (36%); ¹H NMR (300 MHz, acetone-*d*₆) δ 3.90 (3H, s, OCH₃), 6.27 (1H, t, *J* = 2.1 Hz, C4-H), 6.53 (2H, d, *J* = 2.1 Hz, C2, C6-H), 6.81 (1H, d, *J* = 7.8 Hz, C5'-H), 6.94 (1H, d, *J* = 16.5 Hz, olefin H), 7.01 (1H, d, *J* = 15.9 Hz, olefin H), 7.02 (1H, d, *J* = 1.8 Hz, C6'-H), 7.21 (1H, d, *J* = 1.8 Hz, C2'-H), 7.73 (1H, br s, OH), 8.22 (2H, br s, OH); ¹³C NMR (75 MHz, acetone-*d*₆) δ 55.67 (OCH₃), 101.99 (C4), 104.98 (C2, C6), 109.44 (C2'), 115.23 (C5'), 120.52 (C6'), 126.33 (olefinic C), 128.72 (olefinic C), 129.73 (C1'), 140.14 (C1), 146.82 (C4'), 147.83 (C3'), 158.80 (C3, C5).

Synthesis of (E)-3,3',4',5-tetrahydroxystilbene (4) (Method iv). To the stilbene **13g** (0.020 g, 0.038 mmol) and ascorbic acid (0.001 g, 0.008 mmol) was solved in dried CH₂Cl₂ (2 mL) under N₂ gas and cooled to -78 °C. Then boron tribromide (1.0 M in CH₂Cl₂, 0.228 mL, 6 equivalents) was slowly added *via* syringe. Solution was stirred for 1 hr at -78 °C, and then warmed to room temperature and stirred for 2 hr. The reaction was quenched by adding 2 mL water slowly. After stirring for 20 min, the solvent was evaporated and the water-phase was extracted with EtOAc (5 mL × 3). The combined organic layer were dried over anhydrous Na₂SO₄ and concentrated. The residue was chromatographed on silica gel to give pale-yellow solids (0.008 g, 54%); ¹H NMR (300 MHz, acetone-*d*₆) δ 6.31 (1H, t, *J* = 2.1 Hz, C4-H), 6.57 (2H, d, *J* = 1.8 Hz, C3, C5-H), 6.83 (1H, d, *J* = 16.2 Hz, *J* = 8.1 Hz, olefinic H, C5'-H), 6.90 (1H, dd, *J* = 8.4 Hz, 1.8 Hz, C6'-H), 6.95 (1H, d, *J* = 16.5 Hz, olefinic H), 7.10 (1H,

d, $J=1.8$ Hz, C2'-H), 8.15 (4H, br s, OH); ^{13}C NMR (75 MHz, acetone- d_6) δ 102.12 (C4), 105.21 (C2, C6), 113.25 (C2'), 115.71 (C5'), 119.52 (C6'), 126.23 (olefinic C), 128.78 (olefinic C), 130.05 (C1'), 140.17 (C1), 145.24 (C4'), 145 (C3'), 158.64 (C3, C5).

Assay for the Trolox equivalent antioxidative capacity (TEAC). The radical cation was prepared by mixing a 7 mM ABTS $^{+\cdot}$ stock solution with 2.45 mM potassium persulfate (1/1, v/v) and leaving the mixture for 4 - 8 hr until the reaction was complete and the absorbance was stable. The photometric assay was conducted on 0.9 ml of the ABTS $^{+\cdot}$ solution and 0.1 mL of test compounds in a MeOH solution and mixed for 45 sec measurements were taken immediately at 734 nm after 1 min.¹² The TEAC value expresses the numbers of μM of Trolox having an antioxidative capacity corresponding to 1.0 μM of the test substance.

Assay for the tyrosinase inhibitory activity. A 10 μL sample was added to an assay mixture containing with 1 mM L-tyrosine solution, 50 mM phosphate buffer, pH 6.5, and 20 μL of aqueous solution of mushroom tyrosinase (1000 U) was added to a 96-well plate, in a total volume of 200 μL . The assay mixture was incubated at 25 $^{\circ}\text{C}$ for 30 min. After incubation, the optical density at 492 nm was measured (Hewlett-Packard).

Acknowledgments. This work was supported by Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0094072) and in part by Hallym University Research Fund, 2009 (HRF-2009-029).

References

- Howitz, K. T.; Bitterman, K. J.; Cohen, H. Y.; Lamming, D. W.; Lavu, S.; Wood, J. G.; Zipkin, R. E.; Chung, P.; Kisielewski, A. *Nature* **2003**, *425*, 191.
- Jang, M.; Cai, L.; Udeani, G. O.; Slowing, K. V.; Thomas, C. F.; Beecher, C. W. W.; Fong, H. H. S.; Farnsworth, N. R.; Kinghorn, A. D.; Mehta, R. C.; Moon, R. C.; Pezzuto, J. M. *Science* **1997**, *275*, 218.
- (a) Wu, C.-P.; Calcagno, A. M.; Hladky, S. B.; Ambudkar, S. V.; Barrand, M. A. *FEBS J.* **2005**, *272*, 4725. (b) Gledhill, J. R.; Montgomery, M. G.; Leslie, A. G. W.; Walker, J. E. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *272*, 4725.
- Song, S.; Lee, H.; Jin, Y.; Ha, Y. M.; Bae, S.; Chung, H. Y.; Suh, H. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 461.
- Robb, D. A. Tyrosinase In *Copper Proteins and Copper Enzymes*; Lontie, R., Ed.; CRC Press: Boca Raton, FL, 1984; Vol. 2, pp 207-240.
- Kim, Y. M.; Yun, J.; Lee, C. K.; Lee, H.; Min, K. R.; Kim, Y. J. *Biol. Chem.* **2002**, *277*, 16340.
- (a) Meier, H.; Dullweber, U. *Tetrahedron Lett.* **1996**, *37*, 1191. (b) Kim, S.; Ko, H.; Park, J. E.; Jung, S.; Lee, S. K.; Chun, Y.-J. *J. Med. Chem.* **2002**, *45*, 160. (c) Guiso, M.; Marra, C.; Farina, A. *Tetrahedron Lett.* **2002**, *43*, 597. (d) Murias, M.; Handler, N.; Erker, T.; Pleban, K.; Ecker, G.; Saiko, P.; Szekeres, T.; Jäger, W. *Bioorg. Med. Chem.* **2004**, *12*, 5571.
- Han, S. Y.; Lee, H. S.; Choi, D. H.; Hwang, J. W.; Yang, D. M.; Jun, J.-G. *Synth. Commun.* **2009**, *39*, 1425.
- (a) Guiso, M.; Marra, C.; Farina, A. *Tetrahedron Lett.* **2002**, *43*, 597. (b) Choi, S. Z.; Jang, K. U.; Chung, S. H.; Park, S. H.; Kang, H. C.; Yang, E. Y.; Cho, H. J.; Lee, K. R. *Arch Pharm. Res.* **2005**, *28*, 1027. (c) Ko, S. K.; Lee, S. M.; Whang, W. K. *Arch Pharm. Res.* **1999**, *22*, 401.
- Nerya, O.; Musa, R.; Khatib, S.; Tamir, S.; Vaya, J. *Phytochemistry* **2004**, *65*, 1389.
- (a) Likhitwitayawuid, K.; Sritularak, B. *J. Nat. Prod.* **2001**, *64*, 1457. (b) Shimizu, K.; Kondo, R.; Sakai, K. *Planta Med.* **2000**, *66*, 11.
- Lee, B. W.; Lee, J. H.; Gal, S. W.; Moon, Y. H.; Park, K. H. *Biosci. Biotechnol. Biochem.* **2006**, *70*, 427.