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Short communication

Synthesis of dihydroresveratrol glycosides and evaluation of their activity against melanogenesis in B16F0 melanoma cells

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ABSTRACT

Dihydroresveratrol glucoside **1** isolated from *Camellia oleifera* and its xyloside derivative **2** were synthesized for the first time in 5 steps from TBS-protected aldehyde **4**. Natural product **1** is a potent melanogenesis inhibitor in B16F0 melanoma cells (approximately 40 fold more potent than kojic acid). In contrast, the synthetic product **2** stimulates melanogenesis, suggesting that a single hydroxymethyl group in the glycoside substituent of dihydroresveratrols is responsible for inhibition or activation of melanogenesis.

1. Introduction

The abnormal formation of the melanin pigment causes serious dermal problems, including melasma, solar lentigines, and ephelides [1–3]. Initial steps in melanin biosynthesis include two reactions, which are catalyzed by the cupper-containing oxidoreductase tyrosinase (EC 1.14.18.1): hydroxylation of L-tyrosine and oxidation of L-DOPA [4]. Thus, a number of tyrosinase inhibitors have been used for the regulation of melanogenesis [5–7]. In addition, melanin accumulation and related phenomena can be correlated with Parkinson's disease and melanoma development [8,9]. Accordingly, melanogenesis regulating agents possessing novel scaffolds are required for chemical-based functional research for brain and malignant cancer.

In our continuing efforts towards the identification of tyrosinase inhibitors, we have now focused our attention on dihydroresveratrol glucoside **1** isolated from *Camellia oleifera* (Theaceae) [10]. This glucoside has the resorcinol moiety that is frequently found in the structure of potent tyrosinase inhibitors such as 4-hexylresorcinol and cardol [7,11]. Despite containing the pharmacologically active resorcinol motif, there has been no report on the total synthesis of **1** or its development towards the identification of novel anti-melanogenesis agent. This study describes the chemical synthesis of **1**–**3**, along with the evaluation of their activity against melanogenesis observed in B16F0 melanoma cell culture (Fig. 1).

2. Results and discussion

2.1. Synthesis of *1* and *2*

Steps for the chemical synthesis of natural product **1** are illustrated in Scheme 1. TBS-protected aldehyde **4** [12] was reacted with the ylide generated from phosphonium salt **5** [13] in the presence of lithium hexamethyldisilazide (LiHMDS) to obtain stilbene **6** in excellent yield [14]. Analysis by ^{1}H NMR indicates that the ratio of *cis* and *trans* stilbenes is 3:2. Hydrogenation of **6** using Pearlman's catalyst [15] furnished phenol **7** in 80% yield. Although this synthetic scheme is similar to that reported previously [16,17], TBS protection of the starting material led to an improvement in the synthetic route and afforded high yields of the product. Glycosylation of **7** using the glucosyl imidate **8** as the glucosyl donor and trifluoromethanesulfonate (TMSOTf) as the Lewis acid at low

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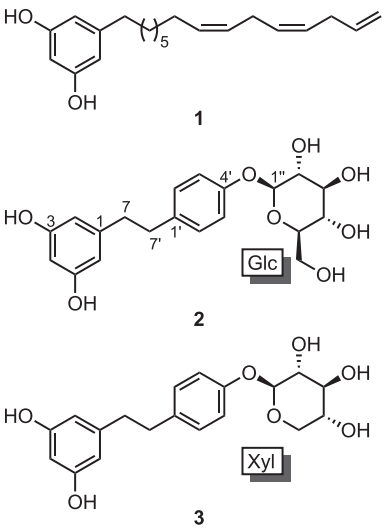
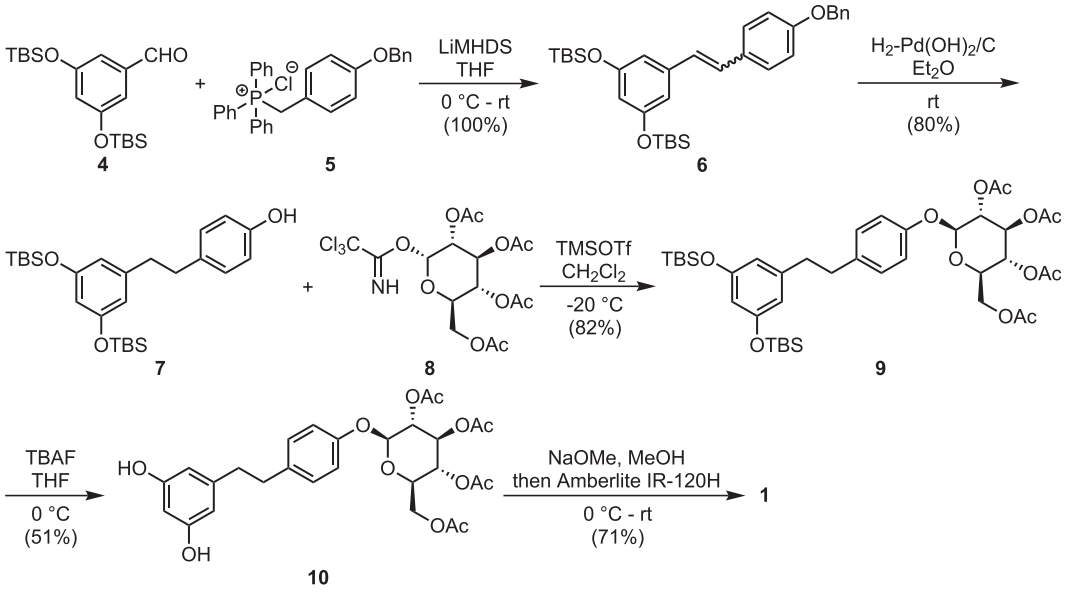


Fig. 1. Structure of dihydroresveratrol derivatives **1**–**3**.

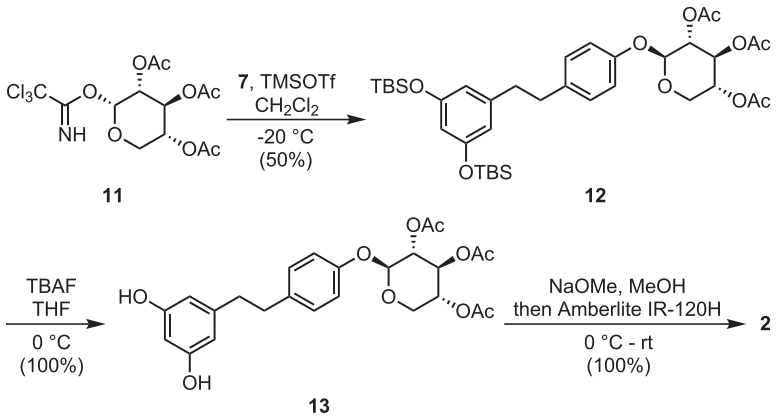
temperatures afforded glycoside **9** in 82% yield. Use of tetrabutylammonium fluoride (TBAF) converted **9** to resorcinol **10** in 51% yield. Transesterification of **10** using sodium methoxide, followed by the treatment with Amberlite IR-120H (to remove sodium ions) provided the natural product **1** in 71% yield. Although analytical data for **1** are nearly consistent with those reported previously [10], NMR assignments have been corrected by careful inspections of NMR data resulting from HMQC and HMBC experiments. Consequently, the natural product **1** was synthesized for the first time via 5 steps from **4** in 24% overall yield.

A previous report describes the synthesis of the bibenzyl glucoside [17], an analogue of **1**, which has been designed on the basis of the structure of the natural bibenzyl xyloside. As a result, tyrosinase inhibitory activity of the bibenzyl glucoside was 1.8 fold weaker than that of the xyloside. Thus, xyloside **2** was prepared in an effort to furnish a potent and simple melanogenesis inhibitor.

Steps for the chemical synthesis of **2** are illustrated in Scheme 2. Glycosylation of **7** with the xylosyl donor **11** in the presence of the catalytic amount of TMSOTf at −20 °C afforded xyloside **12** in 50% yield. Treatment with TBAF transformed **12** to resorcinol **13** quantitatively. The removal of acetyl groups in **12** using sodium



Scheme 1. Synthesis of dihydroresveratrol glucoside **1**.



Scheme 2. Synthesis of dihydroresveratrol xyloside **2**.

methoxide afforded the target compound **2** in excellent yield. Consequently, **2** was synthesized via 5 steps from **4** in 43% overall yield.

2.2. Biological evaluation of **1**–**3** against melanogenesis

Anti-melanogenesis activity of **1** was evaluated in B16F0 melanoma cells (Fig. 2) [18,19]. Kojic acid, a typical melanogenesis inhibitor, was used as the positive control. Melanin content of the cells decreased with the use of increasing concentrations of **1**. When the cultured cells were treated with 25.5 μM (10 μg/mL) of **1**, the melanin amount reduced significantly (74% of negative control). Similar reduction in the melanin content was observed (79% of negative control) when the B16F0 melanoma cells were treated with 1 mM of kojic acid. Therefore, anti-melanogenesis activity of **1** is approximately 40 fold higher than that of kojic acid. The total protein content was estimated by bicinchoninic acid (BCA) protein assay [20]. The protein content after treatment of 25.5 μM of **1** represented 98% of negative control, suggesting that the natural glucoside **1** has an insignificant effect on the protein biosynthesis in B16F0 melanoma cells. In addition, the measurement of cell viability in a 3-(4,5-di-methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [21] revealed no significant decrease in cell viability even at concentrations as high as 25.5 μM (102% cells are viable in the group treated with **1** when compared to negative control group). These results suggested that compound **1** does not have any adverse effects on cell viability.

Likewise, alycone **3** acts as an effective melanogenesis inhibitor because the melanin amount reduced significantly to treat 43.4 μM (10 μg/mL) of **3** in the cell culture experiment (for details, see the Supplementary data). However, the protein content reduced (29% of control), suggesting that **3** possesses an adverse effect on the protein biosynthesis in B16F0 melanoma cells.

Activity of the synthesized compound **2** was evaluated in B16F0 melanoma cells by an assay similar to the one used for evaluating the activity of **1** (Fig. 3). With an increase in the concentration of **2** in the medium, the melanin content in the cells increased. On treatment of 27.6 μM (10 μg/mL) of **2**, melanin production was stimulated to provide 130% of negative control. Furthermore, protein content reduced insignificantly (94% of control), indicating that **2** does not suppress the biosynthesis of the cellular proteins significantly. Concentrations of **2** up to 27.6 μM did not have any influence on the cell viability (102% cells are viable in the group treated with **2** when compared with the negative control group), which was determined by the MTT assay.

Thus, synthetic derivative **2** acts as a melanogenesis activator. While this result is in contrast to our stated objective of seeking to identify potent inhibitors, the discovery of a melanogenesis activator is a remarkable result. Melanogenesis activators, such as the derivatives of coumarin and stilbene isolated from several plants, have been used to treat hypopigmentation [22,23]. In particular, the stilbene derivative, 2,3,5,4′-tetrahydroxystilbene-2-*O*-β-D-glucoside, isolated from the extracts of *Polygonum multiflorum* (Polygonaceae) stimulated melanogenesis by activating mitogen-activated protein (MAP) kinase and inducing microphthalmia-associated transcription factor (MITF) of tyrosinase in B16 melanoma cells [24]. Although additional investigations are required to gain insight into the mode of action of **2**, structural comparison between this stilbene and **2** indicated that the activity of **2** can emerge from similar pathways.

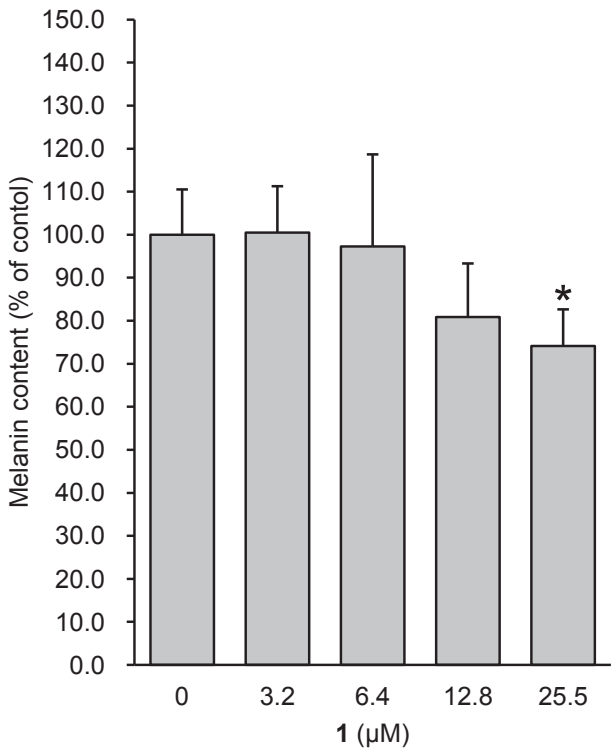


Fig. 2. Melanin content in B16F0 melanoma cells following treatment with **1** for 72 h. The data are expressed as melanin content (% of control) and each column represents the mean ± SD of 3 determinations. Differences are considered significant if $P < 0.05$ (indicated by asterisk).

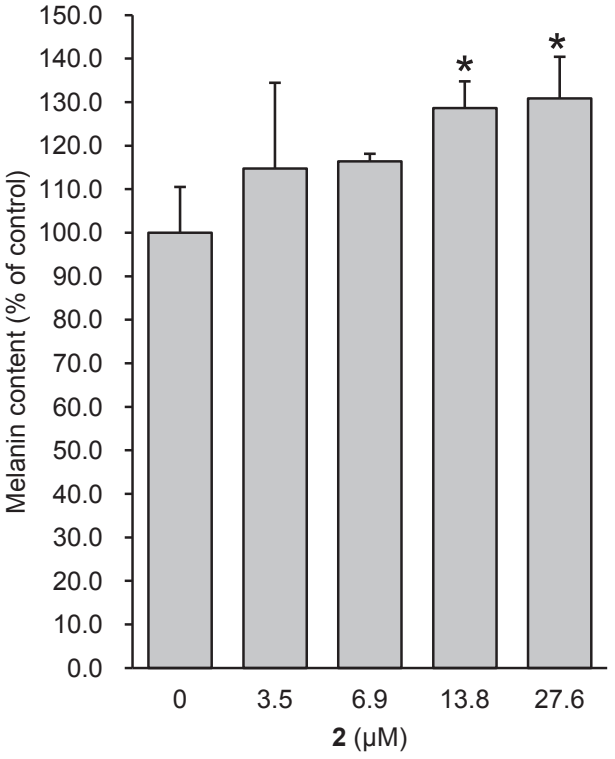


Fig. 3. Melanin content in B16F0 melanoma cells following treatment with **2** for 72 h. The data are expressed as melanin content (% of control) and each column represents the mean ± SD of 3 determinations. Differences are considered significant if $P < 0.05$ (indicated by asterisk).

3. Conclusions

The primary difference in the structure of the two dihydroresveratrol glycosides is the presence or the absence of the hydroxymethyl group in the sugar moiety. This subtle modification drastically changes the molecular property from an inhibitor to an activator of melanogenesis. In addition, the glycosylation of dihydroresveratrol suppresses their effect on the protein biosynthesis in B16F0 melanoma cells.

Since *C. oleifera* has been widely and traditionally used as an ingredient in food, cosmetics, and medicine [25–27], the natural product **1** is likely to be safe for use against hyperpigmentation. Other effective agents commonly used include kojic acid and arbutin [28,29]. In contrast, anti-hypopigmentation agents as the synthetic derivative **2** can be used to remedy dermal disorders [30,31], which can be caused in part due to the use of inappropriate cosmetic ingredients. Moreover, molecules that increase or decrease melanin biosynthesis are useful tools for examining the cellular mechanisms underlying melanogenesis and for potentially revealing previously unknown therapeutic targets for melanin-related diseases [32]. Further investigation for clarifying the molecular logic of the dihydroresveratrol glycosides against melanogenesis is underway.

4. Experimental

4.1. General experimental procedures

NMR spectra were recorded in CD_3OD or $CDCl_3$ on a JEOL EX-400 spectrometer (^1H at 400 MHz and ^{13}C at 100 MHz). Chemical shifts were recorded as ppm relative to the solvent signal for CD_3OD (3.30 ppm for ^1H NMR, 49.0 ppm for ^{13}C NMR) or for $CDCl_3$ (7.24 ppm for ^1H NMR, 77.0 ppm for ^{13}C NMR). HRMS spectra were measured on an AB SCIEX TripleTOF 5600 mass spectrometer fitted with an electrospray ion source in positive ionization mode. IR spectra were measured with a Perkin–Elmer Paragon 1000 PC FT-IR spectrometer. Optical rotations were recorded on a Jasco P-1020 polarimeter. Preparative RP-HPLC was performed on a Hitachi LaChrome Elite instrument equipped with an Inertsil ODS-3 (5 μm, 4.6 × 250 mm) with a flow rate of 1.0 mL/min and detected at 254 nm. Compound **3** was prepared from commercially available resveratrol by catalytic hydrogenation using 5% Pd/C and the spectral data are consisted with those in the previous report [33].

4.2. 4′-Benzyloxy-3,5-di(tert-butyldimethylsilyloxy)stilbene (**6**)

To a suspension of **5** [13] (1.3 g, 2.6 mmol) in THF (10 mL), 1.5 mL of LiHMDS (1.3 M in THF, 2.0 mmol) was added at 0 °C. The resultant solution was stirred at room temperature for 0.5 h. The solution was then cooled to 0 °C, and a THF (2 mL) solution of **4** [12] (0.47 g, 1.3 mmol) was slowly added. After being stirred for 1 h at room temperature, saturated aqueous NH_4Cl solution (30 mL) was poured into the reaction mixture. The resultant mixture was extracted with EtOAc (100 mL). The organic layer was washed with saturated aqueous NH_4Cl solution (30 mL × 2) and brine (30 mL × 2). The aqueous layers were extracted with EtOAc (30 mL × 3), and the combined organic layers were dried over Na_2SO_4. Filtration and concentration followed by silica gel chromatography (0–2% EtOAc in hexane) gave the title compound **6** (0.70 g; *cis*:*trans* = 3:2, as determined by ^1H NMR; 100% from **4**) as a colorless solid. IR (film) ν_{max} 1164, 832, 781 cm^{-1}. *Cis*-**6**; ^1H NMR (400 MHz, $CDCl_3$) δ 7.38 (m, 5H, Bn), 7.16 (d, J = 8.6, 2H, H-2′, H-6′), 6.81 (d, J = 8.6, 2H, H-3′, H-5′), 6.47 (d, J = 12.2, 1H, H-7), 6.39 (d, J = 12.2, 1H, H-7′), 6.35 (d, J = 2.2, 2H, H-2, H-6), 6.18 (t, J = 2.2, 1H, H-4), 5.01 (s, 2H, Bn), 0.92 (s, 18H, TBS), 0.20 (s, 12H, TBS). *Trans*-**6**; ^1H NMR (400 MHz, $CDCl_3$) δ 7.38 (m, 7H, Bn, H-2′, H-6′), 6.95 (d, J = 16.0, 1H, H-7), 6.94 (d, J = 8.6, 2H, H-3′, H-5′), 6.83 (d, J = 16.0, 1H, H-7′), 6.59 (d, J = 2.2, 2H, H-2, H-6), 6.23 (t, J = 2.2, 1H, H-4), 5.07 (s, 2H, Bn), 0.99 (s, 18H, TBS), 0.09 (s, 12H, TBS). ESIHRMS m/z 547.3069 $[M+H]^+$ (calcd for $C_{33}H_{47}O_3Si_2$, 547.3064).

4.3. 3,5-Di(tert-butyldimethylsilyloxy)-4′-hydroxybibenzyl (**7**)

To a solution of **6** (0.71 g, 1.3 mmol) in Et_2O (6 mL) was added 10% $Pd(OH)_2$ on carbon (71 mg). The reaction mixture was stirred for 4 h under a balloon of H_2 at room temperature and filtered through a Celite pad. The filtrate was concentrated, and the residue was purified by silica gel chromatography (5–10% EtOAc in hexane) to give the title compound **7** (0.48 g, 80%) as a white solid. IR (film) ν_{max} 3417, 1160, 836, 781 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 6.99 (d, J = 8.6, 2H, H-2′, H-6′), 6.71 (d, J = 8.6, 2H, H-3′, H-5′), 6.25 (d, J = 2.2, 2H, H-2, H-6), 6.16 (t, J = 2.2, 1H, H-4), 4.79 (bs, 1H, OH), 2.75 (m, 4H, H-7, H-7′), 0.95 (s, 18H, TBS), 0.15 (s, 12H, TBS). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.3 (C-3, C-5), 153.6 (C-4′), 143.7 (C-1), 133.9 (C-1′), 129.5 (C-2′, C-6′), 115.1 (C-3′, C-5′), 113.8 (C-2, C-6), 109.7 (C-4), 38.0 (C-7), 36.7 (C-7′), 25.7 (TBS), 18.2 (TBS), −4.4 (TBS). ESIHRMS m/z 459.2747 $[M+H]^+$ (calcd for $C_{26}H_{43}O_3Si_2$, 459.2751).

4.4. 4′-(2″,3″,4″,6″-Tetra-O-acetyl-β-D-glucopyranosyl)-3,5-di(tert-butyldimethylsilyloxy)bibenzyl (**9**)

To a cold (−20 °C) and stirred solution of **7** (0.20 g, 0.44 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (**8**) (0.40 g, 0.81 mmol) in CH_2Cl_2 (6 mL) was added 73 μL of TMSOTf solution (0.11 M in CH_2Cl_2, 8.0 μmol). After being stirred for 80 min at −20 °C, the resultant solution was quenched with TEA (15 μL) and allowed to warm to room temperature. Concentration in vacuo, followed by silica gel chromatography (20–40% EtOAc in hexane) gave the title compound **9** (0.28 g, 82% from **7**) as a colorless solid. $[\alpha]_D^{23}$ −2.81 (*c* 1.13, $CHCl_3$). IR (film) ν_{max} 1759, 1163, 832, 781 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.05 (d, J = 8.6, 2H, H-2′, H-6′), 6.86 (d, J = 8.6, 2H, H-3′, H-5′), 6.24 (d, J = 2.2, 2H, H-2, H-6), 6.17 (t, J = 2.2, 1H, H-4), 5.26 (t, J = 9.4, 1H, H-3″), 5.23 (dd, J = 7.6, 9.4, 1H, H-4″), 5.14 (dd, J = 7.6, 9.4, 1H, H-2″), 5.01 (d, J = 7.6, 1H, H-1″), 4.27 (dd, J = 5.3, 12.2, 1H, H-6″), 4.14 (dd, J = 2.4, 12.2, 1H, H-6″), 3.82 (ddd, J = 2.4, 5.3, 7.6, 1H, H-5″), 2.77 (m, 4H, H-7, H-7′), 2.05 (s, 3H, Ac), 2.04 (s, 3H, Ac), 2.02 (s, 3H, Ac), 2.01 (s, 3H, Ac), 0.94 (s, 18H, TBS), 0.13 (s, 12H, TBS). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7 (Ac), 170.3 (Ac), 169.5 (Ac), 169.4 (Ac), 156.3 (C-3, C-5), 155.1 (C-4′), 143.4 (C-1), 136.8 (C-1′), 129.5 (C-2′, C-6′), 116.9 (C-3′, C-5′), 113.7 (C-2, C-6), 109.8 (C-4), 99.3 (C-1″), 72.7 (C-5″), 71.9 (C-3″), 71.1 (C-2″), 68.3 (C-4″), 61.9 (C-6″), 37.8 (C-7), 36.7 (C-7′), 25.7 (TBS), 20.7 (Ac), 20.63 (Ac), 20.61 (Ac), 20.59 (Ac), 18.2 (TBS), −4.4 (TBS). ESIHRMS m/z 789.3691 $[M+H]^+$ (calcd for $C_{40}H_{61}O_{12}Si_2$, 789.3702).

4.5. 4′-(2″,3″,4″,6″-Tetra-O-acetyl-β-D-glucopyranosyl)-3,5-dihydroxybibenzyl (**10**)

To a cold (0 °C) and stirred solution of **9** (0.10 g, 0.13 mmol) in THF (2 mL) was added TBAF trihydrate (0.12 g, 0.38 mmol). After being stirred for 80 min at 0 °C, saturated aqueous NH_4Cl solution (30 mL) was poured into the reaction mixture. The resultant mixture was extracted with EtOAc (100 mL). The organic layer was washed with saturated aqueous NH_4Cl solution (30 mL × 2) and brine (30 mL × 2). The aqueous layers were extracted with EtOAc (30 mL × 3), and the combined organic layers were dried over Na_2SO_4. Filtration and concentration followed by silica gel chromatography (50–70% EtOAc in hexane) gave the title compound **10** (37 mg, 51%) as a white solid. $[\alpha]_D^{21}$ −0.21 (*c* 0.23, $CHCl_3$). IR (film) ν_{max} 3405, 1755, 838, 755 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.02 (d, J = 8.6, 2H, H-2′, H-6′), 6.88 (d, J = 8.6, 2H, H-3′, H-5′), 6.17 (t, J = 2.2, 1H, H-4), 6.13 (d, J = 2.2,

2H, H-2, H-6), 5.28 (t, J = 9.1, 1H, H-3″), 5.23 (dd, J = 7.6, 9.1, 1H, H-4″), 5.16 (dd, J = 7.5, 9.1, 1H, H-2″), 5.05 (d, J = 7.5, 1H, H-1″), 4.25 (dd, J = 5.1, 12.2, 1H, H-6″), 4.17 (dd, J = 2.5, 12.2, 1H, H-6″), 3.82 (ddd, J = 2.5, 5.1, 7.6, 1H, H-5″), 2.77 (m, 4H, H-7, H-7′), 2.06 (s, 3H, Ac), 2.05 (s, 3H, Ac), 2.03 (s, 3H, Ac), 2.01 (s, 3H, Ac). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8 (Ac), 170.3 (Ac), 169.6 (Ac), 169.5 (Ac), 156.7 (C-3, C-5), 154.7 (C-4′), 144.4 (C-1), 136.6 (C-1′), 129.6 (C-2′, C-6′), 117.2 (C-3′, C-5′), 108.1 (C-2, C-6), 100.5 (C-4), 99.1 (C-1″), 72.8 (C-5″), 72.0 (C-3″), 71.0 (C-2″), 68.2 (C-4″), 61.9 (C-6″), 37.8 (C-7), 36.6 (C-7′), 20.72 (Ac), 20.65 (Ac), 20.62 (Ac), 20.60 (Ac). ESIHRMS m/z 561.1980 $[M+H]^+$ (calcd for $C_{28}H_{33}O_{12}$, 561.1972).

4.6. 4′-(β-D-glucopyranosyl)-3,5-dihydroxybibenzyl (**2**)

To cold (0 °C) and stirred solution of **10** (34 mg, 61 μmol) in MeOH (4 mL) was added 79 μL of NaOMe (5.2 M in MeOH, 0.41 mmol) slowly. After being stirred for 1 h at room temperature, the resultant solution was neutralized with Amberlite IR-120 (H^+). Filtration and concentration followed by solid phase extraction with Sep-Pak Plus C-18 cartridge (0–40% MeCN in H_2O) gave the crude compound that was further purified by preparative RP-HPLC (28% MeCN in H_2O, t_R = 5.0 min). The title compound **2** (17 mg, 71%) was obtained as a colorless solid. $[\alpha]_D^{23}$ −38.4 (c 0.59, MeOH). IR (film) ν_{max} 3383, 2925, 1602, 1510, 1229, 1076, 837 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 7.08 (d, J = 8.5 Hz, 2H, H-2′, H-6′), 6.99 (d, J = 8.5 Hz, 2H, H-3′, H-5′), 6.11 (bs, 2H, H-2, H-6), 6.07 (bs, 1H, H-4), 4.85 (d, J = 7.3 Hz, 1H, H-1″), 3.89 (dd, J = 11.8, 1.6 Hz, 1H, H-6″), 3.69 (dd, J = 11.8, 5.1 Hz, 1H, H-6″), 3.43 (m, 4H, H-2″–H-5″), 2.80 (dd, J = 9.2, 6.4, 2H, H-7′), 2.70 (dd, J = 9.2, 6.4, 2H, H-7). ^{13}C NMR (100 MHz, CD_3OD) δ 159.3 (C-3, C-5), 157.4 (C-4′), 145.4 (C-1), 137.1 (C-1′), 130.4 (C-2′, C-6′), 117.6 (C-3′, C-5′), 108.1 (C-2, C-6), 102.5 (C-1″), 101.2 (C-4), 78.1 (C-3″), 78.0 (C-5″), 74.9 (C-2″), 71.4 (C-4″), 62.5 (C-6″), 39.3 (C-7), 38.0 (C-7′). ESIHRMS m/z 415.1368 $[M+Na]^+$ (calcd for $C_{20}H_{24}O_8Na$, 415.1369).

4.7. 4′-(2″,3″,4″-Tri-O-acetyl-β-D-xylopyranosyl)-3,5-di(tert-butyldimethylsilyloxy)bibenzyl (**12**)

To a cold (−20 °C) and stirred solution of **7** (0.11 g, 0.24 mmol) and 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl trichloroacetimidate (**11**) (0.20 g, 0.48 mmol) in CH_2Cl_2 (4 mL) was added 43 μL of TMSOTf solution (0.11 M in CH_2Cl_2, 4.7 μmol). After being stirred for 40 min at −20 °C, the resultant solution was quenched with TEA (15 μL) and allowed to warm to room temperature. Concentration in vacuo, followed by silica gel chromatography (20–40% EtOAc in hexane) gave the title compound **12** (86 mg, 50% from **7**) as a white solid. $[\alpha]_D^{22}$ −17.2 (c 2.42, $CHCl_3$). IR (film) ν_{max} 1759, 1162, 832, 781 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.06 (d, J = 8.6, 2H, H-2′, H-6′), 6.87 (d, J = 8.6, 2H, H-3′, H-5′), 6.24 (d, J = 2.2, 2H, H-2, H-6), 6.14 (t, J = 2.2, 1H, H-4), 5,21 (t, J = 7.9, 1H, H-3″), 5.15 (dd, J = 6.1, 7.9, 1H, H-2″), 5.09 (d, J = 6.1, 1H, H-1″), 4.99 (dt, J = 4.8, 7.9, 1H, H-4″), 4.19 (dd, J = 4.8, 12.0, 1H, H-5″), 3.48 (dd, J = 7.9, 12.0, 1H, H-5″), 2.76 (m, 4H, H-7, H-7′), 2.05 (m, 9H, Ac), 0.97 (m, 18H, TBS), 0.16 (m, 12H, TBS). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0 (Ac), 169.9 (Ac), 169.4 (Ac), 156.3 (C-3, C-5), 154.8 (C-4′), 143.5 (C-1), 136.5 (C-1′), 129.5 (C-2′, C-6′), 116.8 (C-3′, C-5′), 113.7 (C-2, C-6), 109.8 (C-4), 98.8 (C-1″), 70.8 (C-3″), 70.2 (C-2″), 68.5 (C-4″), 61.8 (C-5″), 37.8 (C-7), 36.8 (C-7′), 25.7 (TBS), 18.2 (TBS), 20.8 (Ac), 20.73 (Ac), 20.69 (Ac), −4.4 (TBS). ESIHRMS m/z 717.3477 $[M+H]^+$ (calcd for $C_{37}H_{57}O_{10}Si_2$, 717.3490).

4.8. 4′-(2″,3″,4″-Tri-O-acetyl-β-D-xylopyranosyl)-3,5-dihydroxybibenzyl (**13**)

To a cold (0 °C) and stirred solution of **12** (0.13 g, 0.18 mmol) in THF (3 mL) was added TBAF trihydrate (0.24 g, 0.76 mmol). After being stirred for 30 min at 0 °C, saturated aqueous NH_4Cl solution (30 mL) was poured into the reaction mixture. The resultant mixture was extracted with EtOAc (100 mL). The organic layer was washed with saturated aqueous NH_4Cl solution (30 mL × 2) and brine (30 mL × 2). The aqueous layers were extracted with EtOAc (30 mL × 3), and the combined organic layers were dried over Na_2SO_4. Filtration and concentration followed by silica gel chromatography (50–55% EtOAc in hexane) gave the title compound **13** (90 mg, 100%) as a white solid. $[\alpha]_D^{23}$ −25.2 (c 1.67, $CHCl_3$). IR (film) ν_{max} 3441, 1755, 838, 755 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.03 (d, J = 8.5, 2H, H-2′, H-6′), 6.87 (d, J = 8.5, 2H, H-3′, H-5′), 6.18 (bs, 1H, H-4), 6.16 (bs, 2H, H-2, H-6), 5.21 (t, J = 7.7, 1H, H-3″), 5.15 (dd, J = 6.1, 7.7, 1H, H-2″), 5.11 (d, J = 6.1, 1H, H-1″), 4.99 (ddd, J = 4.8, 7.7, 7.9, 1H, H-4″), 4.20 (dd, J = 4.8, 12.1, 1H, H-5″), 3.49 (dd, J = 7.9, 12.1, 1H, H-5″), 2.75 (m, 4H, H-7, H-7′), 2.06 (m, 9H, Ac). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4 (Ac), 170.1 (Ac), 169.9 (Ac), 156.8 (C-3, C-5), 154.6 (C-4′), 144.5 (C-1), 136.5 (C-1′), 129.6 (C-2′, C-6′), 116.9 (C-3′, C-5′), 108.0 (C-2, C-6), 100.5 (C-4), 98.7 (C-1″), 70.8 (C-3″), 70.1 (C-2″), 68.5 (C-4″), 61.8 (C-5″), 37.6 (C-7), 36.5 (C-7′), 20.8 (Ac), 20.74 (Ac), 20.71 (Ac). ESIHRMS m/z 489.1755 $[M+H]^+$ (calcd for $C_{25}H_{29}O_{10}$, 489.1761).

4.9. 4′-(β-D-xylopyranosyl)-3,5-dihydroxybibenzyl (**2**)

To cold (0 °C) and stirred solution of **13** (78 mg, 0.16 mmol) in MeOH (6 mL) was added 0.15 mL of NaOMe (5.2 M in MeOH, 0.78 mmol) slowly. After being stirred for 1 h at room temperature, the resultant solution was neutralized with Amberlite IR-120 (H^+). Filtration and concentration followed by solid phase extraction with Sep-Pak Plus C-18 cartridge (0–40% MeCN in H_2O) gave the crude compound that was further purified by preparative RP-HPLC (30%MeCN in H_2O, t_R = 6.0 min). The title compound **2** (58 mg, 100%) was obtained as a colorless solid. $[\alpha]_D^{23}$ −3.48 (c 0.21, MeOH). IR (film) ν_{max} 3355, 2927, 1601, 1510, 1226, 1039, 837 cm^{-1}. ^{1}H NMR (400 MHz, CD_3OD) δ 7.07 (d, J = 8.2 Hz, 2H, H-2′, H-6′), 6.94 (d, J = 8.2 Hz, 2H, H-3′, H-5′), 6.11 (bs, 2H, H-2, H-6), 6.07 (bs, 1H, H-4), 4.80 (d, J = 7.2 Hz, 1H, H-1″), 3.89 (dd, J = 11.4, 5.4 Hz, 1H, H-5″), 3.55 (m, 1H, H-4″), 3.40 (m, 2H, H-3″, H-5″), 2.79 (dd, J = 9.5, 6.9, 2H, H-7′), 2.69 (dd, J = 9.5, 6.9, 2H, H-7); ^{13}C NMR (100 MHz, CD_3OD) δ 159.3 (C-3, C-5), 157.2 (C-4′), 145.4 (C-1), 137.1 (C-1′), 130.4 (C-2′, C-6′), 117.7 (C-3′, C-5′), 108.1 (C-2, C-6), 103.1 (C-1″), 101.2 (C-4), 77.8 (C-3″), 74.8 (C-2″), 71.1 (C-4″), 66.9 (C-5″), 39.4 (C-7), 38.0 (C-7′). ESIHRMS m/z 385.1251 $[M+Na]^+$ (calcd for $C_{19}H_{22}O_7Na$, 385.1263).

4.10. Biological evaluation against melanogenesis

The assay was performed as reported previously with a few modifications [18,19]. These modifications mainly involve precluding the use of antibiotics and hormones in the assay. Briefly, B16F0 melanoma cells (ATCC CRL-6322), adjusted to 1.0 × 10^4 cells/well, were incubated in Dulbecco's modified Eagle's medium (DMEM) containing 5% fetal bovine serum (FBS) under 5% CO_2 atmosphere at 37 °C for 24 h. The medium was replaced with DMEM containing 5% FBS and an ethanolic solution of the investigated sample. After 72 h of treatment under the conditions described above, the medium was replaced again with the sample-containing medium. After 72 h, trypsin was added to all the wells and the harvested cells were pelleted by centrifugation. The cell pellets were washed with phosphate buffer, 5% trichloroacetic acid, 33% ethanol in ether, and ether. The pellet was then air dried and lysed with 1 M NaOH solution at 100 °C for 10 min and the optical density (475 nm) of the resultant solution was measured. The melanin concentration was determined by comparison with the standard curve (plotted using commercially available melanin). Determination of the protein concentration was performed by BCA protein assay method [20]

using the cell lysate prepared by 0.5% Triton X-100 treatment. Negative and positive controls containing ethanol or kojic acid were conducted in parallel. Cell viability was determined by MTT assay [21]. All the assays were performed in triplicate (or more replicates) on separate occasions, and the Student's *t*-test was used to determine the level of significance between different groups.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.09.092.

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