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Total synthesis of apios isoflavones and investigation of their tyrosinase inhibitory activity

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ABSTRACT

Apios isoflavone glucosides **1** and **2** were synthesized for the first time via Friedel–Crafts reaction, Bischler–Napieralski-type cyclization, and phase-transfer catalyzed glycosylation as the key steps. In addition, aglycones **4** and **5** and related natural isoflavone cajanin (**6**) were synthesized in short steps. Evaluation of the inhibitory activity of these compounds toward tyrosinase indicated that all the compounds were active. In particular, the half-maximal inhibitory concentration of compound **1** toward tyrosinase was measured to be 729 μM.

1. Introduction

Apios americana (American groundnut) is classified as Fabaceae, which is particularly cultivated in the eastern area of Aomori prefecture in Japan. In 2013, novel isoflavone glycosides **1** and **2** were isolated from the edible tuber of *A. americana* along with several derivatives [1–3]. The structure of **1** and **2**, as shown in Fig. 1, displays high similarity to that of bibenzyl glucoside **3** that has been developed as a potent and hydrophilic tyrosinase inhibitor [4,5].

Tyrosinase (EC 1.14.8.1) and its related copper-containing oxidoreductase catalyze the oxidation of monophenol, such as tyrosine, to produce *o*-quinones via the production of *o*-diphenols as reaction intermediates [6,7]. *o*-Quinones are further reacted to generate several biopolymers, including melanin and insect cuticle [8,9]. Thus, the control of this enzymatic oxidation led to the development of effective cosmetics and insecticides [10–15].

To envision the discovery of novel tyrosinase inhibitors, the total synthesis of **1** and **2** was designed, including Friedel–Crafts reaction, a Bischler–Napieralski-type cyclization, and a phase-transfer catalyzed (PTC) glycosylation as the key steps [16–19]. To the best of our knowledge, for the first time, we report herein the chemical synthesis of **1** and **2** and the concise synthesis of their aglycones **4** and **5** and related natural isoflavone cajanin (**6**) [20]. In addition, their tyrosinase inhibitory activities were evaluated and compared with those of known tyrosinase inhibitors, morin (**7**) and kojic acid (**8**) [21,22].

2. Results and discussion

2.1. Synthesis of *1*, *4*, and *6*

As shown in Scheme 1, Friedel–Crafts reaction was performed on commercially available carboxylic acid **9** and 1,3,5-trimethoxybenzene (TMB) at 70 °C using boron trifluoride etherate ($BF_3 \cdot OEt_2$) as the Lewis acid [16]. Consequently, phenyl ketone **10** was solely isolated in 79% yield. Notably, the fully methylated product could not be detected by TLC. Under the described conditions, the removal of the methyl group at 2′-OH preferentially occurred, which may be due to the coordination of Lewis acid between 2′-OMe and carbonyl groups [23]. The chemical synthesis of **10** starting from **9** and TMB was reported to occur in three steps: carboxylic halogenation, Friedel–Crafts reaction, and selective deprotection, although experimental details for those steps were unclear [17]. In our approach, the three reaction steps were reduced to one and the synthetic yield of **10** from **9** was significantly improved from 30% to 79%.

The Bischler–Napieralski-type cyclization of **10** with *N,N*-dimethylformamide dimethyl acetal (DMFDMA) afforded methylated isoflavone **11** in 73% yield [24]. During the treatment of methanesulfonyl chloride, DMF, and $BF_3 \cdot OEt_2$ for the aforementioned step [18], the synthetic yield was poor (30%) and several unidentified side products were observed by TLC. In addition, triethyl orthoformate and morpholine were not utilized to perform

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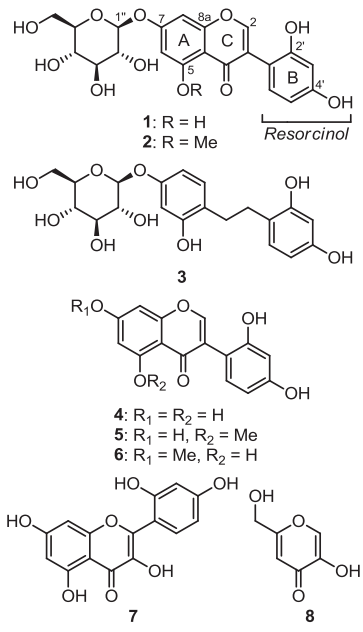
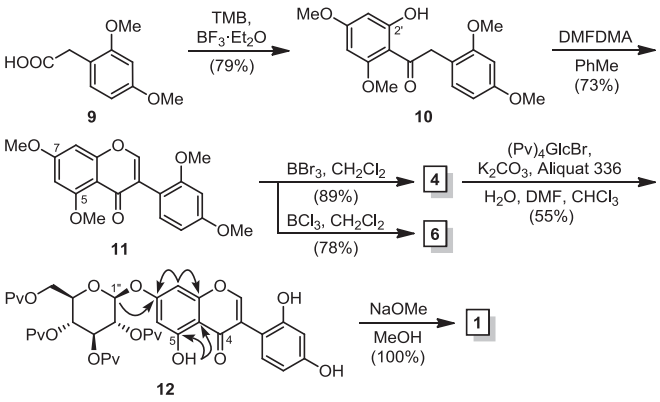


Fig. 1. Structure of compounds **1**–**8**.



Scheme 1. Synthesis of compounds **1**, **4**, and **6**. Curved arrows indicate key HMBC correlations.

this Bischler–Napieralski-type cyclization because of the previously reported low yield (48%) [17].

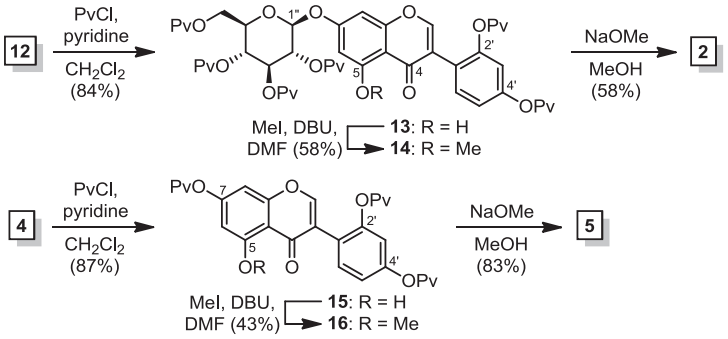
Use of boron tribromide (BBr_3) as an ether-cleaving agent transformed **11** into isoflavone **4** in high yield (89%). Suzuki–Miyaura coupling was previously used as a key step to synthesize **4**; however, this route required four steps [25]. Remarkably, when boron trichloride (BCl_3) was treated under a cooling condition (0 °C), **6** was alternatively synthesized from **11** in 78% yield [26,27]. The presence of the methyl residue of **6** was confirmed via NMR spectroscopy experiments. In contrast, **6** was solely obtained by the BBr_3 treatment of **11**, although the detailed experimental conditions were unclear [17]. The selectivity displayed in this case might be the result of poor nucleophilicity of 7-OMe in **11**, which brought a clue for the next regioselective glycosylation.

PTC glycosylation of **4** was conducted in a two-phase system in the presence of Aliquat 336 and K_2CO_3 [28]. 2,3,5,6-Tetra-*O*-pivaloyl-α-D-glucopyranosyl bromide [$(Pv)_4$GlcBr] was selected as the glucose donor because the pivaloyl (Pv) group showed high resistance under basic conditions [19,29]. As a consequence, monoglycosylated isoflavone **12** was obtained in 55% yield. Thus, no regioisomers of **12** or poly-glycosylated isoflavones were isolated by silica gel chromatography. Schmidt glycosylation of **4** using 2,3,4,6-tetra-*O*-acetyl-α-D-glycosyl trichloroacetimidate as the glucose donor was sluggish and did not proceed completely [4,11]. The glycosylation position in **12** was determined by extensive NMR experiments, especially the significant HMBC correlations depicted in Scheme 1. This regioselective glycosylation was explained on the basis of the high acidity of 7-OH in **4** as implied by the demethylation of **11**.

The removal of the Pv groups from **12** achieved through transesterification with NaOMe afforded the natural glycoside **1** quantitatively. The obtained spectral data were consistent with those reported previously [1]. Thus, total synthesis of **1** was achieved for the first time in 28% yield over five steps starting from **9**. In addition, **4** and **6** were synthesized from **9** over three steps in 51% and 45%, respectively.

2.2. Synthesis of *2* and *5*

Scheme 2 depicts the synthetic route leading to the formation of natural glycoside **2** from glycosylated isoflavone **12**. Because of the hydrogen bond between 5-OH and carbonyl oxygen at C-4 in **12**, 5-OH was less reactive. Thus, the selective acylation at 2′-OH and 4′-OH was achieved using PvCl in the presence of pyridine as a weak base [30]. As a result, the hexa-acylated isoflavone **13** was obtained in 84% yield. After a few unsuccessful attempts to introduce a methyl group at 5-OH [31], the methylation of **13** was achieved using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as an organic base to afford the methyl ether **14** in moderate yield (58%). Finally, the natural product isoflavone **2** was afforded in 58% yield via transesterification with NaOMe. Thus, **2** was synthesized for the first time over seven steps from **9** with a recorded total yield of 8%.



Scheme 2. Synthesis of compounds **2** and **5**.

Table 1
Tyrosinase inhibitory activities of compounds **1**–**8**.

Compounds tested	Inhibition (%)[a,b]	IC_{50} (μM)[b]
1	61.9 ± 0.06	729.3 ± 127.7
2	24.3 ± 0.35	-[c]
3	-[c]	0.77 ± 0.04[d]
4	37.3 ± 0.28	-[c]
5	25.8 ± 0.64	-[c]
6	31.2 ± 0.06	-[c]
7	60.9 ± 0.06	706.7 ± 89.3
8	-[c]	11.3 ± 1.03

[a] The data are expressed as % of control using a solution containing 1 mM of the compound.
[b] The IC_{50} value is represented as mean value ± SE of three different experiments.
[c] This value was not obtained.
[d] This IC_{50} value is drawn from a previous report [4].

The synthesis of methylated isoflavone **5** as the aglycone part of **2** is shown in Scheme 2. Isoflavone **4** was transformed into triacylated aglycone **15** in 87% yield by the action of PvCl and pyridine. The subsequent methylation of **15** with iodomethane in the presence of DBU afforded methyl ether **16** in 43% yield. Finally, **5** was obtained in 83% yield from **16** by the treatment of NaOMe. The aforementioned synthetic route comprised six steps from **9** in 16% overall yield.

2.3. Tyrosinase inhibitory activity

The tyrosinase inhibitory activity of **1**–**8** was evaluated using DOPA as a substrate, as summarized in Table 1. A 1 mM solution of **1** showed 62% inhibition of tyrosinase-catalyzed oxidation. Further dose-response experiments were conducted that identified 729 μM as the half-maximal inhibitory concentration (IC_{50}), which is nearly equivalent to that of **7**, a known flavonoid-type tyrosinase inhibitor [21]. The IC_{50} of methylated glycoside **2** could not be estimated because its 1 mM solution showed only 24% tyrosinase inhibition. These findings indicated that a hydrogen bond between 5-OH and C-4 in the apios isoflavone glucoside might be required for tyrosinase inhibitory activity to be displayed.

Likewise, **5** that is the aglycone part of **2** without the hydrogen bond, possessed very weak inhibitory activity (26% inhibition). Notably, the methylated isoflavone **6** (31% inhibition) proved to be a less effective tyrosinase inhibitor than aglycone **4** (37% inhibition), although both contained the intramolecular hydrogen bond. This suggests that the introduction of a hydrophilic substituent such as glucose at 7-OH may positively influence the tyrosinase inhibitory activity. The IC_{50} of **1** is about a thousand times less than that of bibenzyl glucoside **3**. This gap can be attributed to the structural rigidity of the isoflavone skeleton. The C-ring (see Fig. 1) induced a quasi-planar structure, which may hinder the binding of the resorcinolic B-ring toward the active site of tyrosinase [19]. Further chemical investigations to develop potent tyrosinase inhibitors based on the isoflavone skeleton are underway.

3. Conclusion

To the best of our knowledge, we synthesized **1** and **2** for the first time. The synthetic routes for **1** and **2** comprised five and seven steps from **9**, respectively, and they were characterized by higher overall yields than those of the known processes. In addition, compounds **4** and **5**, the aglycone derivatives of **1** and **2**, and the related isoflavone cajanin **6** were synthesized in three, six, and three steps from **9**, respectively. The isoflavones thus obtained proved weak inhibitors of the tyrosinase-catalyzed DOPA oxidation. Notably, the natural glucoside **1** displayed tyrosinase inhibitory activity that was similar in magnitude to that of **7**, a known flavonoid-type tyrosinase inhibitor.

4. Experimental

4.1. General

RP-HPLC was performed on a Hitachi LaChrome Elite instrument equipped with Inertsil ODS-3 column (7.6 mm × 250 mm). A flow rate and a detection wavelength were used as 2.0 mL/min and 254 nm, respectively. Optical rotations were recorded on a Jasco P-1020 polarimeter. IR spectra were measured with a Perkin-Elmer Frontier FT-IR spectrometer. NMR spectra were recorded in $CDCl_3$, CD_3OD, or DMSO-d_6 on a JEOL EX-400 spectrometer (^1H at 400 MHz and ^{13}C at 100 MHz). Chemical shifts were described as ppm relative to the solvent signal for $CDCl_3$ (7.24 ppm for ^1H NMR, 77.0 ppm for ^{13}C NMR), CD_3OD (3.30 ppm for ^1H NMR, 49.0 ppm for ^{13}C NMR), or DMSO-d_6 (2.49 ppm for ^1H NMR, 39.7 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.

4.2. 1-(2′-Hydroxy-4′,6′-dimethoxyphenyl)-2-(2″,4″-dimethoxyphenyl)ethanone (*10*)

Under Ar atmosphere, **9** (1.20 g, 6.12 mmol) and TMB (6.00 g, 35.7 mmol) were dissolved with $BF_3{\cdot}OEt_2$ (16.0 mL, 127 mmol) at room temperature (rt). After being stirred overnight at 70 °C, the resultant solution was cooled to rt. Saturated aqueous $NaHCO_3$ solution (50 mL) was slowly added into the stirred solution. The resultant mixture was stirred for 1 h and extracted with EtOAc (50 mL × 3). The organic layer was washed with saturated aqueous $NaHCO_3$ solution (50 mL × 3) and brine (50 mL × 3). The resultant aqueous layers were extracted with EtOAc (30 mL × 3). The combined organic layers were dried over Na_2SO_4. Filtration and concentration followed by silica gel chromatography (25% EtOAc in hexane) gave the title compound **10** (1.60 g, 4.81 mmol, 79% yield from **9**) as a colorless solid. IR (film) ν_{max} 2932, 1732, 875, 815 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.98 (d, J = 8.2 Hz 1H, H-6″), 6.48 (d, J = 2.3 Hz 1H, H-3″), 6.45 (dd, J = 8.2, 2.3 Hz 1H, H-5″), 6.06 (d, J = 2.4 Hz, 1H, H-5′), 5.93 (d, J = 2.4 Hz, 1H, H-3′), 4.23 (s, 2H, H-2), 3.85 (s, 3H, OMe), 3.80 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.74 (s, 3H, OMe); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.2 (C-1), 167.4 (C-4′), 165.8 (C-6′), 162.8 (C-4″), 159.9 (C-2′), 158.5 (C-2″), 131.2 (C-6″), 116.7 (C-1′), 105.8 (C-1″), 104.0 (C-5″), 98.6 (C-3″), 93.5 (C-5′), 90.7 (C-3′), 55.51 (OMe), 55.49 (OMe), 55.4 (OMe), 55.3 (OMe), 44.9 (C-2); ESIHRMS m/z 355.1161 $[M+Na]^+$ (calcd for $C_{18}H_{20}O_6Na$, 355.1158).

4.3. 5,7,2′,4′-Tetramethoxyisoflavone (*11*)

Under Ar atmosphere, **10** (1.60 g, 4.81 mmol) and DMFDMA (1.20 mL, 9.06 mmol) were dissolved with anhydrous PhMe (40 mL). The resultant solution was refluxed overnight and cooled to rt. Water (50 mL) was slowly poured into the solution and the resultant mixture was extracted with EtOAc (100 mL). The organic layer was washed with H_2O (50 mL × 3) and brine (50 mL × 3). The resultant aqueous layers were extracted with EtOAc (30 mL × 3). The combined organic layers were dried over Na_2SO_4. Filtration and concentration followed by silica gel chromatography (20–80% EtOAc in hexane) gave the title compound **11** (1.20 g, 3.51 mmol, 73% yield) as a colorless solid. IR (film) ν_{max} 1644, 1028, 864, 821 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.71 (s, 1H, H-2), 7.21 (d, J = 8.9 Hz, 1H, H-6′), 6.50 (m, 2H, H-3′, H-5′), 6.42 (d, J = 2.4 Hz, 1H, H-8), 6.33 (d, J = 2.4 Hz, 1H, H-6), 3.89 (s, 3H, OMe), 3.86 (s, 3H,

OMe), 3.81 (s, 3H, OMe), 3.73 (s, 3H, OMe); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.3 (C-4), 163.7 (C-7), 161.4 (C-5), 160.9 (C-4′), 160.0 (C-8a), 158.5 (C-2′), 151.7 (C-2), 132.5 (C-6′), 123.2 (C-3), 113.6 (C-1′), 110.0 (C-4a), 104.1 (C-5′), 98.8 (C-3′), 96.0 (C-6), 92.5 (C-8), 56.3 (OMe), 55.69 (OMe), 55.67 (OMe), 55.4 (OMe); ESIHRMS *m/z* 343.1185 $[M+H]^+$ (calcd for $C_{19}H_{19}O_6$, 343.1182).

4.4. 2′-Hydroxygenistein (**4**)

To the solution of **11** (0.28 g, 0.82 mmol) in anhydrous CH_2Cl_2 (5 mL), 1 M BBr_3 in CH_2Cl_2 (15.0 mL, 15.0 mmol) was slowly added at −15 °C under Ar atmosphere. After being stirred overnight at rt, the resultant solution was poured into ice water (100 mL) and extracted with EtOAc (50 mL). The organic layer was washed with water (30 mL × 3) and brine (30 mL × 3). The resultant aqueous layers were extracted with EtOAc (30 mL × 3). The combined organic layers were dried over Na_2SO_4. Filtration and concentration followed by silica gel chromatography (3% MeOH in $CHCl_3$) gave the title compound **4** (0.21 g, 0.73 mmol, 89% yield) as a colorless solid. IR (film) ν_{max} 3225, 1650, 1171, 870, 808 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 8.00 (s, 1H, H-2), 7.03 (d, *J* = 8.2 Hz, 1H, H-6′), 6.39 (d, *J* = 2.4 Hz, 1H, H-3′), 6.36 (dd, *J* = 8.2, 2.4 Hz, 1H, H-5′), 6.35 (d, *J* = 2.1 Hz, 1H, H-8), 6.22 (d, *J* = 2.1 Hz, 1H, H-6); ^{13}C NMR (100 MHz, CD_3OD) δ 182.7 (C-4), 166.1 (C-7), 163.7 (C-5), 160.3 (C-4′), 159.8 (C-8a), 157.8 (C-2′), 156.7 (C-2), 133.2 (C-6′), 122.6 (C-3), 110.8 (C-1′), 108.1 (C-5′), 106.2 (C-4a), 104.2 (C-3′), 100.2 (C-6), 94.8 (C-8); ESIHRMS *m/z* 287.0551 $[M+H]^+$ (calcd for $C_{15}H_{11}O_6$, 287.0556). The spectral data (NMR and MS) that mentioned above were consistent with those that were previously reported [25].

4.5. Cajanin (**6**)

To the solution of **11** (0.11 g, 0.32 mmol) in anhydrous CH_2Cl_2 (3 mL), 1 M BCl_3 in CH_2Cl_2 (7.0 mL, 7.0 mmol) was slowly added at −15 °C under Ar atmosphere. After being stirred overnight at rt, the resultant solution was poured into ice water (50 mL) and extracted with EtOAc (30 mL). The organic layer was washed with water (20 mL × 3) and brine (20 mL × 3). The resultant aqueous layers were extracted with EtOAc (20 mL × 3). The combined organic layers were dried over Na_2SO_4. Filtration and concentration followed by silica gel chromatography (3% MeOH in $CHCl_3$) gave the title compound **6** (75 mg, 0.25 mmol, 78% yield) as a colorless solid. IR (film) ν_{max} 3452, 1652, 1503, 1145 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 8.06 (s, 1H, H-2), 7.04 (d, *J* = 8.2 Hz, 1H, H-6′), 6.55 (d, *J* = 2.3 Hz, 1H, H-8), 6.39 (d, *J* = 2.3 Hz, 1H, H-3′), 6.363 (dd, *J* = 8.2, 2.3 Hz, 1H, H-5′), 6.362 (d, *J* = 2.3 Hz, 1H, H-6), 3.88 (s, 3H, OMe); ^{13}C NMR (100 MHz, CD_3OD) δ 182.8 (C-4), 167.3 (C-7), 163.5 (C-5), 160.3 (C-8a), 159.7 (C-4′), 157.8 (C-2′), 157.0 (C-2), 133.2 (C-6′), 122.8 (C-3), 110.6 (C-1′), 108.1 (C-5′), 107.1 (C-4a), 104.2 (C-3′), 99.3 (C-6), 93.2 (C-8), 56.5 (OMe); ESIHRMS *m/z* 301.0717 $[M+H]^+$ (calcd for $C_{16}H_{13}O_6$, 301.0712). The spectral data (NMR and MS) that mentioned above were consistent with those that were previously reported [20].

4.6. 7-(2″,3″,4″,6″-Tetra-O-pivaloyl-β-glucopyranosyl)-2′-hydroxygenistein (**12**)

K_2CO_3 (0.24 g, 1.74 mmol) and **4** (0.25 g, 0.87 mmol) were dissolved with water (10 mL) and DMF (10 mL). To the solution, $(Pv)_4$GlcBr (1.70 g, 2.93 mmol) and Aliquat 336 (0.31 g, 0.77 mmol) in $CHCl_3$ (10 mL) were added. After being stirred for 24 h at 45 °C, water (50 mL) was poured into the solution and the resultant mixture was extracted with EtOAc (20 mL × 3). The organic layer was washed with water (10 mL × 3) and brine (10 mL × 3). The combined organic layers were dried over Na_2SO_4. Filtration and concentration followed by silica gel chromatography (30% EtOAc in hexane) gave the title compound **12** (0.38 g, 0.48 mmol, 55% yield) as a colorless solid. $[\alpha]_D^{23}$-25.7 (c 0.42, $CHCl_3$); IR (film) ν_{max} 2973, 1742, 1135, 753 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.13 (s, 1H, 5-OH), 8.16 (bs, 1H, 2′-OH), 7.94 (s, 1H, H-2), 7.01 (d, *J* = 8.4 Hz, 1H, H-6′), 6.60 (d, *J* = 2.5 Hz, 1H, H-3′), 6.51 (dd, *J* = 8.4, 2.5 Hz, 1H, H-5′), 6.48 (d, *J* = 2.2 Hz, 1H, H-8), 6.40 (d, *J* = 2.2 Hz, 1H, H-6), 6.17 (bs, 1H, 4′-OH), 5.38 (t, *J* = 9.6 Hz, 1H, H-3″), 5.28 (dd, *J* = 9.6, 7.8 Hz, 1H, H-2″), 5.15 (t, *J* = 9.6 Hz, 1H, H-4″), 4.98 (d, *J* = 7.8 Hz, 1H, H-1″), 4.27 (dd, *J* = 12.4, 1.6 Hz, 1H, H-6″), 4.04 (dd, *J* = 12.4, 6.6 Hz, 1H, H-6″), 3.91 (ddd, *J* = 9.6, 6.6, 1.6 Hz, 1H, H-5″), 1.22 (s, 9H, Pv), 1.17 (s, 9H, Pv), 1.14 (s, 9H, Pv), 1.13 (s, 9H, Pv); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.0 (C-4), 178.3 (Pv), 177.6 (Pv), 176.7 (Pv), 176.6 (Pv), 162.9 (C-7), 162.4 (C-5), 158.7 (C-4′), 157.3 (C-8a), 157.1 (C-2′), 155.3 (C-2), 130.8 (C-6′), 123.4 (C-3), 111.7 (C-1′), 109.0 (C-5′), 106.7 (C-4a), 106.6 (C-3′), 100.6 (C-6), 98.4 (C-1″), 94.9 (C-8), 73.0 (C-5″), 71.7 (C-3″), 70.3 (C-2″), 67.6 (C-4″), 62.1 (C-6″), 38.9 (Pv), 38.8 (Pv), 27.12 (Pv), 27.10 (Pv), 27.02 (Pv), 27.00 (Pv); ESIHRMS *m/z* 785.3384 $[M+H]^+$ (calcd for $C_{41}H_{53}O_{15}$, 785.3385).

4.7. 7-(β-Glucopyranosyl)-2′-hydroxygenistein (**1**)

To a solution of **12** (0.10 g, 0.13 mmol) in MeOH (2 mL), 5 M NaOMe in MeOH (0.10 mL, 0.50 mmol) was added. The resultant solution was refluxed for 2 h, cooled to rt, and neutralized with Amberlite IR-120H. Filtration and concentration followed by crystallization from 50% MeOH in H_2O gave the title compound **1** (60 mg, 0.13 mmol, 100% yield) as a colorless solid. $[\alpha]_D^{23}$ -60.1 (c 0.17, MeOH); IR (film) ν_{max} 2973, 1742, 1135, 753 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.00 (s, 1H, 5-OH), 9.39 (s, 1H, 4′-OH), 9.31 (s, 1H, 2′-OH), 8.24 (s, 1H, H-2), 6.98 (d, *J* = 8.3 Hz, 1H, H-6′), 6.70 (d, *J* = 2.2 Hz, 1H, H-8), 6.46 (d, *J* = 2.2 Hz, 1H, H-6), 6.36 (d, *J* = 2.4 Hz, 1H, H-3′), 6.26 (dd, *J* = 8.3, 2.4 Hz, 1H, H-5′), 5.40 (d, *J* = 4.8 Hz, 1H, 2″-OH), 5.13 (d, *J* = 4.6 Hz, 1H, 4″-OH), 5.06 (d, *J* = 5.2 Hz, 1H, 3″-OH), 5.05 (d, *J* = 7.4 Hz, 1H, H-1″), 4.60 (m, 1H, 6″-OH), 3.70 (m, 1H, H-6″), 3.47 (m, 1H, H-6″), 3.43 (m, 1H, H-5″), 3.29 (m, 1H, H-3″), 3.26 (m, 1H, H-2″), 3.15 (m, 1H, H-4″); ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.9 (C-4), 163.1 (C-7), 161.7 (C-5), 158.9 (C-4′), 157.4 (C-8a), 156.6 (C-2′), 156.1 (C-2), 132.4 (C-6′), 121.0 (C-3), 108.6 (C-1′), 106.5 (C-5′), 106.3 (C-4a), 102.8 (C-3′), 100.1 (C-1″), 99.7 (C-6), 94.7 (C-8), 77.4 (C-5″), 76.6 (C-3″), 73.3 (C-2″), 69.8 (C-4″), 60.8 (C-6″); ESIHRMS *m/z* 449.1084 $[M+H]^+$ (calcd for $C_{21}H_{21}O_{11}$, 449.1084). The spectral data (specific rotation, NMR, and MS) that mentioned above were consistent with those that were previously reported [1].

4.8. 7-(2″,3″,4″,6″-Tetra-O-pivaloyl-β-glucopyranosyl)-2′,4′-(di-O-pivaloyl)-2′- hydroxygenistein (**13**)

To a solution of **12** (0.25 g, 0.32 mmol) in CH_2Cl_2 (3 mL), pyridine (1 mL) and PvCl (0.10 mL, 0.81 mmol) were added. After being stirred for 2 h at rt, water (20 mL) was poured into the solution and the resultant mixture was extracted with EtOAc (20 mL × 3). The organic layer was washed with saturated aqueous $CuSO_4$ solution (20 mL × 4), water (20 mL) and brine (20 mL) and was dried over Na_2SO_4. Filtration and concentration followed by silica gel chromatography (10–15% EtOAc in hexane) gave the title compound **13** (0.26 g, 0.27 mmol, 84% yield) as a colorless solid. $[\alpha]_D^{23}$ -22.6 (c 0.38, $CHCl_3$); IR (film) ν_{max} 2973, 1744, 1111, 760 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.58 (s, 1H, 5-OH), 7.77 (s, 1H, H-2), 7.30 (d, *J* = 8.4 Hz, 1H, H-6′), 7.03 (dd, *J* = 8.4, 2.3 Hz, 1H, H-5′), 6.96 (d, *J* = 2.3 Hz, 1H, H-3′), 6.48 (d, *J* = 2.2 Hz, 1H, H-8), 6.40 (d, *J* = 2.2 Hz, 1H, H-6), 5.41 (t, *J* = 9.4 Hz, 1H, H-3″), 5.30 (dd, *J* = 9.4, 7.9 Hz, 1H, H-

2″), 5.18 (d, J = 7.9 Hz, 1H, H-1″), 5.16 (t, J = 9.4 Hz, 1H, H-4″), 4.26 (dd, J = 12.3, 1.7 Hz, 1H, H-6″), 4.06 (dd, J = 12.3, 6.8 Hz, 1H, H-6″), 3.96 (ddd, J = 9.4, 6.8, 1.7 Hz, 1H, H-5″), 1.32 (s, 9H, Pv), 1.21 (s, 9H, Pv), 1.16 (s, 9H, Pv), 1.15 (s, 9H, Pv), 1.12 (s, 9H, Pv), 1.11 (s, 9H, Pv); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.9 (C-4), 178.1 (Pv), 177.1 (Pv), 176.49 (Pv), 176.46 (Pv), 176.2 (Pv), 162.7 (C-7), 162.4 (C-5), 157.5 (C-8a), 154.5 (C-2), 152.0 (C-4′), 149.9 (C-2′), 131.6 (C-6′), 120.8 (C-3), 120.7 (C-1′), 119.1 (C-5′), 116.6 (C-3′), 107.2 (C-4a), 100.0 (C-6), 98.4 (C-1″), 94.9 (C-8), 73.0 (C-5″), 71.7 (C-3″), 70.5 (C-2″), 67.7 (C-4″), 62.1 (C-6″), 39.14 (Pv), 39.06 (Pv), 38.81 (Pv), 38.78 (Pv), 38.7 (Pv), 27.09 (Pv), 27.05 (Pv), 27.0 (Pv), 26.9 (Pv); ESIHRMS m/z 953.4523 $[M+H]^+$ (calcd for $C_{51}H_{69}O_{17}$, 953.4535).

4.9. 5-(O-Methyl)-7-(2″,3″,4″,6″-tetra-O-pivaloyl-β-glucopyranosyl)-2′,4'-(di-O- pivaloyl)-2′-hydroxygenistein (**14**)

MeI (0.20 mL, 3.21 mmol) and DBU (0.60 mL, 4.02 mmol) were added to a solution of **13** (0.15 g, 0.16 mmol) in DMF (3 mL) under Ar atmosphere. After being stirred overnight at rt, water (20 mL) was poured into the solution and the resultant mixture was extracted with EtOAc (30 mL). The organic layer was washed with water (10 mL × 3) and brine (10 mL × 3) and was dried over Na_2SO_4. Filtration and concentration followed by silica gel chromatography (30% EtOAc in hexane) gave the title compound **14** (89 mg, 92 μmol, 58% yield) as a colorless solid. $[\alpha]_D^{23}$ -65.5 (c 0.33, $CHCl_3$); IR (film) ν_{max} 2973, 1745, 1113, 754 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (s, 1H, H-2), 7.32 (d, J = 8.4 Hz, 1H, H-6′), 6.99 (dd, J = 8.4, 2.3 Hz, 1H, H-5′), 6.91 (d, J = 2.3 Hz, 1H, H-3′), 6.55 (d, J = 2.2 Hz, 1H, H-8), 6.34 (d, J = 2.2 Hz, 1H, H-6), 5.42 (t, J = 9.4 Hz, 1H, H-3″), 5.30 (dd, J = 9.4, 7.9 Hz, 1H, H-2″), 5.18 (d, J = 7.9 Hz, 1H, H-1″), 5.17 (t, J = 9.4 Hz, 1H, H-4″), 4.28 (dd, J = 12.2, 1.5 Hz, 1H, H-6″), 4.06 (dd, J = 12.2, 6.6 Hz, 1H, H-6″), 3.97 (ddd, J = 9.4, 6.6, 1.5 Hz, 1H, H-5″), 3.85 (s, 3H, 5-OMe), 1.32 (s, 9H, Pv), 1.20 (s, 9H, Pv), 1.16 (s, 9H, Pv), 1.14 (s, 9H, Pv), 1.120 (s, 9H, Pv), 1.116 (s, 9H, Pv); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.0 (Pv), 177.1 (Pv), 176.48 (Pv), 176.46 (Pv), 176.4 (Pv), 174.1 (C-4), 161.5 (C-5), 160.9 (C-7), 159.4 (C-8a), 151.6 (C-2), 151.5 (C-4′), 149.6 (C-2′), 132.2 (C-6′), 122.6 (C-3), 122.1 (C-1′), 118.8 (C-5′), 116.1 (C-3′), 111.2 (C-4a), 98.7 (C-1″), 97.6 (C-6), 95.3 (C-8), 73.1 (C-5″), 71.6 (C-3″), 70.6 (C-2″), 67.6 (C-4″), 62.1 (C-6″), 56.4 (5-OMe), 39.1 (Pv), 39.0 (Pv), 38.80 (Pv), 38.79 (Pv), 38.7 (Pv), 27.08 (Pv), 27.07 (Pv), 27.05 (Pv), 27.0 (Pv), 26.9 (Pv); ESIHRMS m/z 967.4690 $[M+H]^+$ (calcd for $C_{52}H_{71}O_{17}$, 967.4691).

4.10. 5-(O-Methyl)-7-(β-Glucopyranosyl)-2′-hydroxygenistein (**2**)

To a solution of **14** (0.18 g, 0.19 mmol) in MeOH (4 mL), 5 M NaOMe in MeOH (0.26 mL, 1.30 mmol) was added. The resultant solution was refluxed for 1.5 h, cooled to rt, and neutralized with Amberlite IR-120H. The resin was filtered off and the filtrate was washed with cyclohexane (1 mL × 3). The MeOH layer was concentered and the residue was purified by solid phase extraction using InertSep Slim C18-B cartridge (20–50% MeOH in H_2O) followed by preparative RP-HPLC (35% MeOH in H_2O, t_R = 12.2 min). The title compound **2** (51 mg, 0.11 mmol, 58% yield) was obtained as a colorless solid. $[\alpha]_D^{23}$ -80.3 (c 0.10, 50% MeOH in H_2O); IR (film) ν_{max} 2972, 1745, 1135, 753 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.24 (m, 2H, 2′-OH, 4′-OH), 8.00 (s, 1H, H-2), 6.90 (d, J = 8.3 Hz, 1H, H-6′), 6.72 (d, J = 2.2 Hz, 1H, H-8), 6.59 (d, J = 2.2 Hz, 1H, H-6), 6.32 (d, J = 2.3 Hz, 1H, H-3′), 6.23 (dd, J = 8.3, 2.3 Hz, 1H, H-5′), 5.41 (d, J = 3.9 Hz, 1H, 2″-OH), 5.17 (d, J = 3.4 Hz, 1H, 4″-OH), 5.09 (d, J = 5.3 Hz, 1H, 3″-OH), 5.06 (d, J = 7.4 Hz, 1H, H-1″), 4.63 (m, 1H, 6″-OH), 3.82 (s, 3H, 5-OMe), 3.72 (m, 1H, H-6″), 3.44 (m, 2H, H-5″, H-6″), 3.36 (m, 1H, H-3″), 3.28 (m, 1H, H-2″), 3.15 (m, 1H, H-4″); ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.6 (C-4), 161.5 (C-7), 160.8 (C-5), 159.0 (C-8a), 158.5 (C-4′), 156.6 (C-2′), 152.1 (C-2), 132.3 (C-6′), 123.5 (C-3), 110.5 (C-1′), 109.7 (C-4a), 106.4 (C-5′), 103.0 (C-3′), 100.1 (C-1″), 97.3 (C-6), 95.8 (C-8), 77.5 (C-5″), 76.8 (C-3″), 73.3 (C-2″), 70.0 (C-4″), 60.9 (C-6″), 56.3 (5-OMe); ESIHRMS m/z 463.1243 $[M+H]^+$ (calcd for $C_{22}H_{23}O_{11}$, 463.1240). The spectral data (specific rotation, NMR, and MS) that mentioned above were consistent with those that were previously reported [1].

4.11. 7,2′,4'-(Tri-O-pivaloyl)-2′-hydroxygenistein (**15**)

To a solution of **4** (86 mg, 0.30 mmol) in CH_2Cl_2 (2 mL), pyridine (1 mL) and PvCl (0.23 mL, 1.87 mmol) were added. After being stirred for 2 h at rt, water (20 mL) was poured into the solution and the resultant mixture was extracted with EtOAc (20 mL × 3). The organic layer was washed with saturated aqueous $CuSO_4$ solution (20 mL × 4), water (20 mL) and brine (20 mL) and was dried over Na_2SO_4. Filtration and concentration followed by silica gel chromatography (20% EtOAc in hexane) gave the title compound **15** (0.14 g, 0.26 mmol, 87% yield) as a colorless solid. IR (film) ν_{max} 2975, 1753, 1088, 752 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.58 (s, 1H, 5-OH), 7.84 (s, 1H, H-2), 7.33 (d, J = 8.4 Hz, 1H, H-6′), 7.04 (dd, J = 8.4, 2.2 Hz, 1H, H-5′), 6.98 (d, J = 2.2 Hz, 1H, H-3'), 6.72 (d, J = 2.1 Hz, 1H, H-8), 6.55 (d, J = 2.1 Hz, 1H, H-6), 1.35 (s, 9H, Pv), 1.34 (s, 9H, Pv), 1.16 (s, 9H, Pv); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.3 (C-4), 176.5 (Pv), 176.2 (Pv), 176.0 (Pv), 162.2 (C-5), 156.8 (C-7), 156.7 (C-8a), 154.9 (C-2), 152.1 (C-4′), 149.9 (C-2′), 131.7 (C-6′), 120.8 (C-3), 120.7 (C-1′), 119.1 (C-5′), 116.6 (C-3′), 108.9 (C-4a), 105.7 (C-6), 100.9 (C-8), 39.3 (Pv), 39.2 (Pv), 39.1 (Pv), 27.1 (Pv), 27.0 (Pv), 26.9 (Pv); ESIHRMS m/z 539.2286 $[M+H]^+$ (calcd for $C_{30}H_{35}O_9$, 539.2281).

4.12. 5-(O-Methyl)-7,2′,4'-(tri-O-pivaloyl)-2′-hydroxygenistein (**16**)

MeI (0.10 mL, 1.61 mmol) and DBU (0.30 mL, 2.01 mmol) were added to a solution of **15** (0.20 g, 0.37 mmol) in DMF (2 mL) under Ar atmosphere. After being stirred overnight at rt, water (20 mL) was poured into the solution and the resultant mixture was extracted with EtOAc (30 mL). The organic layer was washed with water (10 mL × 3) and brine (10 mL × 3) and was dried over Na_2SO_4. Filtration and concentration followed by silica gel chromatography (30% EtOAc in hexane) gave the title compound **16** (86 mg, 0.16 mol, 43% yield) as a colorless solid. IR (film) ν_{max} 2975, 1752, 1094, 750 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (s, 1H, H-2), 7.35 (d, J = 8.4 Hz, 1H, H-6′), 7.00 (dd, J = 8.4, 2.3 Hz, 1H, H-5′), 6.92 (d, J = 2.3 Hz, 1H, H-3'), 6.79 (d, J = 2.1 Hz, 1H, H-8), 6.53 (d, J = 2.1 Hz, 1H, H-6), 3.92 (s, 3H, 5-OMe), 1.37 (s, 9H, Pv), 1.33 (s, 9H, Pv), 1.16 (s, 9H, Pv); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.52 (Pv), 176.50 (Pv), 176.1 (Pv), 174.4 (C-4), 161.2 (C-5), 158.6 (C-7), 155.2 (C-8a), 152.1 (C-2), 151.5 (C-4′), 149.6 (C-2′), 132.3 (C-6′), 122.6 (C-3), 122.2 (C-1′), 118.8 (C-5′), 116.2 (C-3′), 112.8 (C-4a), 103.0 (C-6), 101.3 (C-8), 56.6 (5-OMe), 39.4 (Pv), 39.1 (Pv), 39.0 (Pv), 27.1 (Pv), 27.0 (Pv), 26.9 (Pv); ESIHRMS m/z 553.2441 $[M+H]^+$ (calcd for $C_{31}H_{37}O_9$, 553.2438).

4.13. 5-(O-Methyl)-2′-hydroxygenistein (**5**)

To a solution of **16** (0.16 g, 0.29 mmol) in MeOH (3 mL), 5 M NaOMe in MeOH (0.17 mL, 0.85 mmol) was added. The resultant solution was refluxed for 2 h, cooled to rt, and neutralized with Amberlite IR-120H. Filtration and concentration followed by silica gel chromatography (10% MeOH in $CHCl_3$) gave the title compound **5** (72 mg, 0.24 mmol, 83% yield) as a colorless solid. IR (film) ν_{max} 3180, 1573, 1085, 844 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 7.93 (s, 1H, H-2), 7.00 (d, J = 8.1 Hz, 1H, H-6′), 6.42 (d, J = 2.4 Hz, 1H, H-3'), 6.41 (d, J = 2.2 Hz, 1H, H-8), 6.37 (d, J = 2.2 Hz, 1H, H-6), 6.35 (dd, J = 8.1, 2.4 Hz, 1H, H-5'), 3.87 (s, 3H, Me); ^{13}C NMR (100 MHz,

CD_3OD) δ 179.0 (C-4), 166.1 (C-7), 163.0 (C-5), 161.5 (C-8a), 160.2 (C-4′), 158.1 (C-2′), 154.4 (C-2), 133.0 (C-6′), 125.2 (C-3), 112.6 (C-1′), 108.6 (C-4a), 108.3 (C-5′), 104.8 (C-3′), 98.0 (C-6), 96.3 (C-8), 56.4 (5-OMe); ESIHRMS *m/z* 301.0715 $[M+H]^+$ (calcd for $C_{16}H_{13}O_6$, 301.0712).

4.14. Tyrosinase inhibitory assay

The diphenolase assay was performed as previously reported with a slight modification [32,33]. All inhibitors were first dissolved in DMSO and used for the experiment by appropriate dilution with DMSO. First, 0.1 mL of the DMSO solution of inhibitors was mixed with 0.3 mL of a 5.0 mM of DOPA aqueous solution, 0.6 mL of 0.25 M sodium phosphate buffer (pH 6.8), and 1.9 mL of water, incubated at 30 °C for 5 min. Then, 0.1 mL of the 0.05 M sodium phosphate buffer solution (pH 6.8) of the mushroom tyrosinase (0.2 μg/mL) was added. This solution was immediately monitored for the formation of dopachrome by measuring the linear increase in absorbance (475 nm) at 30 °C. Absorption measurements were recorded using a Jasco V-630 spectrophotometer.

The experimental data were delineated and analyzed by using Sigma Plot 12 (Systat Software Inc, San Jose, CA). The IC_{50} was obtained by fitting experiment data to the logistic curve as below [34].

$$\text{Activity}\ (\%) = 100(y_{min} + ((y_{max} - y_{min})/(1 + [I]/IC_{50})))$$

The y is the v_i/v_0 for a given data set. Here v_i and v_0 are the velocities of the enzyme-catalyzed reaction in the presence and absence of inhibitor, respectively.

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Appendix A. Supplementary data

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