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Molecular design of potent tyrosinase inhibitors having the bibenzyl skeleton

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ABSTRACT

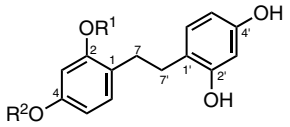
In order to develop water soluble tyrosinase inhibitors, bibenzyl xyloside **1** isolated from *Chlorophytum arundinaceum* (liliaceae), and its derivatives **2** and **3** were synthesized by using Wittig reaction and trichloroimidate glycosylation procedure as key steps. Xylosides **1–3** showed potent tyrosinase inhibitory activity with IC_{50}s of 1.6, 0.43, and 0.73 μM, respectively, although each NMR data of synthetic bibenzyls was not identical to that of naturally occurring xyloside **1**.

The enzymatic oxidation of phenol to *o*-quinone observed in the early stage of various browning phenomena in nature, is mainly catalyzed by tyrosinase (EC 1.14.18.1), a copper containing oxidoreductase.[1,2] In mammals, L-tyrosine is a typical substrate that is enzymatically oxidized to dopaquinone via L-DOPA, and finally transformed to a black pigment, melanin with the aid of enzymatic and non-enzymatic reactions.[3] This pathway is of considerable importance since melanin possesses many functions. Alterations in melanin synthesis occur in many disease states. Melanin pigments are also found in the mammalian brain. Tyrosinase may play a significant role in neuromelanin formation in the human brain and could be central to dopamine neurotoxicity as well as contribute to the neurodegeneration associated with Parkinson's disease.[4,5] Melanoma specific anticarcinogenic activity is also known to be linked with tyrosinase activity.[6]

As prevalent tyrosinase inhibitors, kojic acid and 4-hexylresorcinol are used to prevent deposition of melanin pigments. Kojic acid, a fungal metabolite produced by many species of *Aspergillus* and *Penicillium*, is a good chelator of transition metal ions[7] and a good scavenger of free radicals.[8] Tyrosinase contains binuclear copper ions at its active site. By chelating these copper ions, kojic acid seems to act as an inhibitor. It should be, however, noted that the activity of kojic acid is weaker than that of 4-hexylresorcinol. Recently, the use of 4-hexylresorcinol is considered to be safe in the food industry and is quite effective in the prevention of shrimp melanosis and for browning control in fresh and dried fruit slices.[9] Structure-related activity of flavonoids and stilbenes, obtained from plants and by chemical syntheses, suggested that compounds possessing 4-substituted resorcinol skeleton would exhibit potent tyrosinase inhibitory activity.[10,11]

In 1993, bibenzyl xyloside **1** was tentatively isolated as a secondary metabolite from the methanol extract of *Chlorophytum arundinaceum* (liliaceae) (Fig. 1).[12] Although this plant has been used in folk medicine and is known to possess adaptogenic activity,[13] the synthesis and biological evaluation of **1** have not been carried out. Two 4-substituted resorcinol moieties are found in the structure of **1**, suggesting that this compound would be a potent tyrosinase inhibitor. Therefore, we performed concise syntheses and evaluated the tyrosinase inhibitory activity of **1**, and its derivatives **2**–**4**.

As shown in Scheme 1, bibenzyl xyloside **1** was synthesized via Wittig reaction and trichloroimidate glycosylation procedure as key steps. The starting material, 2,4-dihydroxybenzaldehyde was selectively converted to aldehyde **5** that possessed only a benzyloxy moiety at 4-position, by using benzyl bromide and $NaHCO_3$ as a mild base.[14] In order to demonstrate further selective xylosylation at 2-position in the bibenzyl skeleton, a phenolic hydroxyl at 2-position in **5** was protected as a methoxymethyl



1: R^1 = Xyl, R^2 = H
2: R^1 = H, R^2 = Xyl
3: R^1 = R^2 = Xyl
4: R^1 = R^2 = H

Figure 1. Structure of bibenzyl derivatives **1**–**4**.

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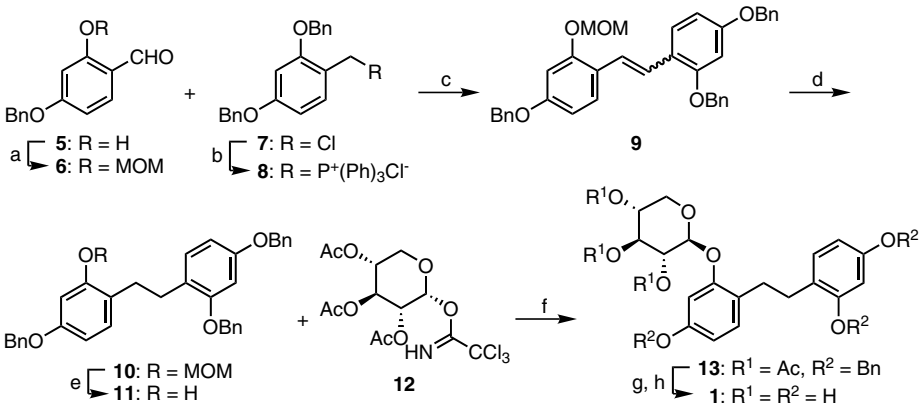


Scheme 1. Synthesis of bibenzyl xyloside **1**. Reagents and conditions: (a) MOMCl, TBAI, DIPEA, CH_2Cl_2, 0 °C to rt, 12 h, 100%; (b) TPP, toluene, reflux, 0.5 h, 59%; (c) LiHMDS, THF, 0 °C to rt, 1 h, 90%; (d) H_2-Pd(en)/C, rt, 12 h, 86%; (e) TsOH, THF, MeOH, reflux, 2 h, 89%; (f) TMSOTf, CH_2Cl_2, 0 °C, 5 min, 100%; (g) H_2-Pd$(OH)_2$/C, rt, 12 h, 100%; (h) NaOMe, MeOH, 0 °C, 0.5 h, then H^+, 94%.

(MOM) group and aldehyde **6** was obtained in quantitative yield. Chloride **7** was prepared from 2,4-dihydroxybenzaldehyde, the same starting material of **6**, through successive three steps involving benzylation of both phenolic hydroxyls, reduction of the aldehyde group, and chlorination of benzylic hydroxyl by using $SOCl_2$, respectively.[15] To obtain phosphonium salt **8**, chloride **7** was refluxed in toluene in the presence of triphenylphosphine (TPP). Wittig reaction took place between an ylide from **8** and aldehyde **6** under basic condition, and, thus, stilbene **9** was furnished in 90% yield. The ratio of *cis* and *trans* stilbenes is 2–1, which was estimated by ^{1}H NMR analysis. Hydrogenation on an olefin of **9** was preferentially performed to obtain bibenzyl **10** by using a Pd/C-ethylenediamine complex (Pd(en)/C) as a catalyst.[16] The removal of MOM group in **10** by tosyl acid led to bibenzyl **11**, a significant intermediate for the total synthesis of **1**, in excellent yield. Bibenzyl **11** and imidate **12** prepared from D-xylose, were coupled in the presence of catalytic amount of trimethylsilyl trifluoromethanesulfonate (TMSOTf). This reaction proceeded as a highly stereo-selective manner, and a β-xyloside **13** was obtained in quantitative yield. Finally, the successive removal of benzyl moieties by hydrogenolysis using Pd$(OH)_2$/C catalyst and acetyl moieties by an ester exchange reaction using sodium methoxide, respectively, gave **1** in 94% yield (two steps). In 65% overall yield, a target compound **1** was synthesized from **5** via seven steps.

The synthetic compound **1** was fully characterized by spectroscopic data (specific rotation, IR, UV, ^{1}H and ^{13}C NMR, DQF-COSY, HMQC, HMBC, and ESIHRMS).[17] As a result, it is clarified that the most of NMR data of **1** were inconsistent with those as previously reported (Tables 1 and 2).[12] For instance, NMR signals of two bibenzyl methylenes observed at δ_H 3.50 and 3.35, and δ_C 31.2 and 31.0, were assigned to δ_H 2.94 (4H), and δ_C 39.5 and 37.2 in the early study. Likewise, two phenyl methines (δ_H 7.42 and δ_C 104.0, and δ_H 7.01 and δ_C 103.2) were placed at C-3 and C-3′ by the HMQC and HMBC experiments, whereas the same signals had emerged as δ_H 7.38 and δ_C 116.9, and δ_H 7.26 and δ_C 119.5, respectively. Thus, the natural occurrence of bibenzyl **1** would be in question.

It should be noted that the IC_{50} value of **1** shows 1.6 μM, which is five times more potent than that of kojic acid (Table 3).[18] Biben-

Table 1
^{1}H NMR data of naturally occurring xyloside **1**, and synthetic derivatives **1** and **2** (400 MHz in pyridine-d_5)

	Position	Natural **1**[a] δ_H (mult., *J* in Hz)	Synthetic **1** δ_H (mult., *J* in Hz)	Synthetic **2** δ_H (mult., *J* in Hz)
Aglycone	3	7.38 (br s)	7.42 (d, 2.0)	7.22 (d, 2.4)
	5	7.03 (d, 8.0)	6.90 (dd, 2.0, 8.0)	6.90 (dd, 2.4, 8.3)
	6	7.20 (d, 8.0)	7.32 (d, 8.0)	7.33 (d, 8.3)
	7	2.94 (t, 7.0)	3.50 (m)	3.44 (m)
	3′	7.26 (br s)	7.01 (d, 2.0)	7.08 (d, 2.4)
	5′	7.03 (d, 8.0)	6.73 (dd, 2.0, 7.8)	6.80 (dd, 2.4, 8.3)
	6′	7.20 (d, 8.0)	7.33 (d, 7.8)	7.35 (d, 8.3)
	7′	2.94 (t, 7.0)	3.35 (m)	3.44 (m)
Xyl	1	5.42 (d, 7.0)	5.43 (d, 7.8)	5.44 (d, 6.8)
	2	3.95–4.95 (m)	4.34 (dd, 7.8, 8.3)	4.31 (m)
	3	3.95–4.95 (m)	4.20 (d, 8.3, 8.8)	4.23 (m)
	4	3.95–4.95 (m)	4.26 (m)	4.26 (m)
	5	3.95–4.95 (m)	4.28 (dd, 4.9, 10.7)	4.28 (m)
			3.66 (m)	3.66 (dd, 9.3, 10.2)

[a] These assignments have been reported previously.[12]

Table 2
^{13}C NMR data of naturally occurring xyloside **1**, and synthetic derivatives **1** and **2** (100 MHz in pyridine-d_5)

	Position	Natural **1**[a] δ_C (mult.)	Synthetic **1** δ_C (mult.)	Synthetic **2** δ_C (mult.)
Aglycone	1	131.4 (s)	122.6 (s)	123.5 (s)
	2	147.6 (s)	157.1 (s)	157.1 (s)
	3	116.9 (d)	104.0 (d)	104.4 (d)
	4	146.6 (s)	157.7 (s)	157.7 (s)
	5	125.1 (d)	109.3 (d)	107.2 (d)
	6	128.0 (s)	130.3 (s)	130.4 (s)
	7	39.5 (t)	31.2 (t)	30.8 (t)
	1′	128.6 (s)	120.1 (s)	120.1 (s)
	2′	146.4 (s)	156.8 (s)	157.0 (s)
	3′	119.5 (d)	103.2 (d)	103.3 (d)
	4′	146.6 (s)	157.6 (s)	157.3 (s)
	5′	120.6 (d)	106.7 (d)	106.6 (d)
	6′	126.1 (s)	130.9 (s)	130.6 (s)
	7′	37.2 (t)	31.0 (t)	30.6 (t)
Xyl	1	104.9 (d)	103.2 (d)	102.8 (d)
	2	74.9 (d)	74.3 (d)	74.2 (d)
	3	78.0 (d)	77.7 (d)	77.8 (d)
	4	71.0 (d)	70.2 (d)	70.3 (d)
	5	62.1 (t)	66.5 (t)	66.6 (t)

[a] These assignments have been reported previously.[12]

Table 3
Tyrosinase inhibitory activities of bibenzyl derivatives **1**–**4** and kojic acid

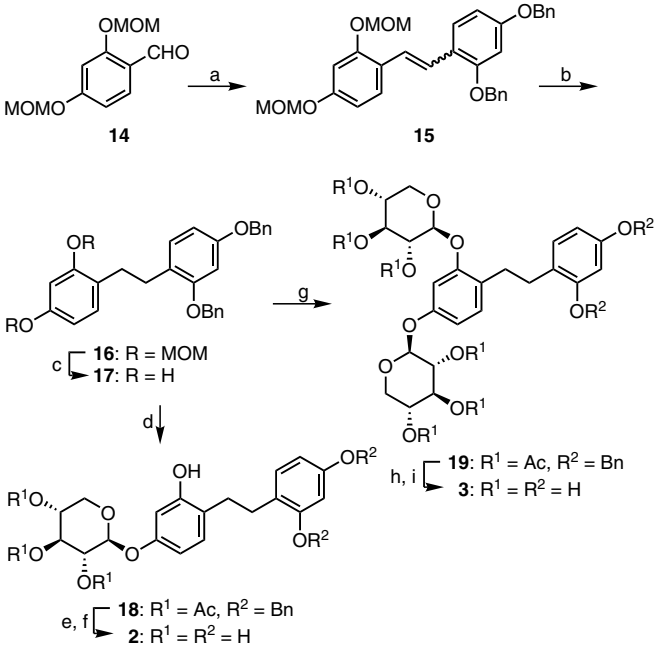
Compounds tested	IC_{50} (μM)
1	1.6 ± 0.43[a]
2	0.43 ± 0.18
3	0.73 ± 0.11
4	0.37 ± 0.06
Kojic acid	7.4 ± 1.4

[a] The IC_{50} values represent means ± SE of three different experiments.

zyl compounds have been isolated from extracts of several organisms including liverwort, algae, and fern,[19] which are known to be possess various biological activities such as antifungal, phytotoxic and anti-HIV effects.[20–22] However, the bibenzyl glycoside having significant tyrosinase inhibitory activity is a novel observation. These structural and biological findings prompted us to further investigate congeners **2**–**4** by a chemical approach.

Bibenzyl derivative **2** was synthesized as illustrated in Scheme 2. With the use of the similar synthetic pathway, derivative **3** could be prepared, effectively. Firstly, both hydroxyl groups of 2,4-dihydroxybenzaldehyde as the same starting materiel of **1** were protected by using MOMCl under basic condition. Phosphonium salt **8** and aldehyde **14** obtained were coupled by Wittig reaction and stilbene **15** yielded in 84%. Sixty percent of **15** were found as *cis*-form, which was calculated by the ^{1}H NMR experiment. Following selective hydrogenation by using Pd(en)/C as a catalyst, **15** was smoothly transformed to bibenzyl **16** in 72% yield. Bibenzyl **17**, a key intermediate was prepared from **16** in quantitative yield by the removal of all MOM groups under acidic condition. In the next reaction, the selectivity of products **2** or **3** was governed by the amount of imidate **12**. When 1.5 equivalent of **12** was used as a glycosylation donor, β-xyloside **18** was obtained in 71% yield and dixyloside **19** was not detected by TLC analyses. Successive removal of the benzyl and acetyl moieties by hydrogenolysis and sodium methoxide, respectively, afforded xyloside **2**, a regioisomer of **1** in 85% yield (two steps). In contrast, **19** was synthesized in 77% by using 4.0 equiv of donor **12**. Removal of all protective groups in **19** gave dixyloside **3** in 84% yield (two steps). Consequently, derivatives **2** and **3** were concisely synthesized from **14** via six steps in 37% and 39% overall yields, respectively.[23,24] Symmetric bibenzyl **4** was readily prepared by the method as previously reported.[25]

Although NMR data of **2** were not identical to that of the bibenzyl reported as a constituent of *C. arundinaceum*, bibenzyls **2**–**4** show remarkable tyrosinase inhibitory activity (Table 3). Especially, IC_{50} of **2** represented 0.43 μM, indicating that this compound



Scheme 2. Syntheses of bibenzyl xylosides **2** and **3**. Reagents and conditions: (a) **8**, LiHMDS, THF, 0 °C to rt, 1 h, 84%; (b) H_2-Pd(en)/C, rt, 12 h, 72%; (c) TsOH, THF, MeOH, reflux, 2 h, 100%; (d) **12**, TMSOTf, CH_2Cl_2, 0 °C, 5 min, 71%; (e) H_2-Pd$(OH)_2$/C, rt, 12 h, 96%; (f) NaOMe, MeOH, 0 °C, 0.5 h, then H^+, 89%; (g) **12** (excess), TMSOTf, CH_2Cl_2, 0 °C, 5 min, 77%; (h) H_2-Pd$(OH)_2$/C, rt, 12 h, 87%; (i) NaOMe, MeOH, 0 °C, 0.5 h, then H^+, 97%.

most effective among all the bibenzyl xylosides synthesized. The xylosyl site on the bibenzyl skeleton has obviously an influence on tyrosinase inhibitory activity. The inhibitory activity of xyloside **2** was comparable to that of bibenzyl **4**. Hence, this resorcinol moiety could be easily to bind on the active site of the tyrosinase because of the absence of the bulky xylosyl substitution at 2-position. The hydrophobic interaction occurred between the tyrosinase inhibitor and the active site of tyrosinase would contribute to the inhibitory activity.[26,27] However, the effect of the hydrophilic substituent on tyrosinase inhibitors had not been well documented. Since derivatives **1–3** were glucosides, they can be lead compounds for the exploring tool of new hydrophilic interaction between the enzyme and inhibitor as well as for the development of water soluble tyrosinase inhibitors with efficiency.

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17. Compound **1**: colorless solid; α_D^{20} −10.6 (*c* 0.09, MeOH); IR (Nujol) ν_{max} 3350, 1603, 1508 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; ESIHRMS *m*/*z* 377.1240 [M−H]$^-$ (Calcd for $C_{19}H_{21}O_8$, 377.1236).
18. The assay was performed as previously reported.[28] The commercial mushroom tyrosinase purchased from Sigma (St. Louis, Mo) was purified by the procedure as previously reported.[29].
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23. Compound **2**: colorless solid; α_D^{20} −5.9 (*c* 0.17, MeOH); IR (Nujol) ν_{max} 3520, 3350, 1595, 1510 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; ESIHRMS *m*/*z* 377.1234 [M−H]$^-$ (Calcd for $C_{19}H_{21}O_8$, 377.1236).
24. Compound **3**: colorless solid; α_D^{20} −48.0 (*c* 0.05, MeOH); IR (Nujol) ν_{max} 3350, 1508 cm^{-1}; ^{1}H NMR (pyridine-d_5, 400 MHz) δ 7.49 (1H, br s), 7.31 (1H, d, *J* = 8.3 Hz), 7.28 (1H, d, *J* = 8.3 Hz), 7.04 (1H, d, *J* = 8.3 Hz), 7.02 (1H, br s), 6.72 (1H, d, *J* = 8.3 Hz), 5.52 (1H, d, *J* = 5.4 Hz), 5.38 (1H, d, *J* = 7.3 Hz), 4.28 (8H, m), 3.85 (1H, t, *J* = 9.8 Hz), 3.67 (1H, dd, *J* = 8.8, 11.2 Hz), 3.43 (2H, m), 3.30 (2H, m); ^{13}C NMR (pyridine-d_5, 100 MHz) δ 157.6, 157.2, 156.8, 156.7, 130.8, 130.1, 126.0, 119.9, 109.6, 106.7, 105.3, 103.2, 103.1, 102.7, 77.8, 77.6, 74.2, 70.3, 70.2, 66.6, 66.5, 30.90, 30.86; ESIHRMS *m*/*z* 509.1649 [M−H]$^-$ (Calcd for $C_{24}H_{29}O_{12}$, 509.1659).
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