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Syntheses of hydroxy substituted 2-phenyl-naphthalenes as inhibitors of tyrosinase

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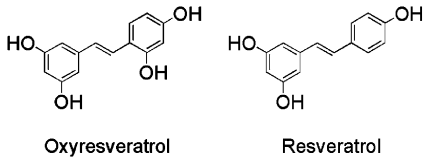
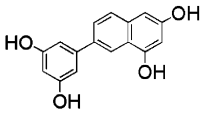
Abstract—Oxyresveratrol and resveratrol, with hydroxy substituted *trans*-stilbene structure, exert potent inhibitory effects on cyclooxygenase, rat liver mitochondrial ATPase activity, and tyrosinase. As the isosteres of oxyresveratrol, a new family of hydroxyl substituted phenyl-naphthalenes were synthesized to show excellent inhibition of mushroom tyrosinase. Compound **10**, which is isostere of resveratrol, showed IC_{50} value of 16.52 μM in mushroom tyrosinase activity. As compared to this, the reference compound, resveratrol, showed IC_{50} value of 55.61 μM. Compound **4**, which is isostere of oxyresveratrol, showed IC_{50} value of 0.49 μM. Among the other three derivatives, compound **13** showed IC_{50} value of 0.034 μM.

Oxyresveratrol (*trans*-2,3′,4,5′-tetrahydroxystilbene), available from mulberry wood (Morus alba L.), has the structure of hydroxy substituted stilbene. It has been known that oxyresveratrol is transported to tissues at high rates resulting in a bioavailability around 50%.[1] Pharmacological studies have demonstrated that oxyresveratrol can be used as an active ingredient in dermatology.[2,3] It also has been revealed that the compound has potent inhibitory effects on cyclooxygenase,[4,5] rat liver mitochondrial ATPase activity,[6] and DOPA oxidase activity.[4] Tyrosinase catalyzes two distinct reactions of the conversion of tyrosine to DOPA.[7] Tyrosinase is responsible for unwanted browning of fruits and vegetables, and coloring of skin, hair, and eyes in animals including human beings.[2] Many tyrosinase inhibitors have been reported, including hydroquinone,[8] vitamin C,[9] kojic acid,[10] albutin,[11] resveratrol,[12] and oxyresveratrol.[4]

In this report, we reveal hydroxy substituted 2-phenyl-naphthalenes as new inhibitors of mushroom tyrosinase.





4 (HS-1713)

For in vivo experiments for the melanin-related disorders, it was necessary to synthesize oxyresveratrol in large quantity. As shown in Scheme 1, for the synthesis of oxyresveratrol, when Wittig reaction was done to generate compound **2**, the *cis*/*trans* mixture was obtained. The wanted compound **2** with *trans*-olefin was separated, by using column chromatography using silica gel, for the synthesis of oxyresveratrol. The byproduct, *cis*-isomer of compound **2**,[14b] was treated with iodine in chloroform to generate *cis*/*trans* mixture again to recover more of the *trans* compound **2**.[14a] But to our surprise, the obtained major product was identified to be 7-(3,5-dimethoxyphenyl)-1,3-dimethoxynaphthalene

Keywords: Tyrosinase; Resveratrol; Oxyresveratrol; X-ray structure.
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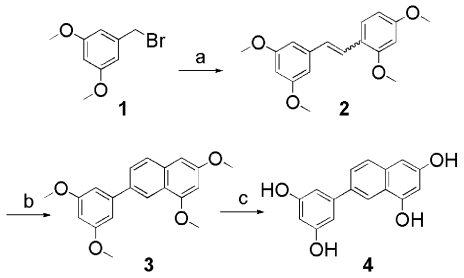


Scheme 1. Reagents and conditions: (a) i—PPh_3, C_6H_6, reflux; ii—*t*-BuOK, EtOH; iii—3,5-dimethoxy-benzaldehyde; (b) I_2, $CHCl_3$; (c) BBr_3, CH_2Cl_2, reflux.

(**3**).[14c] Considerable efforts were directed toward identification of the structure of compound **3**, utilizing HRMS (Korea Basic Science Institute: Daegu) and experiments with 500 MHz NMR including DEPT, COSY, HMQC, and HMBC. In addition to this, the structure of single crystal of compound **3** obtained from a CH_2Cl_2 solution was confirmed by X-ray diffraction as shown in Figure 1. Crystal of compound **3** belongs to the triclinic system, space group P-1; $a = 7.1600$ (8) Å, $b = 10.9250$ (12) Å, $c = 11.8562$ (14) Å, $\alpha = \beta = 105$ °, $\gamma = 107$ °, volume = 798.49 (16) Å, $Z = 2$, $D_{calcd} = 1.349$ Mg/m^3, $m = 0.093$ mm^{-1}, $F(000) = 344$. The final value of $R[I > 2\sigma(I)]$ was 0.0664, $wR_2 = 0.1436$, GooF = 1.000.[13]

After demethylation of compound **3**, the tetrahydroxy substituted compound **4**[14d] was obtained to show excellent inhibition of mushroom tyrosinase with IC_{50} value of 0.49 μM (resveratrol: 55.61 μM). With the structure of the new lead compound in hand, other hydroxy substituted 2-phenyl-naphthalenes were synthesized by using other simple method.

The synthetic routes of hydroxy substituted 2-phenyl-naphthalenes are shown in Schemes 1 and 2. Commercially available 1-bromomethyl-3,5-dimethoxybenzene (**1**) was treated with triphenyl phosphine in *N*,*N*-dimethylformamide (DMF) at reflux to generate the phosphonium salt. The phosphonium salt was reacted with potassium *tert*-butoxide in ethanol and 3,5-dimethoxy-banzaldehyde to generate *cis*/*trans* mixture of 2,3′,4,5′-tetramethoxy-*trans*-stilbene (**2**) in 74% yield. The solution of compound **2** in chloroform was treated with catalytic amount of iodine (0.05 equiv) and stirred

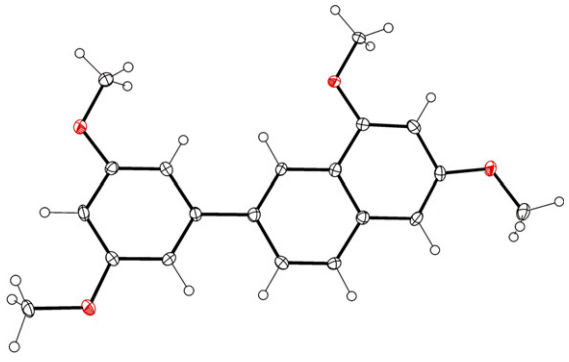
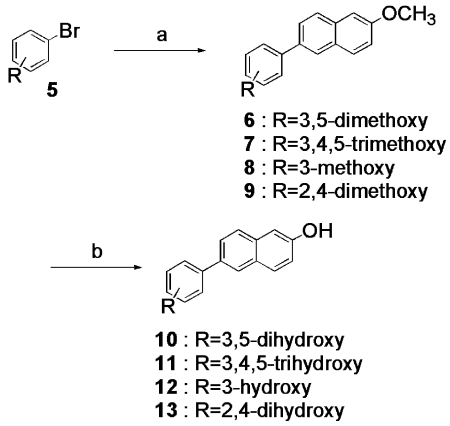


Figure 1. X-ray structure of **3**.



Scheme 2. Reagents and conditions: (a) i—2-bromo-6-methoxy-naphthalene, Mg, I_2, THF; ii—Ni(dppp)Cl_2, THF; iii—**5**; (b) BBr_3, CH_2Cl_2, reflux.

overnight at room temperature to generate 7-(3,5-dimethoxyphenyl)-1,3-dimethoxynaphthalene (**3**) in 72% yield. 7-(3,5-Dimethoxyphenyl)-1,3-dimethoxy-naphthalene (**3**) was treated with boron tribromide in methylene chloride to obtain 7-(3,5-dihydroxyphenyl)-1,3-naphthalenediol (**4**) in 27% yield.

The synthetic route of compounds **10**–**13** is shown in Scheme 2. 2-Bromo-6-methoxy-naphthalene was treated with magnesium turnings, iodine, Ni(dppp)Cl_2, and 1-bromo-3,5-dimethoxy-benzene to generate 2-(3,5-dimethoxyphenyl)-6-methoxy-naphthalene, which was converted to 5-(6-hydroxy-2-naphthyl)-1,3-benzenediol (**10**)[14i] using boron tribromide. 5-(6-Hydroxy-2-naphthyl)-1,2,3-benzenetriol (**11**),[14j] 6-(3-hydroxyphenyl)-2-naphthol (**12**),[14k] and 4-(6-hydroxy-2-naphthyl)-1,3-benzenediol (**13**)[14l] were synthesized using the same method using appropriate starting materials.

Compound **3** contains extended conjugated systems which can react as both diene and dienophile moieties. With hydroxy substituted stilbene, Diels–Alder reactions followed by the fragmentation of the hydroxy substituted benzene have been reported before, which generated di-phenyl substituted naphthalene.[15] The compound which was obtained in our case contains the mono-phenyl substituted naphthalene system, which is isostere of the oxyresveratrol. Fragmentation of two 1,3-dimethoxybenzenes generated mono-phenyl substituted naphthalene system, which was caused by substitution pattern of the methoxy groups on the benzene as compared to the reported case.

While compounds **11** and **13** are new, compounds **4**,[16] **10**,[17,18] and **12**[19] have been illustrated before. But there has been no report about the inhibition of tyrosinase activity Table 1.

Compound **10**, which is isostere of resveratrol, showed IC_{50} value of 16.52 μM in mushroom tyrosinase activity.[20] As compared to this, the reference compound, resveratrol,[12] showed IC_{50} value of 55.61 μM. Compound **4**, which is isostere of oxyresveratrol, showed IC_{50} value

Table 1. Inhibition effects of hydroquinone, kojic acid, resveratrol, and compounds **4** and **10**–**13** on mushroom tyrosinase activity

Compound	HS #	IC_{50}[a] (μM)
Hydroquinone		33.48(±1.70)
Kojic acid		38.24(±1.47)
Resveratrol		55.61(±2.77)
4	HS-1713	0.49(±0.47)
10	HS-1784	16.52(±0.65)
11	HS-1791	2.95(±1.26)
12	HS-1792	6.40(±0.30)
13	HS-1793	0.034(±0.01)

[a] Values are means of three experiments, standard deviation is given in parentheses.

of 0.49 μM. Among the other three derivatives, compound **13** showed IC_{50} value of 0.034 μM.

Acknowledgment

This work was supported for two years by Pusan National University Research Grant (H. Suh).

Supplementary data

Supplementary data associated with compound **3** including $^{1}H/^{13}C$ NMR, DEPT, COSY, HMQC, HMBC, and X-ray diffraction data. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.10.025.

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13. Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 615494. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].
14. (a) *trans*-**2**: white solid: mp 56 °C; R_f 0.22 (SiO_2, 17% EtOAc–Hex); ^{1}H NMR (300 MHz, benzene-d_6) δ 3.29 (s, 3H), 3.35 (s, 6H), 3.38 (s, 3H), 6.40 (d, 1H, J = 2.2 Hz), 6.43 (dd, 1H, J = 2.5, 8.3 Hz), 6.53 (t, 1H, J = 2.2 Hz), 6.88 (d, 2H, J = 2.2 Hz), 7.15 (d, 1H, J = 16.1 Hz), 7.50 (d, 1H, J = 8.3 Hz), 7.82 (d, 1H, J = 16.2 Hz); ^{13}C NMR (75 MHz, benzene-d_6) δ 55.5, 55.6, 55.7, 98.7, 99.6, 104.6, 105.2, 119.5, 124.1, 127.2, 127.6, 140.6, 158.3, 160.8, 161.1; HRMS (EI), *m*/*z* 300.1359 (calculated for $C_{18}H_{20}O_4$ 300.1362).; (b) *cis*-**2**: colorless oil; R_f 0.27 (SiO_2, 17% EtOAc–Hex); ^{1}H NMR (300 MHz, benzene-d_6) δ 3.26 (s, 6H), 3.28 (s, 3H), 3.30 (s, 3H), 6.11 (dd, 1H, J = 2.2, 8.2 Hz), 6.41 (d, 1H, J = 2.2 Hz), 6.48 (t, 1H, J = 2.2 Hz), 6.56 (d, 1H, J = 12.1 Hz), 6.72 (d, 2H, J = 2.2 Hz), 6.96 (d, 1H, J = 12.4 Hz), 7.44 (d, 1H, J = 8.5 Hz); ^{13}C NMR (75 MHz, benzene-d_6) δ 55.3, 55.4, 55.6, 98.2, 99.6, 104.2, 106.7, 118.7, 125.8, 129.0, 130.9, 139.6, 158.3, 160.4, 160.5.; (c) Compound **3**: white solid: mp 89 °C; R_f 0.27 (SiO_2, 17% EtOAc–Hex); ^{1}H NMR (300 MHz, benzene-d_6) δ 3.35 (s, 6H), 3.37 (s, 3H), 3.51 (s, 3H), 6.54 (d, 1H, J = 1.8 Hz), 6.63 (t, 1H, J = 2.2 Hz), 6.66 (d, 1H, J = 2.2 Hz), 7.07 (d, 2H, J = 2.2 Hz), 7.69 (d, 1H, J = 8.8 Hz), 7.80 (dd, 1H, J = 1.8, 8.4 Hz), 8.84 (d, 1H, J = 1.5 Hz); ^{13}C NMR (75 MHz, benzene-d_6) δ 55.2, 55.3, 55.4, 98.6, 98.9, 100.4, 106.3, 121.2, 123.0, 127.6, 127.8, 135.6, 137.0, 144.7, 157.7, 158.4, 162.3; HRMS (EI), *m*/*z* 324.1359 (calculated for $C_{20}H_{20}O_4$ 324.1362).; (d) Compound **4**: dark brown solid: mp 119 °C; R_f 0.22 (SiO_2, 66% EtOAc–Hex); ^{1}H NMR (300 MHz, benzene-d_6) δ 6.76 (s, 1H), 6.92 (s, 1H), 7.00 (s, 1H), 7.11 (s, 2H), 7.60 (d, 1H, J = 8.5 Hz), 7.74 (d, 1H, J = 8.5 Hz), 8.77 (s, 1H); ^{13}C NMR (75 MHz, benzene-d_6) δ 101.7, 101.9, 102.2, 106.8, 121.0, 121.7, 126.9, 127.8, 135.6, 136.8, 145.0, 155.9, 156.7, 159.6; HRMS (EI), *m*/*z* 268.0731 (calculated for $C_{16}H_{12}O_4$ 268.0736); (e) Compound **6**: dark brown solid: mp 77 °C; R_f 0.25 (SiO_2, 20% EA–Hex); ^{1}H NMR (300 MHz, benzene-d_6) δ 3.39 (s, 6H), 3.40 (s, 3H), 6.64 (t, 1H, J = 5.2 Hz), 6.96 (d, 1H, J = 2.5 Hz), 7.03 (d, 2H, J = 2.2 Hz), 7.21 (dd, 1H, J = 2.5, 8.8 Hz), 7.55 (d, 1H, J = 9.1 Hz), 7.66 (d, 1H, J = 8.5 Hz), 7.75 (dd, 1H, J = 1.9, 8.5 Hz), 7.99 (d, 1H, J = 1.6 Hz); ^{13}C NMR (75 MHz, benzene-d_6) δ 55.3, 55.3, 100.1, 106.2, 106.4, 119.9, 126.6, 126.8, 127.8, 130.1, 130.5, 134.9, 137.4, 144.3, 158.7, 162.2; HRMS (EI), *m*/*z* 294.1257 (calculated for $C_{19}H_{18}O_3$ 294.1256); Compound (f) **7**: dark brown solid: mp 74 °C; R_f 0.20 (SiO_2, 20% EA–Hex); ^{1}H NMR (benzene-d_6) δ(ppm) : 3.43 (s, 3H), 3.46 (s, 6H), 3.94 (s, 3H), 6.90 (s, 2H), 7.02 (d, 1H, J = 2.4 Hz), 7.25 (dd, 2H, J = 2.5, 9.1 Hz), 7.63 (d, 1H, J = 9.1 Hz), 7.76 (d, 1H, J = 2.2 Hz), 8.01 (s, 1H); ^{13}C NMR (75 MHz, benzene-d_6) δ 54.7, 55.8, 60.5, 105.4, 105.9, 119.6, 125.8, 126.4, 127.5, 127.6, 129.6, 129.9, 134.3, 137.2, 137.3, 154.3, 158.2; HRMS (EI), *m*/*z* 324.1359 (calculated for $C_{20}H_{20}O_4$ 324.1362); (g) Compound **8**: dark brown solid: mp 84 °C; R_f 0.28 (SiO_2, 20% EA–Hex); ^{1}H NMR (300 MHz, benzene-d_6) δ 3.38 (s, 3H), 3.40 (s, 3H), 6.85 (d, 1H, J = 6.5 Hz), 6.95 (d, 1H, J = 2.5 Hz), 7.19 (m, 2H), 7.28 (t, 1H, J = 6.8 Hz), 7.35 (s, 1H), 7.53 (d, 1H, J = 8.9 Hz), 7.65 (d, 1H, J = 8.5 Hz), 7.71 (d, 1H, J = 8.5 Hz), 7.94 (s, 1H); ^{13}C NMR (75 MHz, benzene-d_6) δ 55.1, 106.1, 113.3, 113.7, 119.9, 120.4, 120.5, 126.6, 126.8, 120.5, 120.5, 124.8, 127.2, 130.1, 143.6, 158.6, 161.1; HRMS (EI), *m*/*z* 264.1149 (calculated for $C_{18}H_{16}O_2$ 264.1150); (h) Compound **9**: dark brown solid: mp 99 °C; R_f 0.25 (SiO_2, 20% EA–Hex); ^{1}H NMR (300 MHz, benzene-d_6) δ 3.26 (s, 3H), 3.40 (s, 3H), 3.41(s, 3H), 6.48 (dt, 1H, J = 2.5, 8.3 Hz), 6.58 (t, 1H, J = 3.3 Hz), 7.00 (s, 1H), 7.22 (dt, 1H, J = 8.9, 2.8 Hz), 7.37 (dd, 1H, J = 8.5, 3.6 Hz), 7.59 (dd, 1H, J = 9.0, 3.0 Hz), 7.72 (dd, 1H, J = 8.5, 3.0 Hz), 7.89 (dt, 1H, J = 1.6, 8.5 Hz), 8.00 (s, 1H);

^{13}C NMR (75 MHz, benzene-d_6) δ 55.1, 55.3, 55.4, 100.0, 105.4, 106.3, 119.5, 124.8, 126,8, 128.7, 129.7, 130.0, 130.3, 132.3, 134.3, 135.0, 158.4, 158.6, 161.3; HRMS (EI), *m*/*z* 294.1255 (calculated for $C_{19}H_{18}O_3$ 294.1256); (i) Compound **10**: dark brown solid: mp 189 °C; R_f 0.22 (SiO_2, 5% MeOH–MC); ^{1}H NMR (300 MHz, benzene-d_6) δ 6.85 (t, 1H, J = 2.2 Hz), 7.12 (d, 2H, J = 2.2 Hz), 7.30 (dd, 1H, J = 2.5, 8.8 Hz), 7.40 (d, 1H, J = 2.5 Hz), 7.60 (s, 1H), 7.63 (s, 1H), 7.74 (dd, 1H, J = 1.6, 8.5 Hz), 8.04 (d, 1H, J = 1.4 Hz); ^{13}C NMR (75 MHz, benzene-d_6) δ 102.0, 106.6, 109.8, 119.1, 126.0, 126.3, 127.0, 129.3, 130.8, 135.0, 138.5, 144.8, 155.9, 159.9; HRMS (EI), *m*/*z* 252.0785 (calculated for $C_{16}H_{12}O_3$ 252.0786); (j) Compound **11**: dark brown solid: mp 215 °C; R_f 0.19 (SiO_2, 10% MeOH–MC); ^{1}H NMR (benzene-d_6) δ(ppm) : 7.13 (s, 2H), 7.28 (dd, 1H, J = 2.5, 8.8 Hz), 7.37 (d, 1H, J = 2.5 Hz), 7.62 (dd, 2H, J = 1.9, 8.8 Hz), 7.71 (dd, 1H, J = 1.9, 8.5 Hz), 7.98 (d, 1H, J = 1.6 Hz); ^{13}C NMR (75 MHz, benzene-d_6) δ 107.4, 109.8, 119.8, 125.7, 126.6, 127.8, 129.8, 130.5, 133.4, 134.2, 134.9, 136.9, 147.0, 155.8; HRMS (EI), *m*/*z* 268.0733 (calculated for $C_{16}H_{12}O_4$ 268.0736); (k) Compound **12**: dark brown solid: mp 174 °C; R_f 0.22 (SiO_2, 3% MeOH–MC); ^{1}H NMR (300 MHz, benzene-d_6) δ 7.08 (dt, 1H, J = 7.1, 1.9 Hz), 7.24 (s, 1H), 7.26 (t, 1H, J = 8.4 Hz), 7.33 (dd, 1H, J = 2.5, 8.8 Hz), 7.41 (d, 1H, J = 2.5 Hz), 7.50 (d, 1H, J = 1.6 Hz), 7.65 (d, 2H, J = 8.8 Hz), 7.68 (dd, 1H, J = 1.6, 8.2 Hz), 7.98 (s, 1H); ^{13}C NMR (75 MHz, benzene-d_6) δ 109.8, 114.8, 115.0, 119.5, 126.4, 126.6, 127.5, 129.7, 130.6, 130.7, 135.3, 136.7, 143.8, 156.2, 158.5; HRMS (EI), *m*/*z* 236.0834 (calculated for $C_{16}H_{12}O_2$ 236.0837); (l) **13**: dark brown solid: mp 186 °C; R_f 0.22 (SiO_2, 5% MeOH–MC); ^{1}H NMR (300 MHz, benzene-d_6) δ 6.72 (dd, 1H, J = 2.2, 8.5 Hz), 6.86 (d, 1H, J = 2.5 Hz), 7.23 (dd, 1H, J = 2.5, 8.8 Hz), 7.28 (d, 1H, J = 8.5 Hz), 7.37 (d, 1H, J = 2.5 Hz), 7.60 (d, 1H, J = 5.8 Hz), 7.62 (d, 1H, J = 5.5 Hz), 7.78 (dd, 1H, J = 1.6, 8.5 Hz), 7.98 (s, 1H); ^{13}C NMR (75 MHz, benzene-d_6) δ 104.0, 108.4, 109.8, 119.1, 121.9, 126.7, 127.4, 129.3, 129.7, 130.4, 132.5, 134.6, 134.7, 155.8, 156.1, 158.5; HRMS (EI), *m*/*z* 252.0790 (calculated for $C_{16}H_{12}O_3$ 252.0786).

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20. Tyrosinase enzymatic assay: briefly 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 microplate (Nunc, Denmark), in a total volume of 200 μL. The assay mixture was incubated at 25 °C for 30 min After incubation, the amount of dopachrome produced in the reaction mixture was determined as the optical density at 492 nm (OD_{492}) in a microplate leader (Hewlett-Packard).