http://dx.doi.org/10.5012/bkcs.2011.32.12.4411

Studies on Tyrosinase Inhibitory and Antioxidant Activities of Benzoic Acid Derivatives Containing Kojic Acid Moiety

Ho Sik Rho,[*] Chang Seok Lee, Soo Mi Ahn,[†] Yong Deog Hong, Song Seok Shin, Young-Ho Park, and Soo Nam Park[‡,*]

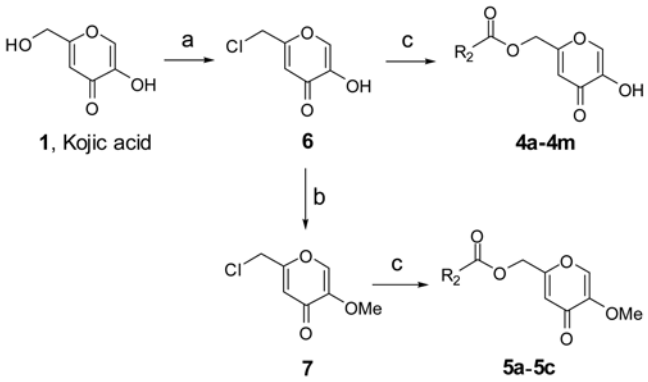
R & D Center, AmorePacific Corporation, Yongin 446-729, Korea. [*]E-mail: thiocarbon@hanmail.net
[†]Kyung Hee University Skin Biotechnology Center, Suwon 443-270, Korea
[‡]Department of Fine Chemistry, Seoul National University of Science and Technology, Seoul 139-743, Korea
[*]E-mail: snpark@seoultech.ac.kr
Received June 21, 2011, Accepted October 3, 2011

Key Words : Tyrosinase, DPPH, Hydroxyl benzoic acid, Kojic acid, Ester

Tyrosinase[1] is widely distributed in microorganisms, animals, and plants and is responsible for melanization in animals and browning in plants. Melanin plays a crucial protective role against the photodamage of skin. However, the production of abnormal melanin pigmentation is a serious esthetic problem for human beings. In the food industry, tyrosinase is responsible for enzymatic browning reactions in damaged fruit during post-harvest handling and processing. Therefore, the development of tyrosinase inhibitors that can be used as skin whitening agents[2] or preservatives[3] for fresh food has been pursued. Kojic acid[4] (**1**) is produced by various fungi and bacteria and is widely used as a skin whitening agent because of its tyrosinase inhibitory activity. Kojic acid has also been shown to prevent photodamage due to its free radical scavenging activity.[5] However, its inhibitory activity and storage properties are insufficient for use in cosmetics and as an *anti*-browning agent for food. To increase its activity, many semi-synthetic kojic acid derivatives have been synthesized. Many of these compounds were formed by modifying the C-2 hydroxyl group to form esters,[6] ethers,[7] sulfides[8] and peptide[9] derivatives because C-5 enolic hydroxyl group is considered as a pharmacophore for tyrosinase inhibition. Recently, we synthesized a 2,4-dihydroxy benzoate ester of kojic acid containing an adamantane moiety (**2**) and evaluated its tyrosinase activity.[10] Although compound **2** has a kojic acid moiety for chelation to copper in the active site of tyrosinase, tyrosinase inhibitory activity is not found. We also synthesized a benzoate ester of kojic acid without an adamantane moiety and investigated its tyrosinase inhibitory and radical scavenging activities.

Scheme 1 shows the synthetic pathways for the benzoate ester of kojic acid. The reaction of kojic acid with thionyl chloride produces compound **6**, which is conveniently converted to compound **7** using dimethylsulfate and potassium carbonate in acetone under reflux conditions. Compounds **6** and **7**, which are both chlorides, react with potassium salts of benzoic acids in dimethylformamide at 110 °C - 120 °C to give the corresponding ester derivatives **4a-4m** and **5a-5c**.

First, we evaluated the tyrosinase inhibitory activities of the kojic acid and benzoate ester derivatives. Tyrosinase is known to be the enzyme responsible for the oxidation of tyrosine. The inhibitory activities were evaluated by measuring the transformation rate of L-tyrosine to L-dopaquinone. Compounds **4a-4m** exhibited potent inhibitory activities against tyrosinase. The hydrophobic benzoate increased the inhibitory activity of kojic acid. However, the numbers and



Scheme 1. Reaction conditions: (a) thionyl chloride, DMF, room temperature; (b) dimethylsulfate, potassium carbonate, acetone, reflux; (c) potassium salts of benzoic acids, DMF, 110 °C - 120 °C.

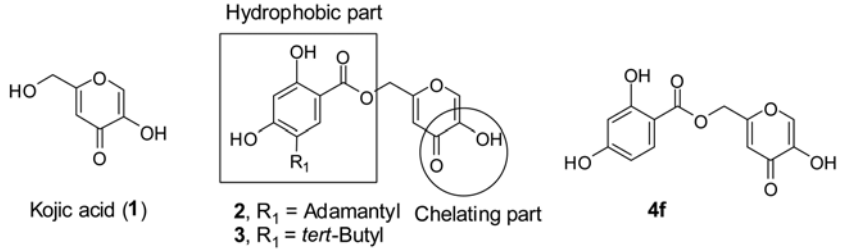
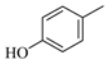
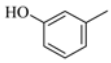
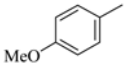
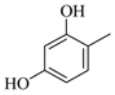
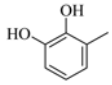
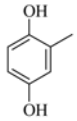
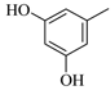
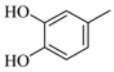
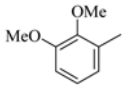
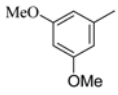
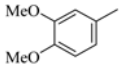
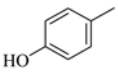
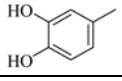


Figure 1. Structures of kojic acid and benzoate ester derivatives.

Table 1. Inhibitory activities of kojic acid and benzoate ester derivatives.on mushroom tyrosinase and DPPH radical

Compounds	R_2	Inhibitory activity [IC_{50}[a] (μM)]	
		Tyrosinase (IC_{50})	DPPH (IC_{50})
Kojic acid		30.0	> 500
2		> 200	> 500
3		20.0	> 500
4a		3.2	> 500
4b		2.8	> 500
4c		3.3	> 500
4d		4.5	> 500
4e		2.0	> 500
4f		3.5	> 500
4g		5.7	4.37
4h		4.4	5.97
4i		3.6	> 500
4j		3.3	5.7
4k		2.0	> 500
4l		8.1	> 500
4m		2.4	> 500
5a		> 200	> 500
5b		> 200	> 500
5c		> 200	5.5

[a]Values were determined from logarithmic concentration-inhibition curves and are the means of three experiments.

positions of the phenolic hydroxyl groups in benzoate had only a minor effect on their inhibitory activities. Although there is no phenolic hydroxyl group in, **4a**, **4e**, **4k**, **4l**, and **4m**, these compounds exhibited the potent inhibitory activities. Compound **4e**, (5-hydroxy-4-oxo-4*H*-pyran-2-yl)methyl 4-methoxybenzoate, exhibited the most potent inhibitory activity (IC_{50} = 2.0 μM). In the case of the methyl-protected derivatives of the 5-enolic hydroxyl group, inhibitory activities were lost completely (**5a**, **5b**, and **5c**). From these results, we conclude that the kojic acid moiety is a more important pharmacophore than the phenolic hydroxyl groups in benzoate for the inhibition of tyrosinase.

To explain the different tyrosinase inhibitory activities of compounds **2**, **3**, and **4f**, we conducted a modeling study. Figure 2 shows the selected docked conformation of compounds **2**, **3**, and **4f** in the tyrosinase binding site.

The docking results from the crystal structure of tyrosinase in the modeling study agreed well with the observed *in vitro* data, which indicated that compound **4f** (IC_{50} = 3.5 μM) was a potent inhibitor of tyrosinase while compound **2** (IC_{50} > 200 μM) exhibited no tyrosinase inhibitory activity. The docked score of **4f** (6.22 kcal/mol) indicated tight binding to the active site of tyrosinase. From the docking pose of **4f**, we concluded that the π-π stacking interaction between kojic acid moiety and His194 is the most important interaction for the inhibition of tyrosinase. Moreover, these docked conformations also formed H-bond interactions with peroxide in the active site (Fig. 1(a)). However, compound **2** (14.90 kcal/mol) exhibited a different binding pattern due to the adamantane moiety. The bulky adamantane group caused a steric hindrance that changed its binding to the active site of tyrosinase (Fig. 1(c)). To confirm the size effect on tyrosinase inhibitory activity, we synthesized compound **3** (11.70 kcal/mol) containing a *tert*-butyl moiety and evaluated its tyrosinase activity (IC_{50} = 20.0 μM). The *tert*-butyl group also showed negative effect on binding to the active site of tyrosinase which is similar to that of adamantane group.

After evaluating the tyrosinase inhibitory activity, we investigated the radical scavenging activities of kojic acid and benzoate ester derivatives. One of the reactions catalyzed by the tyrosinase is the oxidation of L-dopa to L-dopaquinone by using molecular oxygen. Thus it is obvious that antioxidants may inhibit the oxidation step, without interacting with the tyrosinase. All compounds were evaluated for their free radical scavenging activity by using the DPPH assay. In contrast to the result of tyrosinase, numbers and position of phenolic hydroxyl groups in benzoate showed major effect on their inhibitory activities. Compounds **4a**, **4e**, **4k**, **4l**, and **4m** which contains no hydroxyl group showed no inhibitory activity although they has kojic acid moiety. Monohydroxyl compounds **4b**, **4c**, and **4d** also exhibited no inhibitory activities. In the case of dihydroxyl compounds, potent inhibitory activities were observed. However, only compounds containing specific hydroxyl position exhibited inhibitory activities. Compounds **4g** (2,3-dihydroxy), **4h** (2,5-dihydroxy), and **4j** (3,4-dihydroxy) exhibited inhibitory activities, whereas compounds **4f** (2,4-dihydroxy) and **4i** (3,5-dihydroxy) exhibited no inhibitory activities. From these results, we conclude that the

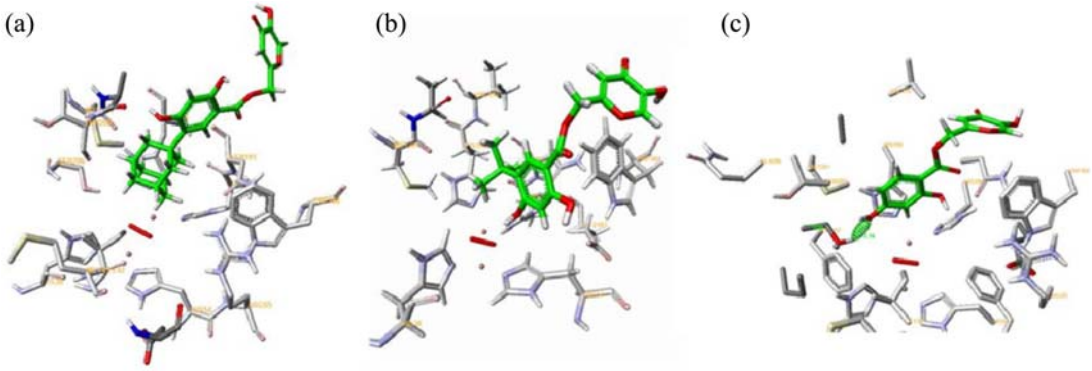


Figure 2. Docked conformation of compounds **2**, **3**, and **4f** in the binding site of tyrosinase: (a) compound **2**, (b) compound **3**, (c) compound **4f**.

position of both of the hydroxyl groups is important for radical scavenging activity. Only *ortho* and *para* positions of the dihydroxyl group can stabilize the anion of the hydroxyl group. Compound **5c** exhibited potent inhibitory activity although a methyl group protected its kojic acid moiety. Its activity was similar to that of compound **4j**. From these results, we conclude that dihydroxyl moiety is a more important pharmacophore than kojic acid for a DPPH assay.

In conclusion, we synthesized a series of kojic acid derivatives containing ester linkages (**4a-4m**) and (**5a-5c**). Their inhibitory activities against tyrosinase and DPPH were evaluated. Their structure is composed of three parts: kojic acid, an ester linkage, and a benzoate moiety. Tyrosinase inhibitory activity originates from the kojic acid moiety rather than the hydroxyl group in benzoate (**3a-3j**). However, compound **2**, which contained a bulky adamantane group in the benzoate moiety exhibited no inhibitory activity. Our modeling study showed that the adamantane moiety interrupted the binding to the active site of tyrosinase. A slightly different experimental result was obtained for the DPPH assay. Radical scavenging activities of the kojic acid derivatives may be due to their 1,2 or 1,4 dihydroxyl groups. On the basis of two assay results, compounds **4g**, **4h**, and **4j** can be considered as good candidates for *anti*-tyrosinase and antioxidative agents.

Experimental Section

(5-Hydroxy-4-oxo-4*H*-pyran-2-yl)methyl benzoate (4a). To a stirred solution of kojyl chloride **2** (4.80 g, 30.0 mmol) in DMF (100 mL) under N_2 was added potassium salt of benzoic acid (4.79 g, 30.0 mmol) with benzoic acid (2.73 g, 2.25 mmol). The reaction mixture was stirred for 2 h at 110 °C - 120 °C, after which DMF was evaporated *in vacuo*. The residue was extracted with ethyl acetate (500 mL), washed with water. The organic layer was dried with anhydrous $MgSO_4$ and concentrated to give a crude product. The resultant was purified by crystallization from ethyl acetate-hexane to give **4a** (5.37 g) in 73% yields.

^{1}H NMR (300 MHz, DMSO-d_6): δ 9.29 (s, 1H), 8.11 (s, 1H), 8.02 (m, 2H), 7.71 (m, 1H), 7.58 (m, 2H), 6.55 (s, 1H), 5.22 (s, 2H). ^{13}C-NMR (125 MHz, DMSO-d_6): 173.5, 164.9, 161.4, 146.0, 139.9, 133.7, 129.3, 128.8, 128.7, 112.6, 61.9. FABMS, *m/e* 245.1 $[M-H]^+$.

(5-Hydroxy-4-oxo-4*H*-pyran-2-yl)methyl 4-hydroxybenzoate (4b). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.08 (s, 1H), 9.25 (s, 1H), 8.10 (s, 1H), 7.87 (d, 2H, J = 8.4 Hz), 6.88 (d, 2H, J = 8.4 Hz), 6.51 (s, 1H), 5.14 (s, 2H). ^{13}C-NMR (125 MHz, DMSO-d_6): 173.5, 164.7, 162.4, 161.8, 146.0, 139.8, 131.7, 119.1, 115.4, 112.3, 61.4. FABMS, *m/e* 261.2 $[M-H]^+$.

(5-Hydroxy-4-oxo-4*H*-pyran-2-yl)methyl 3-hydroxybenzoate (4c). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.90 (s, 1H), 9.22 (s, 1H), 8.11 (s, 1H), 7.38 (m, 3H), 7.04 (d, 1H, J = 8.4 Hz), 6.53 (s, 1H), 5.19 (s, 2H). ^{13}C-NMR (125 MHz, DMSO-d_6): 173.5, 164.9, 161.5, 157.5, 146.0, 139.9, 129.99, 129.88, 120.8, 120.0, 115.6, 112.5, 61.8. FABMS, *m/e* 261.2 $[M-H]^+$.

(5-Hydroxy-4-oxo-4*H*-pyran-2-yl)methyl 2-hydroxybenzoate (4d). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.24 (s, 1H), 9.30 (s, 1H), 8.11 (s, 1H), 7.80 (d, 1H, J = 8.4 Hz), 7.53 (m, 1H), 6.95 (m, 2H), 6.58 (s, 1H), 5.23 (s, 2H). ^{13}C-NMR (125 MHz, DMSO-d_6): 173.5, 167.2, 161.2, 159.7, 146.0, 139.9, 135.7, 130.3, 119.4, 117.4, 113.0, 112.6. 61.9. FABMS, *m/e* 261.2 $[M-H]^+$.

(5-Hydroxy-4-oxo-4*H*-pyran-2-yl)methyl 4-methoxybenzoate (4e). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.24 (s, 1H), 8.11 (s, 1H), 7.97 (d, 2H, J = 8.4 Hz), 7.08 (d, 2H, J = 8.4 Hz), 6.53 (s, 1H), 5.17 (s, 2H), 3.84 (s, 3H). ^{13}C-NMR (125 MHz, DMSO-d_6): 173.5, 164.6, 163.5, 161.6, 146.0, 139.8, 131.5, 120.8, 114.2, 112.4, 61.5, 55.5. FABMS, *m/e* 275.0 $[M-H]^+$.

(5-Hydroxy-4-oxo-4*H*-pyran-2-yl)methyl 2,4-dihydroxybenzoate (4f). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.54 (s, 1H), 10.42 (s, 1H), 9.28 (s, 1H), 8.12 (s, 1H), 7.70 (d, 1H, J = 8.4 Hz), 6.55 (s, 1H), 6.39 (d, 2H, J = 8.4 Hz), 6.31 (s, 1H), 5.19 (s, 2H). ^{13}C-NMR (125 MHz, DMSO-d_6): 173.5, 167.6, 164.5, 162.6, 161.3, 146.0, 139.9, 132.0, 112.5, 108.5, 103.6, 102.6, 61.5. FABMS, *m/e* 277.2 $[M-H]^+$.

(5-Hydroxy-4-oxo-4*H*-pyran-2-yl)methyl 2,3-dihydroxybenzoate (4g). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.01 (s, 1H), 9.51 (s, 1H), 9.25 (s, 1H), 8.11 (s, 1H), 7.27 (d, 1H, J = 8.4 Hz), 7.06 (d, 1H, J = 8.4 Hz), 6.79 (m, 1H), 5.23 (s, 2H). ^{13}C-NMR (125 MHz, DMSO-d_6): 173.5, 167.9, 161.1, 149.1, 146.19, 146.06, 139.9, 120.8, 119.85, 119.08, 113.03, 112.71, 62.0. FABMS, *m/e* 277.2 $[M-H]^+$.

(5-Hydroxy-4-oxo-4*H*-pyran-2-yl)methyl 2,5-dihydroxybenzoate (4h). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.69 (s, 1H), 9.26 (s, 1H), 9.22 (s, 1H), 8.12 (s, 1H), 7.17 (s, 1H),

6.97 (d, 1H, J = 8.4 Hz), 6.82 (d, 1H, J = 8.4 Hz), 6.57 (s, 1H), 5.21 (s, 2H). ^{13}C-NMR (125 MHz, DMSO-d_6): 173.5, 167.3, 161.2, 152.9, 149.6, 146.0, 139.9, 124.0, 118.3, 114.3, 112.62, 112.34, 61.9. FABMS, *m/e* 277.2 $[M-H]^+$.

(5-Hydroxy-4-oxo-4*H*-pyran-2-yl)methyl 3,5-dihydroxybenzoate (4i). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.76 (bs, 2H), 9.25 (s, 1H), 8.11 (s, 1H), 6.85 (s, 2H), 6.51 (s, 1H), 6.44 (s, 1H), 5.15 (s, 2H). ^{13}C-NMR (125 MHz, DMSO-d_6): 173.5, 164.9, 161.5, 158.6, 146.0, 139.9, 130.3, 112.4, 107.67, 107.23, 61.8. FABMS, *m/e* 277.2 $[M-H]^+$.

(5-Hydroxy-4-oxo-4*H*-pyran-2-yl)methyl 3,4-dihydroxybenzoate (4j). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.84 (bs, 2H), 9.23 (s, 1H), 7.38 (s, 1H), 7.36 (d, 1H, J = 8.4 Hz), 6.84 (d, 1H, J = 8.4 Hz), 6.50 (s, 1H), 5.12 (s, 2H). ^{13}C-NMR (125 MHz, DMSO-d_6): 173.5, 164.8, 161.8, 150.9, 145.99, 145.17, 139.8, 122.1, 119.3, 116.3, 115.4, 112.2, 61.3. FABMS, *m/e* 277.2 $[M-H]^+$.

(5-Hydroxy-4-oxo-4*H*-pyran-2-yl)methyl 2,3-dimethoxybenzoate (4k). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.24 (s, 1H), 8.12 (s, 1H), 7.18-8.37 (m, 3H), 6.55 (s, 1H), 5.18 (s, 2H), 3.84 (s, 3H), 3.74 (s, 3H). ^{13}C-NMR (125 MHz, DMSO-d_6): 173.6, 164.9, 161.4, 153.1, 148.0, 146.0, 139.9, 124.8, 124.3, 121.3, 116.8, 112.7, 62.0, 60.9, 56.0. FABMS, *m/e* 305.3 $[M-H]^+$.

(5-Hydroxy-4-oxo-4*H*-pyran-2-yl)methyl 3,5-dimethoxybenzoate (4l). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.25 (s, 1H), 8.11 (s, 1H), 7.09 (s, 1H), 6.82 (s, 1H), 6.53 (s, 1H), 5.21 (s, 2H), 3.80 (s, 6H). ^{13}C-NMR (125 MHz, DMSO-d_6): 173.6, 164.7, 161.3, 160.5, 146.0, 139.9, 130.6, 112.6, 107.0, 105.6, 62.1, 55.5. FABMS, *m/e* 305.3 $[M-H]^+$.

(5-Hydroxy-4-oxo-4*H*-pyran-2-yl)methyl 3,4-dimethoxybenzoate (4m). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.24 (s, 1H), 8.11 (s, 1H), 7.66 (d, 1H, J = 8.4 Hz), 7.465 (s, 1H), 7.11 (d, 1H, J = 8.4 Hz), 6.52 (s, 1H), 5.18 (s, 2H), 3.84 (s, 3H), 3.81 (s, 3H). ^{13}C-NMR (125 MHz, DMSO-d_6): 173.6, 164.7, 161.7, 153.4, 148.5, 146.0, 139.9, 123.6, 120.7, 112.4, 111.8, 111.2, 61.6, 55.77, 55.60. FABMS, *m/e* 305.3 $[M-H]^+$.

(5-Methoxy-4-oxo-4*H*-pyran-2-yl)methyl benzoate (5a). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.18 (s, 1H), 8.02 (m, 2H), 7.70 (m, 1H), 7.56 (m, 1H), 6.52 (s, 1H), 5.23 (s, 2H), 3.65 (s, 3H). ^{13}C-NMR (125 MHz, DMSO-d_6): 172.9, 165.5, 161.9, 148.6, 140.1, 134.3, 129.87, 129.40, 129.20, 113.9, 62.3, 56.6. FABMS, *m/e* 261.1 $[M+H]^+$.

(5-Methoxy-4-oxo-4*H*-pyran-2-yl)methyl 4-hydroxybenzoate (5b). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.42 (s, 1H), 8.16 (s, 1H), 7.86 (d, 2H, J = 8.4 Hz), 6.87 (d, 2H, J = 8.4 Hz), 6.47 (s, 1H), 5.15 (s, 2H), 3.64 (s, 3H). ^{13}C-NMR (125 MHz, DMSO-d_6): 172.4, 164.7, 162.4, 161.8, 148.1, 139.5, 131.7, 119.1, 115.5, 113.2, 61.3, 56.1. FABMS, *m/e* 275.2 $[M-H]^+$.

(5-Methoxy-4-oxo-4*H*-pyran-2-yl)methyl 3,4-dihydroxybenzoate (5c). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.84 (bs, 2H), 9.23 (s, 1H), 7.38 (s, 1H), 7.36 (d, 1H, J = 8.4 Hz), 6.84 (d, 1H, J = 8.4 Hz), 6.50 (s, 1H), 5.12 (s, 2H), 3.64 (s, 3H). ^{13}C-NMR (125 MHz, DMSO-d_6): 172.4, 164.8, 161.9, 150.9, 148.1, 145.1, 122.2, 119.3, 116.3, 115.4, 113.1, 61.2, 56.1. FABMS, *m/e* 291.1 $[M-H]^+$.

Mushroom Tyrosinase Assay. Mushroom tyrosinase, L-tyrosine were purchased from Sigma Chemical. The reaction mixture for mushroom tyrosinase activity consisted of 150 μL of 0.1 M phosphate buffer (pH 6.5), 3 μL of sample solution, 8 μL of mushroom tyrosinase (2,100 unit/mL, 0.05 M phosphate buffer at pH 6.5), and 36 μL of 1.5 mM L-tyrosine. Tyrosinase activity was determined by reading the optical density at 490 nm on a microplate reader (Bio-Rad 3550, Richnmond, CA, U.S.A.) after incubation for 20 min at 37 °C. The inhibitory activity of the sample was expressed as the concentration that inhibits 50% of the enzyme activity (IC_{50}).

DPPH Radical Scavenging Assay. DPPH radical-scavenging assay was carried out using the following procedure. The reaction mixture containing various concentrations of the test samples and DPPH methanolic solution (0.2 mM) was incubated at room temperature for 30 min and the absorbance was measured at 517 nm. The scavenging activity was expressed as a percent compared to control DPPH solution (100%). The synthetic antioxidant trolox and L-ascorbic acid were included in experiments as a positive control.

Molecular Modeling. Molecular modeling study was carried out on linux system using SYBYL-X version 1.2. To prepare the tyrosinase structure, the crystal structure of the oxy form of S. castaneoglobisporus tyrosinase was taken from the Protein Data Bank (PDB cod 1 wx2) because there is no crystal structure of mushroom tyrosinase published yet. The caddie protein and water molecules were removed. Hydrogen atoms were added to the enzyme using the SYBYL. For the molecular docking method, Surflex-Dock version 2.5 was used using standard parameters and allowing the hydrogen of protein movement.

Acknowledgments. This work was supported by Department of Chemistry, Hanyang University, Seoul, Korea.

References and Notes

1. Sanchez-Ferrer, A.; Rodriguez-Lopez, J. N.; Garcia-Canavas, F.; Garcia-Carmona, F. *Biochim. Biophys. Acta* **1995**, *1247*, 1.
2. (a) Solano, F.; Briganti, S.; Picardo, M.; Ghanem, G. *Pigment Cell Res.* **2006**, *19*, 550. (b) Brigati, S.; Camera, E.; Picardo, M. *Pigment Cell Res.* **2003**, *16*, 101.
3. Lunadei, L.; Galleguillo, P.; Diezma, B.; Lleo, L.; Ruiz-Garcia, L. *Postharvest Biol. Technol.* **2011**, *60*, 225.
4. Ohyama, Y.; Mishima, Y. *Fragrance J.* **1990**, *6*, 53.
5. (a) Mitani, H.; Koshiishi, I.; Sumita, T.; Imanari, T. *Eur. J. Pharmacol.* **2001**, *411*, 169. (b) Rho, H. S.; Baek, H. S.; You, J. W.; Kim, S.; Lee, J. Y.; Kim, D. H. *Bull. Korean Chem. Soc.* **2007**, *28*, 471.
6. Manosroi, A.; Wongtrakul, P.; Manosroi, J.; Midorikawa, U.; Hanyu, Y.; Yuasa, M.; Sugawara, F.; Sakai, H.; Abe, M. *Int. J. Pharm.* **2005**, *298*, 13.
7. Kodokawa, J.; Nishikura, T.; Muraoka, R.; Tagaya, H.; Fukuoka, N. *Synth. Commun.* **2003**, *33*, 1081.
8. (a) Rho, H. S.; Ahn, S. M.; Yoo, D. S.; Kim, M. K.; Cho, D. H.; Cho, J. Y. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6569. (b) Rho, H. S.; Baek, H. S.; Kim, M. K.; Ghimeray, A. K.; Cho, D. H. *Bull. Korean Chem. Soc.* **2010**, *31*, 2375. (c) Rho, H. S.; Baek, H. S.; Ahn, S. M.; Kim, D. H.; Chang, I. S. *Bull. Korean Chem. Soc.* **2008**, *29*, 1569.
9. Kwak, S.-Y.; Noh, J.-M.; Park, S.-H.; Byun, J.-W.; Choi, H.-R.; Park, K..-C.; Lee, Y.-S. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 738.
10. Rho, H. S.; Goh, M. J.; Lee, J.; Ahn, S. M.; Yeon, J.; Yoo, D. S.; Kim, D. H.; Kim, H. G.; Cho, J. Y. *Bull. Korean Chem. Soc.* **2011**, *32*, 1411.