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Tyrosinase Inhibitory Effects of Derivatives of (*E*)-2-(Substituted Benzylidene)-3,4-Dihydronaphthalen-1(2*H*)-One

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Tyrosinase plays an essential role in melanin biosynthesis, and as such it has received great attention as a key target for the treatment of pigmentation disorders. In our earlier studies, we explored analogs with the β-phenyl-α,β-unsaturated carbonyl template, and the results obtained indicated that this template confers potent tyrosinase inhibitory activity. Thus, in the present study, (*E*)-2-(substituted benzylidene)-3,-4-dihydronaphthalen-1(2*H*)-one derivatives (compounds **1**–**6**) with this template were synthesized and investigated with respect to their mushroom tyrosinase inhibitory effects. Derivative **4** with a 3-hydroxy-4-methoxyl substituent on the β–phenyl ring of the template inhibited mushroom tyrosinase fourfold more than kojic acid (IC_{50} = 15.60 ± 0.32 μM vs. 57.06 ± 0.46 μM). *In silico* docking simulation supported this potent inhibitory activity of compound **4** by demonstrating a binding energy of −6.4 kcal/mol at the active site of mushroom tyrosinase. Kinetic results imply that **4** competitively inhibits mushroom tyrosinase.

Keywords: Tyrosinase, (*E*)-2-Benzylidene-3,4-dihydronaphthalen-1(2*H*)-one, β-Phenyl-α,β-unsaturated carbonyl, Tyrosinase inhibitor, Kojic acid

Introduction

Melanins are a family of dark macromolecular insoluble pigments found widely in nature and are classified as eumelanins, pheomelanins, neuromelanins, allomelanins, and pyomelanins.[1] Eumelanins and pheomelanins are animal pigments and are responsible for brown-black and a yellow-red colors, respectively.[2] In animals, melanins are produced by melanocytes in the basal layer of dermis via a series of oxidation and polymerization reactions of L-tyrosine.[3] Although melanin protects skin from UV radiation, toxic chemicals, and environmental toxicants,[4] excessive melanogenesis causes a variety of skin disorders such as melasma, lentigo, age spots, freckles, and senile pigment spots.[5]

Tyrosinase (polyphenol oxidase, EC 1.14.18.1) has received considerable attention because it catalyzes the rate-determining step in melanin biosynthesis. Tyrosinase catalyzes two steps, that is, the conversion of L-tyrosine to L-3,4-dihydroxyphenylalanine (L-dopa) and of L-dopa to L-dopaquinone, which is in turn, is converted to eumelanin or pheomelanin by chemical and/or enzymatic processes, depending on the participation of glutathione and cysteine.[6] In their presence, pheomelanin is produced, whereas in their absence, eumelanin is biosynthesized. Tyrosinase plays many important roles in microorganisms, plants, and animals. It contributes to the defense systems of bacteria and insects[7] and to developmental functions in insects.[8] In plants, it oxidizes various phenolics and is responsible for the browning of fruits and crops,[9] and as mentioned above, in vertebrates, it plays a key role in melanin synthesis.

Over the past 10 years, we have synthesized many compounds to find potent tyrosinase inhibitors[10-18] and have shown that several derivatives with the β-phenyl-α,-β-unsaturated carbonyl template are effective tyrosinase inhibitors. The present study represents a continuation of this work and involved the synthesis and an investigation of the mushroom tyrosinase inhibitory activities of (*E*)-2-benzylidene-3,4-dihydronaphthalen-1(2*H*)-one derivatives, which all contained the β-phenyl-α,β-unsaturated carbonyl template. Since chalcones have the above template, the synthesized compounds can be classified as a group of chalcone tyrosinase inhibitors.

Experimental Section

General. The chemicals were purchased and used directly. TLC (thin column chromatography) plates ($60F_{245}$) were obtained from Merck and MP Silica (Darmstadt, Germany,

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40–63 μm, 60 Å) were used for column chromatography. Water was removed from solvents by distillation over CaH_2 or Na/benzophenone. Mass data in low resolution was obtained in ESI negative mode via Expression CMS (advion, NY 14850, USA). Nuclear magnetic resonance (NMR) data were obtained through Varian Unity AS500 spectrometer (Agilent Technologies, Santa Clara, CA, USA) or a Varian Unity INOVA 400 spectrometer for ^{1}H NMR (500 MHz and 400 MHz) or on a Varian Unity INOVA 400 spectrometer for ^{13}C NMR (100 MHz). DMSO-d_6 and chloroform-d ($CDCl_3$) solvents were used for ^{1}H and ^{13}C NMR data recording. Coupling constants and chemical shifts frequency were measured in Hz (hertz) and ppm (parts per million), respectively. These abbreviations singlet (s), doublet (d), triplet (t) and multiplet (m) were used in the analysis of ^{1}H NMR data.

General Synthetic Procedure of (*E*)-2-(substituted benzylidene)-3,4-dihydronaphthalen-1(2*H*)-one Analogs (1–6). A solution of α-tetralone and an appropriate benzaldehyde (0.9 equiv.) in 1.0 M HCl acetic acid (0.5 mL/0.1 mL of α-tetralone) was stirred at room temperature for 72–96 h. Water (5 mL/0.1 mL of α-tetralone) and methanol (1 mL/0.1 mL of α-tetralone) were added to the reaction mixture, and the mixture was stirred for 1 min. The methanol was then removed *in vacuo*. The resulting solid was filtered through a Buchner funnel and washed with water and/or dichloromethane to give (*E*)-2-(substituted benzylidene)-3,4-dihydronaphthalen-1(2*H*)-one analogs **1**–**6** as solids in yields of 16.2–73.8%.

(*E*)-2-(4-Hydroxybenzylidene)-3,4-dihydronaphthalen-1(2*H*)-one (1). ^{13}C NMR (100 MHz, DMSO-d_6) δ 187.2, 159.1, 143.8, 137.0, 133.9, 133.8, 133.0, 132.8, 129.0, 127.9, 127.6, 126.7, 116.2, 28.5, 27.4; LRMS (ESI-) *m/z* 249 $(M-H)^-$.

(*E*)-2-(3,4-Dihydroxybenzylidene)-3,4-dihydronaphthalen-1(2*H*)-one (2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 187.2, 147.6, 145.8, 143.8, 137.4, 133.9, 133.8, 132.8, 129.0, 127.9, 127.6, 127.2, 123.6, 118.0, 116.4, 28.5, 27.4; LRMS (ESI-) *m/z* 265 $(M-H)^-$.

(*E*)-2-(4-Hydroxy-3-methoxybenzylidene)-3,4-dihydronaphthalen-1(2*H*)-one (3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.1, 146.6, 146.6, 143.3, 137.3, 133.8, 133.8, 133.3, 128.4, 128.4, 128.3, 127.2, 124.0, 114.6, 113.0, 56.2, 29.0, 27.5; LRMS (ESI-) *m/z* 279 $(M-H)^-$, 264 $(M-H-CH_3)^-$.

(*E*)-2-(3-Hydroxy-4-methoxybenzylidene)-3,4-dihydronaphthalen-1(2*H*)-one (4). ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.1, 147.3, 145.6, 143.4, 136.9, 134.2, 133.8, 133.3, 129.5, 128.4, 128.3, 127.2, 123.5, 116.0, 110.7, 56.2, 29.0, 27.4; LRMS (ESI-) *m/z* 279 $(M-H)^-$, 264 $(M-H-CH_3)^-$.

(*E*)-2-(4-Hydroxy-3,5-dimethoxybenzylidene)-3,4-dihydronaphthalen-1(2*H*)-one (5). ^{13}C NMR (100 MHz, $CDCl_3$) δ 187.9, 147.1, 143.2, 137.5, 136.0, 134.0, 133.8, 133.4, 128.4, 128.3, 127.3, 127.2, 107.5, 56.6, 29.0, 27.5; LRMS (ESI-) *m/z* 309 $(M-H)^-$, 294 $(M-H-CH_3)^-$, 279 $(M-H-2CH_3)^-$.

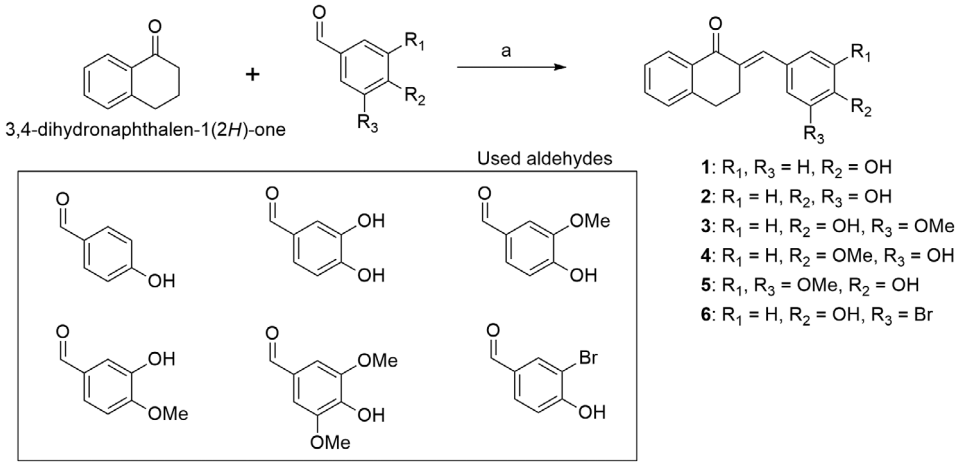
(*E*)-2-(3-Bromo-4-hydroxybenzylidene)-3,4-dihydronaphthalen-1(2*H*)-one (6). ^{13}C NMR (100 MHz, $CDCl_3$) δ 187.2, 155.5, 143.9, 135.3, 134.5, 134.1, 133.6, 131.7, 129.1, 128.5, 128.0, 127.6, 116.9, 110.1, 28.4, 27.3; LRMS (ESI-) *m/z* 327 $(M-H)^-$, 329 $(M+2-H)^-$.

^{1}H NMR data for compounds **1**–**6** are presented in Table 1.

Mushroom Tyrosinase Inhibition Assay. The tyrosinase inhibitory effects of synthesized compounds **1**–**6** were determined following the existing procedure with minor modification.[19] Briefly, tyrosinase solution (20 μL; 1000 U/mL) and the synthesized compounds (**1**–**6**, 10 μL, 50 μM) were added to 170 μL of a solution of 50 mM phosphate buffer (pH 6.5) containing 293 μM of L-tyrosine in the wells of a 96-well plate and then incubated for 0.5 h at 37 °C. Percentage tyrosinase inhibitions were calculated by

Table 1. ^{1}H NMR data for compounds **1**–**6.**

	1 (400 MHz, DMSO-d_6)	2 (500 MHz, DMSO-d_6)	3 (500 MHz, $CDCl_3$)	4 (500 MHz, $CDCl_3$)	5 (500 MHz, $CDCl_3$)	6 (500 MHz, DMSO-d_6)
position	δ_H (*J* in Hz)					
3	3.04, t (6.4)	3.08, t (6.0)	3.16, t (6.0)	3.16, t (6.5)	3.17, t (6.0)	3.06, t (6.0)
4	2.88, t (6.4)	2.92, t (6.0)	2.96, t (6.0)	2.95, t (6.5)	2.96, t (6.0)	2.92, t (6.0)
5	7.37–7.31, m	7.35, d (8.0)	7.26, d (7.5)	7.25, d (7.5)	7.25, d (7.5)	7.35, d (7.5)
6	7.51, t (7.6)	7.54, t (7.5)	7.49, t (7.5)	7.49, t (7.5)	7.49, t (7.5)	7.55, t (7.5)
7	7.37–7.31, m	7.38, t (7.5)	7.37, t (7.5)	7.36, t (7.5)	7.36, t (7.5)	7.39, t (7.5)
8	7.89, d (7.6)	7.92, d (8.0)	8.13, d (7.5)	8.13, d (8.0)	8.12, d (7.5)	7.93, d (7.5)
vinylic	7.62, s	7.57, s	7.83, s	7.80, s	7.81, s	7.69, s
2′	7.38, d (7.6)	6.98, s	6.98–6.58, m	7.09, s	6.72, s	7.60, s
3′	6.82, d (7.6)	9.45 or 9.15, s, *OH*	3.93, s, *OCH₃*	5.69, s, *OH*	3.92, s, *OCH₃*	
4′	9.91, s, *OH*	9.45 or 9.15, s, *OH*	5.87, s	3.94, s, *OCH₃*	5.77, s, *OH*	10.75, s, *OH*
5′	6.82, d (7.6)	6.80, d (8.0)	6.98–6.58, m	6.91, d (8.5)	3.92, s, *OCH₃*	7.02, d (8.5)
6′	7.38, d (7.6)	6.88, d (8.0)	7.06, d (8.0)	7.01, d (8.5)	6.72, s	7.40, d (8.5)



Scheme 1. Synthesis of the (*E*)-2-(substituted benzylidene)-3,4-dihydronaphthalen-1(2*H*)-one derivatives **1**–**6** and details of the aldehydes used. Reagents and conditions: (a) 1 M HCl acetic acid, rt., 72–96 h.

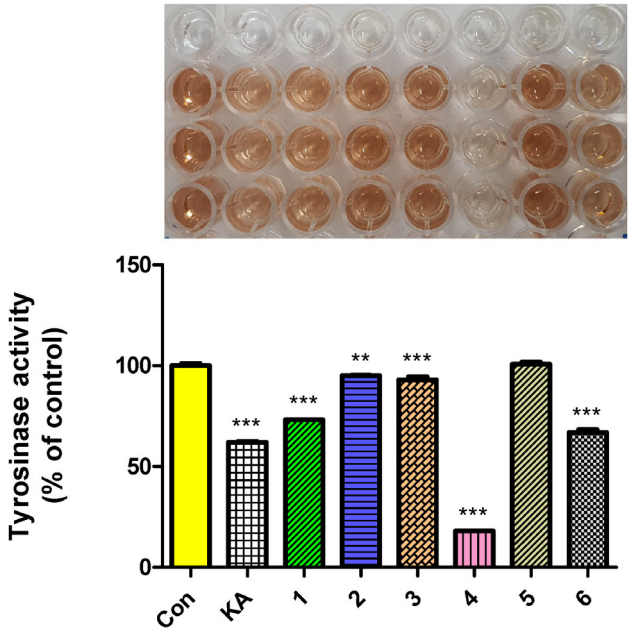


Figure 1. Mushroom tyrosinase inhibitory effects of (*E*)-2-(substituted benzylidene)-3,4-dihydronaphthalen-1(2*H*)-one derivatives **1**–**6**. Experiments were performed in triplicate at a derivative concentration of 50 μM. Con: Control, KA: Kojic acid.

measuring optical densities at 475 nm using a shaking microplate reader (VersaMax™, Molecular Devices, San Jose, CA, USA). Kojic acid (50 μM) was used as the positive control. To ensure consistency in the results, all experiments were performed three times and the percentage inhibition was obtained by the following formula.

% Tyrosinase inhibition = [1−(A/B) × 100].

A is optical density of the treated compound while B the optical density of the nontreated compound. Compound **4** exhibited greatest inhibitory activity and was subjected to further study. Three concentration-dependent inhibition experiments were carried out in triplicate at compound

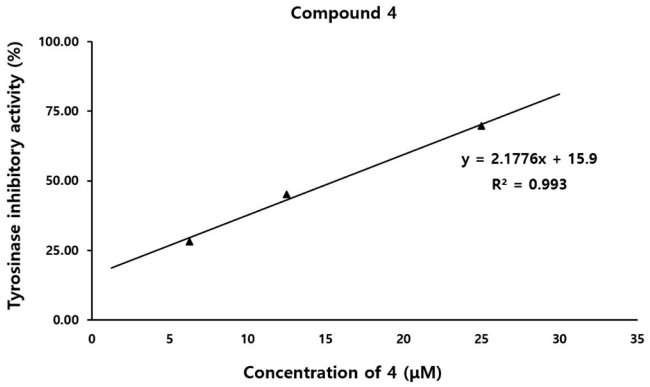


Figure 2. Tyrosinase inhibitory activities of compound **4** at concentrations of 6.25, 12.5, and 25 μM. The IC_{50} value of **4** was calculated from these data.

4 concentrations of 6.25, 12.5, or 25 μM and kojic acid concentrations of 12.5, 25, or 50 μM. Linear plots were obtained by plotting Log percent inhibition versus concentration, and IC_{50} values (defined as the concentration corresponding to 50% inhibition) were calculated.

***In silico* Docking Simulation of the Interaction between Compound 4 and Tyrosinase.** Docking studies were conducted using the Schrodinger Suite (release 2020–2).[20,21] The protein structure of tyrosinase was obtained from the Protein Data Bank of ID 2Y9X. With Maestro 12.4, unwanted chains were removed from the protein structure and hits were regenerated. In the receptor grid wizard, receptor grid coordinates were generated in the ligand-binding site of tyrosinase. The ligand structures of kojic acid and compound **4** were then imported into the entry list of Maestro project table in CDXML format and optimized with LigPrep using the OPLS3e force field. Glide extra precision (XP) was used to determine docking scores and protein-ligand interactions.

Table 2. IC_{50} values of kojic acid and compound **4.**

Compound	Conc. (μM)	Tyrosinase inhibition[a] (%)	IC_{50}[b] (μM)
Kojic acid	—	—	57.06 ± 0.46
4	6.25	28.19 ± 1.51	15.60 ± 0.32
	12.5	45.11 ± 0.71	
	25	70.42 ± 0.75	

[a] Results are the means ± SEs of three experiments.
[b] 50% inhibitory concentration.

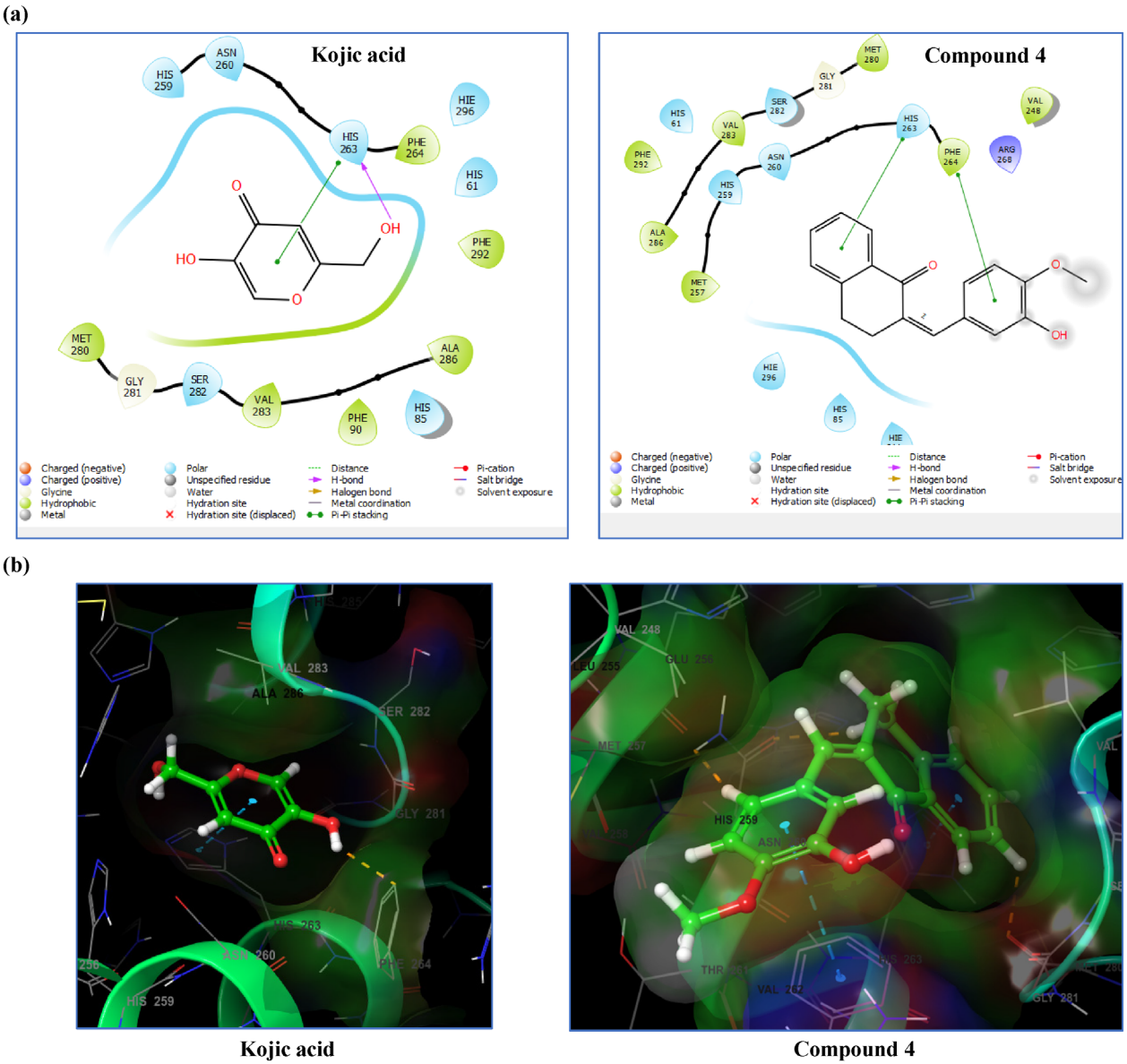


Figure 3. Binding interactions between tyrosinase (PDB ID = 2Y9X) and compound **4** or kojic acid. Pharmacophore results and binding interactions with tyrosinase for the two compounds were obtained using maestro 12.4. Figure 3(a) and (b) are 2D and 3D representations of tyrosinase-ligand interactions.

Kinetic Experiments of Tyrosinase Inhibition. To a 96-well plate, 20 μL of mushroom tyrosinase (1000 units/mL) aqueous solution, and 170 μL containing L-tyrosine (0, 2, 4, and 8 mM) as a substrate, and 50 mM potassium phosphate buffer (pH 6.5) were added with or without compound **4** (0, 7.5, 15, and 30 μM, each 10 μL). The initial

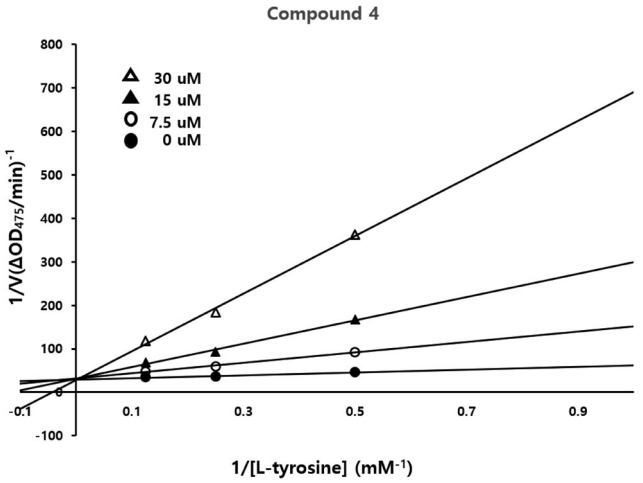


Figure 4. Lineweaver-Burk plot of mushroom tyrosinase at four different concentrations of **4**. The plot is presented as average values of 1/V of three times independent experiments with four different L-tyrosine concentrations (0, 2, 4, and 8 mM).

rate of dopachrome generation was examined by measuring the changes of optical density at 475 nm per minute (ΔOD_{475}/min). The optical density was measured using a shaking microplate reader (VersaMax™, Molecular Devices, San Jose, CA, USA). All kinetic experiments were independently repeated three times.

Results and Discussion

Synthesis. Syntheses of (*E*)-2-(substituted benzylidene)-3,-4-dihydronaphthalen-1(2*H*)-one derivatives **1**–**6** were accomplished by condensing 3,4-dihydronaphthalen-1(2*H*)-one with an appropriate benzaldehyde in 1 M HCl acetic acid solution, as shown in Scheme 1. According to our accumulated structure–activity relationship data, derivatives with at least one hydroxyl group on the β–phenyl ring of the β-phenyl-α,β-unsaturated carbonyl template exhibited tyrosinase inhibitory activity, and thus, we used benzaldehydes with one or more hydroxyl groups. The structures of the six derivatives produced were confirmed by ^{1}H and ^{13}C NMR and mass spectroscopy. Compared to chalcones, the synthesized compounds have two more carbons in their structure, providing a higher lipophilicity. When these compounds are used as whitening agents, the increased lipophilicity can improve the absorption of these compounds into the skin.

Tyrosinase Inhibition. The inhibitory effects of the six (*E*)-2-(substituted benzylidene)-3,4-dihydronaphthalen-1(2*H*)-one derivatives **1**–**6** on mushroom tyrosinase were examined. Kojic acid was used as a positive reference control. The mushroom tyrosinase inhibitory activities of all compounds were evaluated at 50 μM. The results are shown in Figure 1. Of the six compounds, compound **4** with a 3-hydroxy-4-methoxyl substituent on the β–phenyl ring of the template exhibited the greatest inhibitory effect, and at this concentration, its inhibitory effect was considerably stronger than that of kojic acid (81.92% *vs*. 37.95%). Compound **6** (33.10% inhibition) with a 3-bromo-4-hydroxyl substituent on the β–phenyl ring inhibited tyrosinase to the same extent as kojic acid, whereas compound **1** with a 4-hydroxyl substituent inhibited tyrosinase by 26.78%. The other compounds (**2**, **3**, and **5**) inhibited mushroom tyrosinase by less than 10%.

Because compound **4** exhibited greatest inhibitory activity, it was investigated in further detail. The IC_{50} value of **4** (15.60 ± 0.32 μM) was fourfold less than that of kojic acid (57.06 ± 0.46 μM) (Table 2), and at concentrations of 6.25, 12.5, and 25 μM, it inhibited tyrosinase by 28.19%, 45.11%, and 70.42%, respectively (Figure 2).

***In silico* Docking Simulation.** To investigate whether compound **4** binds to the active site of tyrosinase, *in silico* docking simulation was performed using Schrodinger Suite. Kojic acid was used as the reference control. Compound **4** and kojic acid both occupied the same binding pocket of mushroom tyrosinase (Figure 3(b)). The two-dimensional (2D) figures of tyrosinase-ligand interactions showed that compound **4** and kojic acid interacted with the amino acid residues of tyrosinase (Figure 3(a)). The pyran-4-one of kojic acid and the 3,4-dihydronaphthalenone of compound **4** both interacted with His263 via π-π stacking. Kojic acid also formed a hydrogen bond with His263. Although compound **4** did not form hydrogen bonds, it interacted via an additional pi-pi stacking interaction (blue dashed lines) with Phe264 of tyrosinase and formed several hydrophobic interactions (yellow dashed lines) with amino acid residues in the active site (Figure 3(b)). As a result of these additional interactions compound **4** (−6.4 kcal/mol) showed higher binding affinity for tyrosinase than kojic acid (−5.3 kcal/mol).

Kinetic Studies. To investigate the mechanism of tyrosinase inhibition, the initial rate of dopachrom production by tyrosinase was measured at different concentrations (0, 7.5, 15, and 30 μM) of compound **4** in the presence of L-tyrosine of various concentrations (0, 2, 4, and 8 mM). As shown in Lineweaver-Burk plot of Figure 4, four lines with different slops merged at one point on the y-axis, indicating that compound **4** competitively inhibits mushroom tyrosinase by noncovalently binding to the active site of the enzyme.

Conclusion

Based on considerations of our accumulated structure–activity relationship data, we synthesized six (*E*)-2-(substituted benzylidene)-3,4-dihydronaphthalen-1(2*H*)-one derivatives **1**–**6** containing the β-phenyl-α,β-unsaturated carbonyl template and then assayed their mushroom tyrosinase inhibitory activities. Compound **4** showed much stronger tyrosinase inhibitory activity than kojic acid, and this was supported by our docking simulation results, which showed the binding energies of

compound **4** and kojic acid were −6.4 and −5.3 kcal/mol, respectively. Kinetic studies have demonstrated that **4** is an inhibitor that competes with tyrosinase substrates. Our results also support the notion that the β-phenyl-α,-β-unsaturated carbonyl template confers tyrosinase inhibition and demonstrate that the types and positions of substituents on the β-phenyl ring of the template greatly influence tyrosinase inhibitory activity.

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Supporting Information. Additional supporting information may be found online in the Supporting Information section at the end of the article.

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