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# Synthesis and structure–activity relationships of phenylpropanoid amides of serotonin on tyrosinase inhibition

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ABSTRACT

In this manuscript, we synthesized a series of phenylpropanoid amide of serotonin **1**–**9**, analyzed their structural importance for two biologic activities (antioxidant activity and tyrosinase inhibitory activity). While the serotonin moiety and the amide linkage of serotonin derivatives affect antioxidant activity strongly, the serotonin moiety, the amide linkage and the cinnamic acid moiety affect tyrosinase inhibitory activity. Among tested compounds, compound **4** which contains cathechol moiety exhibited the most antioxidant activity ($EC_{50}$ = 19.4 ± 2.0 μM), and compound **6** exhibited significant tyrosinase inhibitory activity ($IC_{50}$ = 5.4 ± 3.6 μM). Our data suggests that a useful clue for the design and development of new tyrosinase inhibitors.



Tyrosinase (EC 1.14.18.1) is known to be a key enzyme for melanin biosynthesis and is responsible for melanization in animals and browning in plants.[1] Melanin plays a crucial protective role against skin phytocarnogenesis, however, the production of abnormal melanin pigmentation is serious esthetic problem in human beings. In the food industry, tyrosinase is responsible for enzymatic browning reactions in damaged fruit during post-harvest handling and processing. The browning reaction produces undesirable changes in color, flavor, and nutritive value of the product. Therefore, the development of tyrosinase inhibitors that can be used as preservatives for fresh food or as skin-whitening agents have been pursued. In particular, there is a concerned effort to research for naturally occurring tyrosinase inhibitors from plants, because plants constitute a rich source of bioactive chemicals and many of them are free from harmful adverse effects.[2,3] Several hydroxycinnamic acid derivatives have been found to possessed strong antioxidant activities as radical scavengers their antioxidant activity being strongly related to their structural features and the presence of a hydroxyl function in the aromatic structure[4], cinnamic acid derivatives have been reported to act as tyrosinase inhibitors.[5–7] In a recent study, the tyrosinase inhibitory effects of amides derived from coupling of caffeic acid, ferulic acid and derivatives with phenylalkylamines (tyramine, dopamine) have been reported.[8,9]

On the other hand, antioxidants, used to prevent or inhibit the natural phenomena of oxidation, have a broad application in diverse industrial fields as they have a huge importance either as industrial additives or health agents[10,11], in addition, applications of antioxidants as preservative in food industry[12] and skin-protective ingredients in cosmetics have also received considerable attention.[13]

Serotonin derivatives were identified as the major unique phenolic compound of safflower seeds, there are members of a family of indole hydroxycinnamic acid amides.[14–17] These compounds have a number of biologic effect, such as, antioxidative activity[18], anti-tumor activity[19], fibroblasts growth promoting activity[20], in particular, melanogenesis-inhibitory effects[21] of *N*-(*p*-coumaroyl serotonin) and *N*-feruloyl serotonin and tyrosinase inhibition of *N*-caffeoyl serotonin[22] have been reported. Though these compounds were important substance as a new class of potent tyrosinase inhibitors, to our knowledge, there is no report on the correlation between tyrosinase inhibition and structures of serotonin derivatives in detail. Therefore, we further investigated the structure–activity relationship of serotonin derivatives **1–9** on the inhibition of tyrosinase[23] and anti oxidant activity[24] In this study, we synthesized a series of serotonin derivatives **1–9**[25,26] and analyzed their structural importance for two biologic activities. The synthetic pathways are shown Scheme 1.

The antioxidative activity of serotonin derivatives **1–9** and cinnamic acid derivatives **1a–9a** (Fig. 1) was investigated and compared with that of dibutylhydroxytoluene (BHT). The results are shown in Table 1. All serotonin derivatives **1–9** showed a more active DPPH radical scavenging than BHT ($EC_{50}$ = 87.5 ± 2.6 μM). In particular, compound **4** which contains cathechol moiety exhibited the most potent DPPH radical scavenging activity ($EC_{50}$ = 19.4 ± 2.0 μM). On the other hand, among cinnamic acid derivatives **1a–8a**, compound **4a** which contains cathecohol moiety showed the

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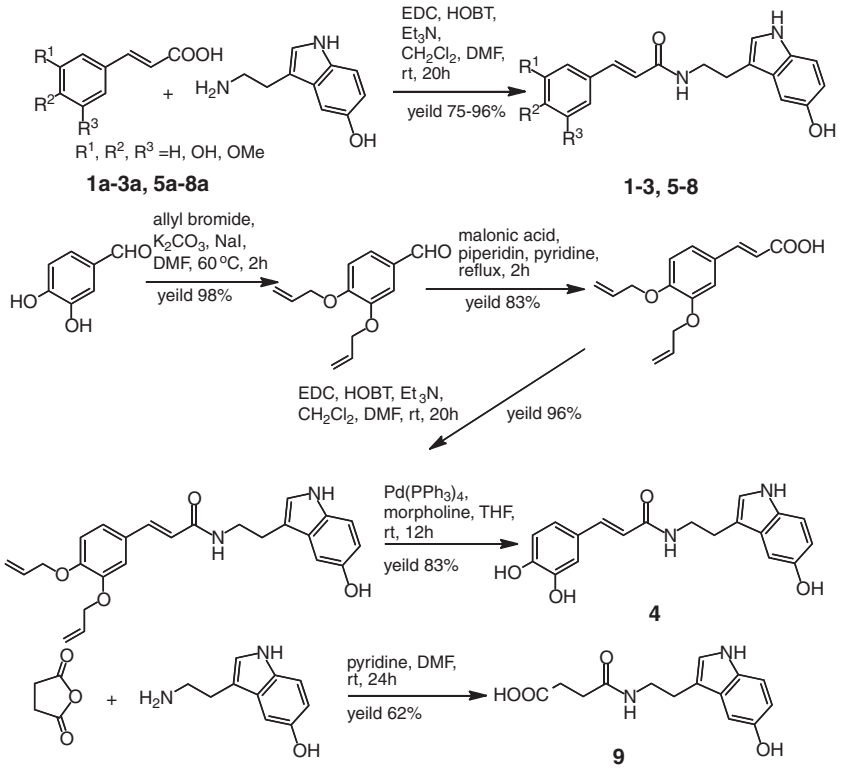


**Scheme 1.** Synthesis of serotonin derivatives **1**–**9**.

| Compound | $R^1$ | $R^2$ | $R^3$ | Compound | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|---|---|---|---|
| **1** | H | H | H | **1a** | H | H | H |
| **2** | H | OH | H | **2a** | H | OH | H |
| **3** | H | OMe | H | **3a** | H | OMe | H |
| **4** | H | OH | OH | **4a** | H | OH | OH |
| **5** | H | OH | OMe | **5a** | H | OH | OMe |
| **6** | H | OMe | OH | **6a** | H | OMe | OH |
| **7** | H | OMe | OMe | **7a** | H | OMe | OMe |
| **8** | OMe | OH | OMe | **8a** | OMe | OH | OMe |

Compound **9** (bufobutanoic acid)

**Figure 1.** Structure of serotonin derivatives and cinnamic acid derivatives.

antioxidant activity ($EC_{50}$ = 136.5 ± 3.5 μM). Whereas, all other cinnamic acid derivatives (**1a–3a**, **5a–8a**) showed low antioxidant activities. These results indicate that the serotonin moiety affect the antioxidant activity. Furthermore, serotonin did not exhibit radical scavenging activity, to the contrary, compound **9** exhibited antioxidant activity ($EC_{50}$ = 38.3 ± 1.8 μM). In antioxidant assays, the amide linkage and the serotonin moiety of serotonin derivatives are essential for the antioxidant activity. The cinnamic moiety

**Table 1**
Effects of serotonin derivatives and cinnamic acid derivatives against DPPH radical

| Compound | $EC_{50}$ [a] (μM) | Compound | $EC_{50}$ [a] (μM) |
|---|---|---|---|
| **1** | 22.2 ± 2.3 | 1a | >250 |
| **2** | 24.7 ± 1.9 | 2a | >250 |
| **3** | 34.2 ± 2.0 | 3a | >250 |
| **4** | 19.4 ± 2.0 | 4a | 136.5 ± 3.5 |
| **5** | 22.8 ± 2.6 | 5a | >250 |
| **6** | 35.1 ± 2.2 | 6a | >250 |
| **7** | 28.1 ± 2.5 | 7a | >250 |
| **8** | 30.7 ± 2.0 | 8a | >250 |
| **9** | 38.3 ± 1.8 | BHT | 87.5 ± 2.6 |
| Serotonin | >250 | | |

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

**Table 2**
Inhibitory effects of serotonin derivatives and cinnamic acid derivatives against tyrosinase

| Compound | $IC_{50}$ [a] (μM) | Compound | $IC_{50}$ [a] (μM) |
|---|---|---|---|
| **1** | 40.4 ± 3.7 | 1a | >350 |
| **2** | 8.8 ± 3.7 | 2a | 121.3 ± 4.0 |
| **3** | 23.8 ± 3.7 | 3a | >350 |
| **4** | 278.1 ± 4.6 | 4a | >350 |
| **5** | 8.0 ± 3.8 | 5a | >350 |
| **6** | 5.4 ± 3.6 | 6a | 115.2 ± 4.1 |
| **7** | 321.8 ± 3.7 | 7a | >350 |
| **8** | >350 | 8a | >350 |
| **9** | >350 | Kojic acid | 51.4 ± 3.6 |
| Serotonin | >350 | Arbutin | 201.2 ± 2.3 |

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

of serotonin derivatives did not affect antioxidant activity strongly. Therefore, we considered that the serotonin moiety and the amide linkage of serotonin derivatives have a great influence on the antioxidant activity.

After evaluating the antioxidant activity, we investigated the tyrosinase inhibitory activity of serotonin derivatives. The inhibitory activity of compounds **1, 2, 3, 5** and **6** was more potent than kojic acid ($IC_{50}$ = 51.4 ± 3.6 μM). Among tested serotonin derivatives, compound **6**[27] showed the most inhibitory activity ($IC_{50}$ = 5.4 ± 3.6 μM), whereas, compounds **4**, **7**, **8**, **9** and serotonin exhibited low inhibitions (Fig. 2 and Table 2).

Inhibitory mechanism of compound **6** on mushroom tyrosinase was analyzed by a Lineweaver–Burk plot as shown in Fig. 3. Three lines representing the uninhibited enzyme and different concentrations of compound **6** intersected on the horizontal axis. Therefore, this compound is regarded as a non-competitive inhibitor of mushroom tyrosinase. The inhibitory activity of tyrosinase was found to be similar to antioxidant activity, that is to say, inhibitory activities of serotonin derivatives were more potent than cinnamic acid derivatives. To identify the structural importance of serotonin derivatives, we evaluated compound **9** and serotonin. These compounds exhibited decreased inhibitory activity, thus, we assumed that the serotonin moiety and the cinnamic acid moiety of serotonin derivatives are essential for tyrosinase inhibitory activity. From the structural–activity point of view, compound **2** and **3** which have OH or OMe group at 4′ position exhibited a increase in tyrosinase inhibitory activity relative to compound **1**. In the case of

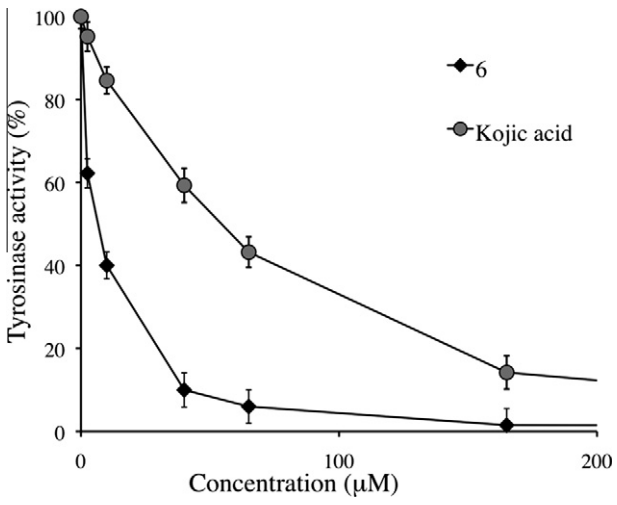


**Figure 2.** Dose-dependent inhibitory effects of compound **6** (diamond shape) on tyrosinase activity. Tyrosinase activity was measured using L-tyrosine as the substrate.

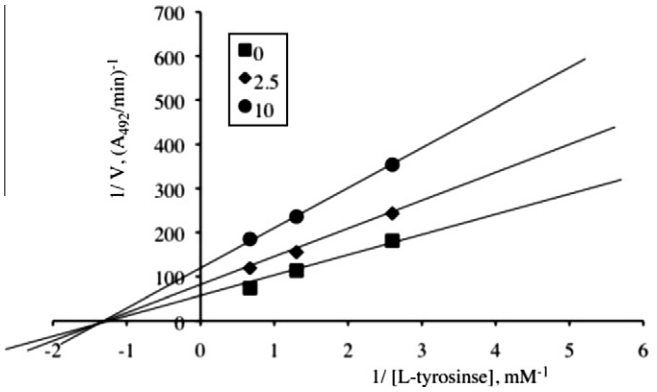


**Figure 3.** Lineweaver–Burk plot of compound **6** on tyrosinase. Concentrations of **6** were 0 mM (square), 2.5 mM (diamond), 10 mM (circle), respectively.

compound **2**, 3′ position replacement with OH group (compound **4**) decreases the inhibitory activity very much, to the contrary, in the case of compound **3**, 3′ position replacement with OH group (compound **6**) increased the activity. When the *meta*-hydroxy function of compound **2** was substituted by a methoxy group (compound **5**), the activity was similar inhibitory potency to compound **2**, on the other hand, in the case of compound **3**, compound **7** showed a significant loss in inhibitory activity. Furthermore, the introduction of another methoxy group in an *ortho*-position to a hydroxy group (compound **8**) led to less in inhibitory activity relative to compound **5**. Compound **9** exhibited a low inhibitory activity, thus, the cinnamic acid moiety of serotonin derivatives is critical factor for tyrosinase inhibitory activity. Among cinnamic acid derivatives, compounds **2a** and **6a** exhibited inhibitory potency with $IC_{50}$ values of 121.3 and 115.2 μM respectively. On the other hand, these serotonin derivatives (**2** and **6**) also exhibited inhibitory potency, therefore, we considered that the cinnamic moiety of serotonin derivatives also affect tyrosinase inhibitory activities. These results indicate the cinnamic moiety, the serotonin moiety and the amide linkage of serotonin derivatives are essential for the tyrosinase inhibitory activity.

In conclusion, we synthesized a series of serotonin derivatives (**1–9**). Compound **4** (*N*-caffeoyl serotonin) showed the most potent activity in DPPH assay ($EC_{50}$ = 19.4 μM) and compound **6** showed significant tyrosinase inhibitory activity ($IC_{50}$ = 5.4 μM). In the two biologic activity (antioxidant activity and tyrosinase inhibitory activity), while, the serotonin moiety and the amide linkage of serotonin derivatives affect the antioxidant activity strongly, the serotonin moiety, the amide linkage and the cinnamic acid moiety of serotonin derivatives also affect tyrosinase inhibitory activity. These results indicate that there is no evidence for direct correla-

tion between free radical scavenging activity and tyrosinase inhibition, however, suggest that serotonin derivatives may serve as the designed development of novel tyrosinase inhibitors.

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.02.028.

## References and notes


1. Perez-Gilabert, M.; Garcia-Carmona, F. *Biochem. Biophys. Res. Commun.* **2001**, *285*, 257.
2. Kubo, I.; Yokokawa, Y.; Kinst-Hori, I. *J. Nat. Prod.* **1995**, *58*, 739.
3. Lee, G. C.; Lee, C. Y. *Food Chem.* **1997**, *60*, 231.
4. Bran-Williams, W.; Cuvelier, ME.; Berset, C. *Lebensm-Wiss-Technol.* **1995**, *28*, 25.
5. Gomez-Cordové, C.; Bartolomé, B.; Vieira, W.; Virador, V. M. *J. Agric. Food. Chem.* **2001**, *49*, 1620.
6. Masamoto, Y.; Murata, Y.; Baba, K.; Shimoishi, Y.; Tada, M.; Takahata, K. *Biol. Pharm. Bull.* **2004**, *27*, 422.
7. Khan, K. M.; Maharvi, G. M.; Abbaskhan, A.; Hayat, S.; Khan, M. T. H.; Makhmoor, T.; Choudhary, M. I.; Shaheen, F.; Atta-ur-Rahman. *Helv. Chim. Acta* **2003**, *86*, 457.
8. Okombi, S.; Kival, D.; Bonnet, S.; Moriotte, A. M.; Pervier, E.; Boumendzel, A. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2252.
9. Mellay-Hamon, V. L.; Criton, M. *Biol. Pharm. Bull.* **2009**, *32*, 301.
10. Aruoma, O. I.; Halliwell, B. *Free Radical Food Addit.* **1991**, 121.
11. Halliwell, B.; Murcia, M. A.; Chirico, S.; Aruoma, O. I. *Crit. Rev. Food Sci. Nutr.* **1995**, *35*, 7.
12. Winkler, C.; Frick, B.; Schroecksnadelm, K.; Schennach, H.; Fuchs, D. *Food Chem. Toxicol.* **2006**, *44*, 2003.
13. Lupo, M. P. *Clin. Dermatol.* **2001**, *19*, 467.
14. Sakamura, S.; Terayama, Y.; Kawakatsu, S.; Ichihara, A.; Saito, H. *Agric. Biol. Chem.* **1978**, *42*, 1805.
15. Sato, H.; Kawagishi, H.; Nishimura, T.; Yoneyama, S.; Yoshimoto, Y.; Sakamura, S. *Agric. Biol. Chem.* **1985**, *49*, 2969.
16. Park, J. B. *Phytomedicine* **2008**, *15*, 1093.
17. Kang, K.; Park, S.; Kim, Y. S.; Lee, S.; Back, K. *Appl. Microbiol. Biotechnol.* **2009**, *83*, 27.
18. Zhang, H. L.; Nagatsu, A.; Watanabe, T.; Sakakibara, J.; Okuyama, H. *Chem. Pharm. Bull.* **1997**, *45*, 1910.
19. Nagatsu, A.; Zhang, H. L.; Mizukami, H.; Okuyama, H.; Sakakibara, J.; Tokuda, H.; Nishino, H. *Nat. Prod. Lett.* **2000**, *14*, 153.
20. Takii, T.; Hayashi, M.; Hiroma, H.; Chiba, T.; Kawashima, S.; Zhang, H. L.; Nagatsu, A.; Sakakibara, J.; Onozaki, K. *J. Biochem.* **1999**, *125*, 910.
21. Roh, J. S.; Han, Y. J.; Kim, J. H.; Hwong, J. K. *Biol. Pharm. Bull.* **2004**, *27*, 1976.
22. Yamazaki, Y.; Kawano, Y.; Yamanaka, A.; Maruyama, S. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4178.
23. *Method of tyrosinase assay*: The tyrosinase assay was performed by using L-tyrosine as the substrate. 140 μL of 0.1 M phosphate buffer (pH 7.0), 36 μL of 1.5 mM L-tyrosine and 13 μL of sample solution were added to each well of a 96-well plate and then incubated at 37 °C for 10 min. Then 16 μL of mushroom tyrosinase (500 unit/ml, 0.1 M phosphate buffer at pH 7.0) was added, and the assay mixture was incubated at 37 °C for 25 min. Before and after incubation, the amount of dopachrome produced in the reaction mixture was measured at 492 nm in a microplate reader (Corona Electric Co. Ltd.). Arbutin and kojic acid were used as a positive control. The extent of tyrosinase inhibition by the addition of the different compounds was calculated and expressed as the percentage necessary for 50% inhibition concentration ($IC_{50}$). The percentage of tyrosinase activity was calculated as follows: Tyrosinase activity (%) = $[(C-D)/(A-B)] \times 100$, where $A$ is the absorbance at 492 nm without test sample, $B$ is the absorbance at 492 nm without test sample and substrate, $C$ is the absorbance at 492 nm with test sample, $D$ is the absorbance at 492 nm with test sample, but without substrate.
24. The scavenging activity for the DPPH radical was performed according to the follow method. An amount of 500 μL of a 0.5 mM methanoic DPPH solution was mixed in a cuvette with 500 μL of cinnamic acid derivatives at different concentration levels. These cuvettes were shaken vigorously. The cuvettes were allowed to stand at 27 °C for 30 min, the absorbance was measured at 517 nm using a U-1500 spectrophotometer. The percentage of radical scavenging activity was calculated using the equation. Radical scavenging activity (%) = (Control OD−Sample OD/Control OD) × 100. All tests were performed in triplicate. BHT was used as a reference standard for the investigation of radical scavenging activity.
25. Koyama, N.; Kuribayashi, K.; Seki, T.; Kobayashi, K.; Furuhata, Y.; Suzuki, K.; Arisaka, H.; Nakano, T.; Amino, Y.; Ishii, K. *J. Agric. Food. Chem.* **2006**, *54*, 4970.
26. Somei, M.; Yamada, F.; Kurauchi, T.; Nagahama, Y.; Hasegawa, M.; Yamada, K.; Teranishi, S.; Sato, H.; Kaneko, C. *Chem. Pharm. Bull.* **2001**, *49*, 87.
27. *N-Isoferuloyl serotonin* (**6**): This compound was obtained with a yield of 77.0%; mp: 99–103 °C; IR (KBr): 3390, 1652, 1584, 1509 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.47 (1H, d, $J$ = 2.0 Hz, H-1), 9.16 (1H, s, 3′-OH) 8.59 (1H, s, 5-OH), 8.10 (1H, t, $J$ = 6.0 Hz, –CONH), 7.27 (1H, d, $J$ = 15.6 Hz, H-7′), 7.10 (1H, d, $J$ = 8.0 Hz, H-7), 7.04 (1H, d, $J$ = 2.0 Hz, H-2), 6.97 (1H, d, $J$ = 1.2 Hz, H-2′), 6.95 (1H, dd, $J$ = 8.4 and 1.2 Hz, H-6′), 6.92 (1H, d, $J$ = 8.4 Hz, H-5′), 6.86 (1H, d, $J$ = 1.6 Hz, H-4), 6.58 (1H, dd, $J$ = 8.0 and 1.6 Hz, H-6), 6.39 (1H, d, $J$ = 15.6 Hz, H-8′), 3.78 (3H, s, 4′-OMe), 3.41 (2H, dt, $J$ = 6.0 and 7.2 Hz, H-11), 2.77 (2H, t, $J$ = 7.2 Hz, H-10). $^{13}C$ NMR (100 MHz, DMSO-$d_6$) $\delta$ 165.1 (C-9′), 150.2 (C-5), 149.1 (C-4′), 146.7 (C-3′), 138.5 (C-7′), 130.8 (C-8), 127.9 (C-9), 127.8 (C-1′), 123.1 (C-2), 120.1 (C-6′), 119.7 (C-8′), 113.3 (C-5′), 112.1 (C-7), 111.6 (C-6), 111.3 (C-3), 110.8 (C-2′), 102.4 (C-4), 55.6 (4′-OMe), 39.4 (C-11), 25.4 (C-10).