Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 17 (2007) 5462–5464

Synthetic tyrosyl gallate derivatives as potent melanin formation inhibitors

Chan Woo Lee,[a] Eun-Mi Son,[b] Han Sung Kim,[a] Pan Xu,[b] Tuyagerel Batmunkh,[c] Burm-Jong Lee[b,c,*,†] and Kyung Ah Koo[b,*,†]

[a]*R&D Center, AmorePacific Corporation, Kyounggi-do 449-729, Republic of Korea*
[b]*Biohealth Products Research Center, Inje University, Gimhae 621-749, Republic of Korea*
[c]*Department of Chemistry, Inje University, Gimhae 621-749, Republic of Korea*

Received 11 April 2007; revised 14 June 2007; accepted 9 July 2007
Available online 25 July 2007

Abstract—Three tyrosyl gallate derivatives (**1**–**3**) with variable hydroxyl substituent at the aromatic ring of tyrosol were synthesized and evaluated as potent inhibitors on tyrosinase activity and melanin formation in melan-a cells. Among three tyrosyl gallate derivatives, 4-hydroxyphenethyl 3,4,5-trihydroxybenote (**1**) (IC_{50} = 4.93 μM), 3-hydroxyphenethyl 3,4,5-trihydroxybenote (**2**) (IC_{50} = 15.21 μM), and 2-hydroxyphenethyl 3,4,5-trihydroxybenote (**3**) (IC_{50} = 14.50 μM) exhibited significant inhibitory effect on tyrosinase activity. Compound **1** was the most active compound, though it did not show the inhibitory effect on melanin formation in melan-a cells. However, compounds **2** (IC_{50} = 8.94 μM) and **3** (IC_{50} = 13.67 μM) significantly suppressed the cellular melanin formation without cytotoxicity. This study shows that the position of hydroxyl substituent at the aromatic ring of tyrosol plays an important role in the intracellular regulation of melanin formation in cell-based assay system.

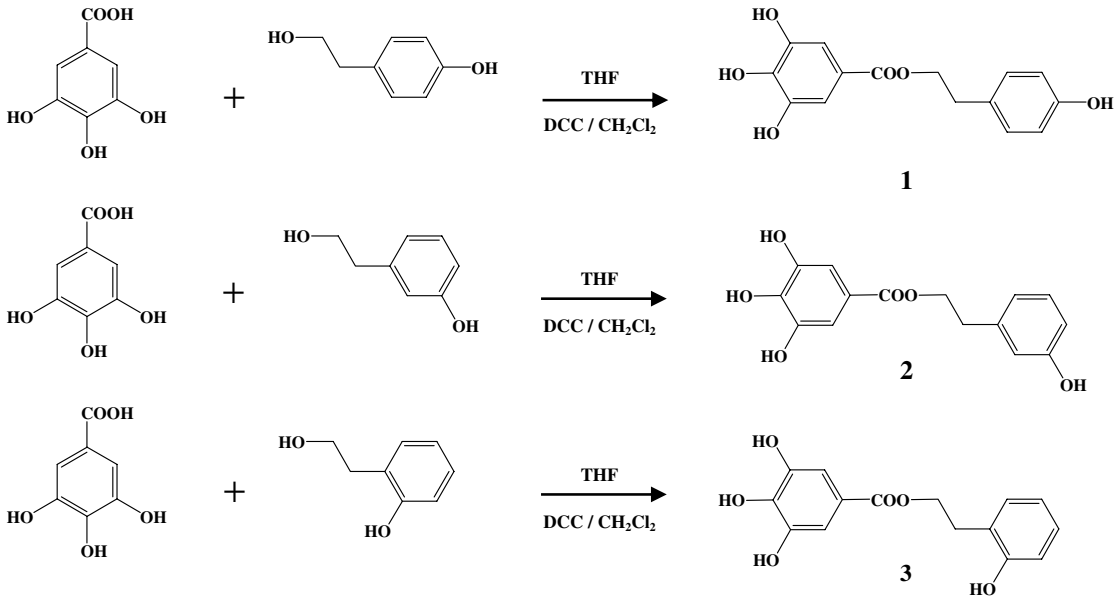
Tyrosinase is the key enzyme that converts the amino acid L-tyrosine finally to melanin and its inhibitors are target molecules to develop depigmentation agents for treatment skin after sunburn.[1] The melanin formation by the tyrosinase activity after sunlight exposure causes some dermatological disorders associated with freckles, melasma, and senile lentigines.[2] Therefore, potent inhibitors on tyrosinase and melanin formation should be clinically and cosmetically useful for the treatment of dermatological disorders.[3,4]

To develop depigmentation agents, we designed and synthesized three natural product derivatives using tyrosol and gallate as basic skeletons, because many phenolic compounds such as caffeic acid, ferulic acid, resveratrol, and *p*-coumaric acid have been reported to act as tyrosinase inhibitors.[5,6] And also, some antioxidant and anti-inflammatory compounds were reported to inhibit the melanin formation in the melanogenic process.[7] Tyrosol and gallate are present in other dietary sources and have various biological properties such as antioxidative, cancer preventive, and anti-inflammatory activities.[8]

Tyrosyl gallate derivatives were synthesized in one step through the reaction of esterification using *N*,*N*′-dicyclohexylcarbodiimide (DCC) together with 4-hydroxyphenyl alcohol, 3-hydroxyphenyl alcohol, and 2-hydroxyphenyl alcohol, respectively, in the presence of tetrahydrofuran (THF) and dichloromethane (CH_2Cl_2) at the room temperature. The synthetic routes of tyrosol gallate derivatives are shown in Scheme 1. The synthetic tyrosol gallate derivatives, 4-hydroxyphenethyl 3,4,5-trihydroxybenote (**1**), 3-hydroxyphenethyl 3,4,5-trihydroxybenote (**2**), and 2-hydroxyphenethyl 3,4,5-trihydroxybenote (**3**), were successfully obtained by silica gel column chromatography using mixture solvent of ethyl acetate and *n*-hexane in 31.3%, 28.5%, and 26% yields, respectively (Scheme 1). The chemical structures of the synthesized compounds were determined by different spectroscopic techniques, like ^{1}H NMR, ^{13}C NMR, IR, and EA.[15] 4-Hydroxyphenethyl 3,4,5-trihydroxybenote (**1**) is an already reported compound, but 3-hydroxyphenethyl 3,4,5-trihydroxybenote (**2**) and 2-hydroxyphenethyl 3,4,5-trihydroxybenote are newly reported in synthetic and natural constituents.[9]

Keywords: Tyrosyl gallate derivative; Tyrosinase inhibitor; Melan-a cell; Melanin formation inhibition.
* Corresponding authors. Tel.: +82 55 320 3802; fax: +82 55 331 0422 (K.A.K.); e-mail: kakoo@inje.ac.kr
† These authors contributed equally to this work.

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.07.032



Scheme 1. Synthesis of tyrosyl gallate derivatives (**1**–**3**).

All the synthesized tyrosyl gallate derivatives were subjected to tyrosinase inhibition assay using melan-a cell line as cell-based assay system.[16] After incubation for 3 days with various concentrations of compounds in cultured cell, a cell lysate containing tyrosinase enzyme was prepared and the inhibitory activity with L-tyrosine and L-Dopa measured. Arbutin isolated from the bearberry is well-known tyrosinase inhibitor and it is therefore used as a skin-lightening agent and a positive standard.[10] Compounds **1**, **2**, and **3** exhibited significant inhibitory effect on tyrosinase activity. As shown in Table 1, compound **1** (IC_{50} = 4.93 μM), which has a hydroxyl group of *para*-configuration, showed the strongest inhibitory activity among compounds **2** (IC_{50} = 15.21 μM) having hydroxyl group of *meta*-configuration and **3** (IC_{50} = 14.50 μM) having hydroxyl group of *ortho*-configuration. The inhibitory activities of tyrosyl gallate derivatives were more potent than that of which arbutin showed tyrosinase activity with IC_{50} values of 30.26 μM. From the structural-activity point of view, we have found that 4-hydroxyphenethyl 3,4,5-trihydroxybenote (**1**) with a *para*-configuration of hydroxyl group may contribute to the inhibitory activity against tyrosinase enzyme.

The tyrosinase enzyme catalyzes the oxidation of L-Dopa to *ortho*-dopaquinone, and antioxidants may prevent the oxidation step in the process of melanogenesis.[11] Such as, we measured their DPPH radical scavenging activity.[17] The derivatives (**1**–**3**) showed significant antioxidant activity with IC_{50} values of 7.52, 7.78, and 8.31 μM, respectively, like α-tocopherol (IC_{50} = 9.89 μM) and vitamin C (IC_{50} = 13.60 μM). This result demonstrates that the inhibitory effect of tyrosol gallate derivatives against tyrosinase reaction is evidence for direct correlation with antioxidant activity.

Table 1. Inhibitory effects of the synthetic tyrosyl gallate derivatives on tyrosinase and melanin formation in melan-a cells

Compound	Tyrosinase IC_{50} (μM)	Melanin formation IC_{50} (μM)	Cytotoxicity IC_{50} (μM)
1	4.93	—	>10
2	15.21	8.94	>50
3	14.50	13.67	>50
Arbutin	30.26	118.05	>1500

To evaluate the inhibitory potency against the melanin formation, three tyrosol gallate derivatives were assessed for their cytotoxicity and the inhibitory effect in the cultured melan-a cells.[19] After treatment of compound for 5 days, a melanin fraction from a cell lysate was prepared and the melanin contents determined.[18] A standard curve for melanin determination was prepared using synthetic melanin purchased from Sigma Co. Among three derivatives, compounds **2** (IC_{50} = 8.94 μM) and **3** (IC_{50} = 13.67 μM) significantly suppressed the cellular melanin formation as compared to the inhibitory activity of arbutin (IC_{50} = 118.05 μM). Compound **1**, which exhibited the highest activity on tyrosinase inhibition, however, it did not show the inhibitory activity in melanin formation. As shown in the results for cytotoxicity of these compounds, compounds **2** and **3** did not show cytotoxic effects at concentrations ranging from 1 to 50 μM in the cultured melan-a cells, while compound **1** showed cytotoxic effect over the concentration of 10 μM.

From these results, tyrosol gallate derivatives can be utilized for the development of new candidate for treatment of disorders related to tyrosinase.

Acknowledgments

This study was supported by a grant from the Ministry of Commerce, Industry and Energy (MOCIE) and the Korea Institute of Industrial Technology Evaluation &

Planning (ITEP) through the Biohealth Products Research Center (BPRC) of Inje University and in partly Brain Korea 21 (BK21) of Korea Research Foundation.

References and notes

1. Briganti, S.; Camera, E.; Picardo, M. *Pigment Cell Res.* **2003**, *16*, 101.
2. Frenk, E. *Melasma: New Approaches to Treatment*; Martin Dunitz: London, 1995, pp. 9–15.
3. Hearing, V. J. In *Methods in Enzymology*; Academic Press: New York, 1987; Vol. 142, pp 631–643.
4. Kligman, A. M.; Willis, I. *Arch. Dermatol.* **1975**, *111*, 40.
5. Go'mez-Cordove's, C.; Bartolome', B.; Vieira, W.; Virador, V. M. *J. Agric. Food Chem.* **2001**, *49*, 1620.
6. Ohguchi, K.; Tanaka, T.; Ito, T.; Iinuma, M.; Matsumoto, K.; Akao, Y.; Nozawa, Y. *Biosci. Biotechnol. Biochem.* **2003**, *67*, 1587.
7. Kimaand, Y.-J.; Uyama, H. *Cell Mol. Life Sci.* **2005**, *621*, 707.
8. Tuck, K. L.; Hayball, P. J. *J. Nutr. Biochem.* **2002**, *13*, 636.
9. Viranga Tillekeratne, L. M.; Sherette, A.; Fulmer, J. A.; Hupe, L.; Hupe, D.; Gabbara, S.; Peliska, J. A.; Hudson, R. A. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 525.
10. Maeda, K.; Fukuda, M. *J. Pharmacol. Exp. Ther.* **1995**, *276*, 765.
11. Hearing, V. J.; Tsukamoto, K. *FASEB J.* **1991**, *5*, 2902.
12. Jiménez-Cervantes, C.; Solano, F.; Kobayashi, T.; Urabe, K.; Hearing, V. J.; Lozano, J. A.; García-Borrón, J. C. *J. Biol. Chem.* **1994**, *269*, 17993.
13. Wright, S. *J. Dermatolog. Treat.* **1990**, *1*, 47.
14. Kalb, V. F.; Bernlohr, R. W. *Anal. Biochem.* **1977**, *82*, 362.
15. (a) Compound **1**: white solid; mp 205 °C; R_f 0.69 (SiO_2, 100% EtOAc); ^{1}H NMR (300 MHz, acetone-d_6) δ 2.98 (t, 2H), 4.39 (t, 2H), 6.8 (d, 2H), 7.15–7.35 (d, 4H), 8.19 (s, 3H); ^{13}C NMR (75 MHz, acetone-d_6) δ 34.93, 66.04, 109.62, 109.86, 115.89, 116.17, 121.93, 129.86, 130.88, 138.64, 145.95, 156.79, 166.57; EA found for $C_{15}H_{14}O_6$: C 61.42, H 5.34, O 33.00% (calculated analysis for $C_{15}H_{14}O_6$: C 62.07, H 4.86, O 33.07%); IR (KBr): ν (cm^{-1}) 3334, 3050, 2980, 1687, 1604, 1456, 1244; (b) Compound **2**: white solid; mp 148 °C; R_f 0.67 (SiO_2, 100% EtOAc); ^{1}H NMR (300 MHz, acetone-d_6) δ 3.04 (t, 2H), 4.41 (t, 2H), 6.78 (t, 1H), 6.81 (d, 1H), 7.04 (t, 1H), 7.11 (s, 2H), 7.18 (d, 1H), 8.22 (s, 3H); ^{13}C NMR (75 MHz, acetone-d_6) δ 30.55, 64.48, 109.78, 115.83, 120.38, 122.03, 125.16, 128.48, 131.69, 138.59, 145.94, 156.18, 166.69; EA found for $C_{15}H_{14}O_6$: C 61.89, H 4.64, O 32.74% (calculated analysis for $C_{15}H_{14}O_6$: C 62.07, H 4.86, O 33.07%); IR (KBr): ν (cm^{-1}) 3473, 3203, 2974, 1693, 1625, 1452, 1259; (c) Compound **3**: white solid; mp 118 °C; R_f 0.71 (SiO_2, 100% EtOAc); ^{1}H NMR (300 MHz, acetone-d_6): δ 3.04 (t, 2H), 4.41 (t, 2H), 6.78 (t, 1H), 6.81 (d, 1H), 7.04 (t, 1H), 7.11 (s, 2H), 7.18 (d, 1H), 8.22 (s, 3H); ^{13}C NMR (75 MHz, acetone-d_6) δ 166.69, 156.18, 145.94, 138.59, 131.69, 128.48, 125.16, 122.03, 120.38, 115.83, 109.78, 64.48, 30.55; EA found for $C_{15}H_{14}O_6$: C 61.89, H 4.64, O 32.74% (calculated analysis for $C_{15}H_{14}O_6$: C 62.07, H 4.86, O 33.07%); IR (KBr): ν (cm^{-1}) 3473, 3203, 2974, 1693, 1625, 1452, 1259.
16. *Tyrosinase inhibition assay*. The tyrosinase activity was measured with a modification of the method reported by Martinez-Esparza et al.[12] Melan-a cells were cultured for 3 days with various concentrations of the synthetic compounds. The cells were collected by centrifugation, washed with ice-cold PBS (pH 7.4), and resuspended in 1 ml of homogenization buffer (80 mM PO_4 buffer + 1% Triton X-100 + 100 μg/ml PMSF). The cell lysate was centrifuged at 12,500 rpm for 15 min to remove insoluble material. The protein concentration was determined by the Bradford method using BSA as a standard. The cell homogenate was mixed and incubated with 0.8 mM L-Dopa for 1 h at 37 °C and then the activity was assayed at 475 nm using ELISA micro plate reader.
17. *DPPH radical scavenging assay*. One hundred microliters of 60 μM DPPH in ethanol was mixed with 100 μl of the different concentrations of the tested compounds in 96-well plate. After 30 min at room temperature, the absorbance was recorded at 520 nm using ELISA micro plate reader.
18. *Melanin formation assay*. The melanin content for inhibition assay on melanin formation was measured using a modified method.[13] After incubation for 5 days with the compounds, the cells were washed with ice-cold PBS (pH 7.4) and the pellets were collected by trypsinization and centrifugation. The pellets were dissolved in 1 N NaOH solution and the melanin was measured for absorbance at 400 nm using an ELISA reader. The melanin content was determined in μg from a synthetic melanin standard curve and correlated to mg protein using method of Kalb and Bernlohr (1977).[14]
19. *Cytotoxicity assay*. Cell viability of compounds was determined using the MTT method. The melan-a cell was plated in 96-well plates and after 24 h, the test compounds were added to each well and incubated during indicated time. Cell survival was determined in a colorimetric assay using mitochondrial dehydrogenase activity in active mitochondria to form purple formazan. The absorbance was measured at 570 nm using an ELISA reader.