# New 5-Hydroxy-2-(hydroxymethyl)-4H-pyran-4-one Derivative Has Both Tyrosinase Inhibitory and Antioxidant Properties

**Ho Sik Rho,* Heung Soo Baek, Jae Won You, Sujong Kim, Jin Young Lee, Duck Hee Kim, and Ih Seop Chang**

*R & D Center, AmorePacific Corporation, Yongin, Gyeonggi 446-729, Korea. *E-mail: thiocarbon@freechal.com*
*Received November 18, 2006*

**Key Words :** 5-Hydroxy-2-(hydroxymethyl)-4H-pyran-4-one, Derivative, Tyrosinase, Antioxidant

Kojic acid,[1] 5-hydroxy-2-(hydroxymethyl)-4H-pyran-4-one, is produced from carbohydrate sources in an aerobic process by a variety of microorganisms. It showed broad biological activities such as inhibition of tyrosinase,[2] scavenging of the free radicals,[3] chelating activity of metal ions[4] and prevention of photodamage.[3] Its various activities are due to γ-pyranone structure having enolic hydroxyl group. Recently, enolic hydroxyl group of kojic acid has been focused as an alternative of carboxylic acid in retinoid structure.[5] We synthesized 3,4-methylenedioxy cinnamic acid ester of kojic acid as a new retinoidal compound. In this study, we evaluated biological activities of new kojic acid derivative **1**, 2-((3*E*)-4(2H,3H-benzo[3,4-d]1,3-dioxolan-5-yl)-2-oxo-but-3-enyloxy)-5-hydroxy-4H-pyran-4-one.

## Experimental Section

**Synthesis.** Compound **1** was synthesized by the condensation of kojyl chloride with potassium salt of 3,4-methylenedioxy cinnamic acid. Structures of compounds and synthetic pathways are shown in Figure 1. Kojic acid was reacted with thionyl chloride to afford a kojyl chloride **2**. Then, kojyl chloride **2** was reacted with potassium salt of 3,4-(methylenedioxy) cinnamic acid to afford the final compound **1**.

TLC, $SiO_2$, EtOAc/hexanes 2 : 1, $R_f$ = 0.41 $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$9.20 (bs, 1H), 8.05 (s, 1H), 7.58 (d, 1H, $J$ = 15.9 Hz), 7.39 (s, 1H), 7.19 (d, 1H, $J$ = 8.4 Hz), 6.90 (d, 1H, $J$ = 8.4 Hz), 6.55 (d, 1H, $J$ = 15.9 Hz), 6.45 (s, 1H), 6.02 (s, 2H), 5.00 (s, 2H). IR $\nu_{max}$ (KBr) 3206, 1726 cm$^{-1}$. Ms-FAB (m/e) 317 ($M^+$+1).

**Mushroom tyrosinase assay.** Mushroom tyrosinase, L-tyrosine, and L-DOPA were purchased from Sigma Chemical (St. Louis, MO, USA). Tyrosinase activity was determined using the method of Pomerantz[6] with minor modification. Twenty-five μL of 0.5 mM L-DOPA, 25 μL of 10 mM L-tyrosine, 875 μL of 50 mM phosphate buffer (pH 6.5), and 25 μL of test sample solution were mixed. Then 50 μL of mushroom tyrosinase (1600 U/mL) was added. The amount of dopachrome produced in the reaction mixture was determined against a blank (solution without enzyme) at 475 nm ($OD_{475}$) using a spectrophotometer (Shimadzu Corporation, Kyoto, Japan).

**MTT growth assay.** HaCaT keratinocytes were maintained in DMEM (Gibco, Grand Island, NY, USA) supplemented 10% fetal bovine serum, previously inactivated at 56 °C for 20 min. The cytotoxic effects of test materials were monitored by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as dose dependent manner.

**Lipid peroxidation.** HaCaT keratinocytes were grown in DMEM medium containing 10% fetal bovine serum and 1% antibiotic and antimycotic solution. For experiments, cells were maintained in DMEM supplemented with 1% fetal bovine serum (FBS) and test materials for 18 h. After HaCaT keratinocytes were incubated with test materials for 18 h, the cells were exposed to 4 mM *t*-BOOH for 4 h. Following incubation, the cell were washed twice with phosphate-buffered saline (PBS), and lysed by repetitive freeze/thawing in distilled water. To establish the levels of lipid peroxidation, malondialdehyde (MDA) and 4-hydroxy-2(*E*)-nonenal (4-HNE) levels were quantified using a commercial colorimetric lipid peroxidation assay kit (Calbiochem, San Diego, CA). This method analyzes MDA and 4-HNE by their reaction with a chromogen (*N*-methyl-2-phenylindole) at 45 °C to produce a stable chromogen. The reaction products were measured by spectrophotometry at 586 nm. The procedure was performed in accordance with the manufacturer's specifications and data were expressed in mmol/mg protein.

## Results and Discussion

Compound **1** is a kojic acid derivative which possesses an ester linker between kojic acid and 3,4-(methylenedioxy)-

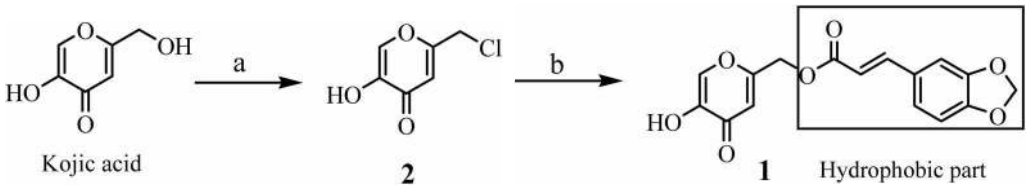


**Figure 1.** Reaction conditions; (a) $SOCl_2$, DMF; (b) Potassium salt of 3,4-(methylenedioxy)cinnamic acid, DMF.

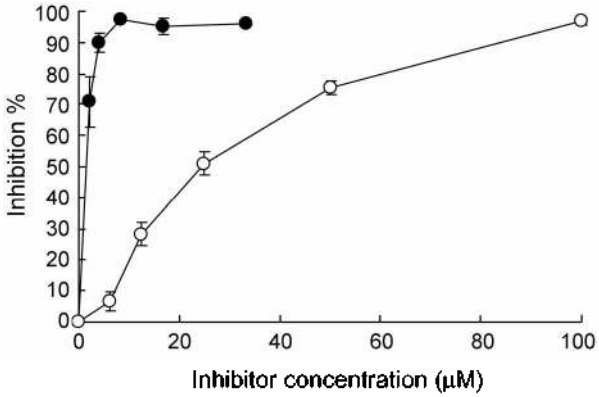


**Figure 2.** Dose-dependent inhibitory effects on mushroom tyrosinase by compound **1** and kojic acid. Samples shown are compound **1** (closed circle) and kojic acid (open circle). Effect on tyrosinase activity by samples as a function of concentration are represented as inhibition %, means ± S.E. of the three independent tests.

cinnamate moiety. Cinnamate group was introduced as a hydrophobic moiety to increase tyrosinase inhibitory activity of kojic acid. The mushroom tyrosinase inhibitory activities of compound **1** and kojic acid were determined using L-tyrosine as substrate. When L-tyrosine was used as a substrate, compound **1** showed stronger inhibitory activity than that of kojic acid. $IC_{50}$ of compound **1** is 1.4 $\mu$M (Fig. 2).

A kinetic study of L-tyrosine oxidation catalyzed by mushroom tyrosinase was accomplished in the presence of compound **1** and kojic acid (Fig. 3). Compound **1** and kojic acid showed the same Michaelis-Menten constant ($K_m$ value). It means that the same moiety was used for inhibitory effects on the mushroom tyrosinase. Through Lineweaver-Burk plot data, compound **1** was a competitive inhibitor.

Another expected biological activity of compound **1** is an antioxidant effect. Recently, kojic acid showed inhibitory activity in lipid peroxidation.[3] 5-Hydroxyl group of kojic acid is regarded as a hydrogen donor that results in radical scavenging activity. Cytotoxicity and inhibitory potency of compound **1** in lipid peroxidation was compared with known antioxidant agents such as trolox,[7] EGCG[8] and kojic acid. Cell viability was assessed by the MTT reduction assay. HaCat cells were resistant to up to 10 $\mu$M concentration for all test materials.

After confirming cell viability, we evaluated inhibitory activity of compound **1** and known antioxidants. Their activities were examined in terms of ability to reduce the oxidative factors such as malondialdehyde (MDA) and 4-hydroxy-2(*E*)-nonenal (4-HNE), generated by TBHP (*tert*-butylhydroperoxide) in HaCaT cell line.[9] Treatment of 4 mM of TBHP increased lipid peroxide level up to about three times as compared with untreated sample. When 10 $\mu$M of compounds were treated, trolox, EGCG and compound **1** were active (Fig. 4). Compound **1** decreased the level of lipid peroxidation by about 47% in contrast with TBHP-treated control. However, kojic acid showed no inhibitory activity at 10 $\mu$M concentration.

Compound **1** showed more potent biological activities

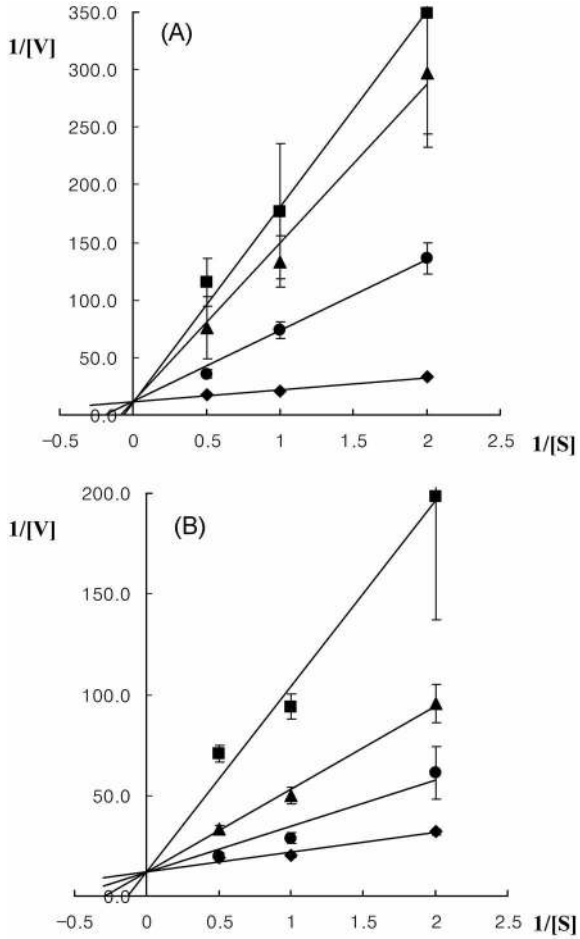


**Figure 3.** Lineweaver-Burk plot of mushroom tyrosinase on the presence of compound **1** and kojic acid. Data were obtained as mean value of 1/[V], inverse of the increase of optical density at 450 nm per min. (OD450/min), of three independent tests with different concentrations of L-tyrosine as a substrate. (A) with 10 $\mu$M (rectangle), 5 $\mu$M (triangle), 2 $\mu$M (circle), or no compound **1** (diamond) and kojic acid (B) with 100 $\mu$M (rectangle), 50 $\mu$M (triangle), 20 $\mu$M (circle), or no kojic acid

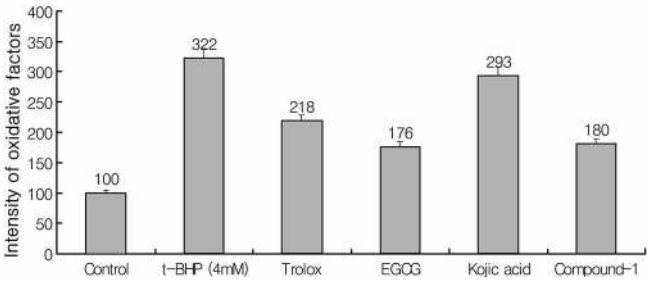


**Figure 4.** Inhibitory effects on lipid peroxidation induced by TBHP in HaCat cell line. All compounds were tested at 10 $\mu$M concentration.

than those of kojic acid in two tested methods. These results suggest that biological activities of kojic acid were increased by the addition of 3,4-(methylenedioxy)cinnamate moiety as a hydrophobic part. Kojic acid is hydrophilic compound because it has two hydroxyl groups in 2 and 5 positions. Compound **1** is believed to be more adequate in cell permeation than kojic acid because of its balance in hydrophilic

**Table 1**. Calculation of Log P values

| Compound | Log P[a] |
|---|---|
| Kojic acid | −1.111 |
| Compound **1** | 1.169 |

[a]Log P: Log[octanol/water] partition coefficient

and hydrophobic character. To compare hydrophobic character of compound **1** with kojic acid, we calculated lop P value (Table 1).

In conclusion, pharmacophore of kojic acid is enolic hydroxyl group in 5-position. To enhance biological activities of kojic acid, we increased hydrophobicity by introduction of 3,4-methylenedioxy cinnamate moiety in 2-position which is not pharmacophore. Its potent activities may be due to balance between hydrophilic and hydrophobic character.

## References

1. (a) Burdock, G. A.; Soni, M. G.; Carabin, I. G. *Regulatory Toxicology and Pharmacology* **2001**, *33*, 80. (b) Futamura, T.; Okabe, M.; Tamura, T.; Toda, K.; Matsunobu, T.; Park, Y. S. *J. Biosci. Bioeng.* **2001**, *91*, 272. (c) Ohyama, Y.; Mishima, Y. *Fragrance J.* **1990**, *6*, 53.
2. Kobayashi, Y.; Kayahara, H.; Tadasa, K.; Tanaka, H. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1303.
3. Mitani, H.; Koshiishi, I.; Sumita, T.; Imanari, T. *Eur. J. Pharmacol.* **2001**, *411*, 169.
4. Yuen, V. G.; Caravan, P.; Gelmini, L.; Glover, N.; Mcneill, J. H.; Setyawati, I. A.; Zhou, Y.; Orvig, C. *J. Inorg. Biochem.* **1997**, *68*, 109.
5. Kim, M. S.; Lee, S.; Rho, H. S.; Kim, D. H.; Chang, I. S.; Chung, J. H. *Clinica. Chimica. Acta* **2005**, *362*, 161.
6. Pomerantz, S. H. *J. Biol. Chem.* **1963**, *238*, 2351.
7. (a) Peus, D.; Meves, A.; Pott, M.; Beyerle, A.; Pittelkow, M. R. *Free Radic. Biol. Med.* **2001**, *30*, 425. (b) Delicado, E. N.; Ferrer, A. S.; Carmona, F. G. *Biochim. Biophys. Acta. General Subjects* **1997**, *1335*, 127.
8. (a) Saffari, Y.; Hossein Sadrzadeh, S. M. *Life Sciences* **2004**, *74*, 1513. (b) Bors, W.; Michel, C. *Free Radic. Biol. Med.* **1999**, *27*, 1413.
9. Rho, H. S.; Baek, H. S.; Lee, B. S.; Kim, J. H.; Kim, D. H.; Chang, I. S. *Bull. Korean Chem. Soc.* **2006**, *27*, 115.