Bioorganic & Medicinal Chemistry Letters 20 (2010) 6569–6571

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Kojyl thioether derivatives having both tyrosinase inhibitory and anti-inflammatory properties

Ho Sik Rho [a,*], Soo Mi Ahn [a], Dae Sung Yoo [c], Myung Kyoo Kim [b], Dong Ha Cho [c], Jae Youl Cho [c,*]

[a] R & D Center, AmorePacific Corporation, Yongin 446-729, Republic of Korea
[b] Samkyung Costech Co., Ltd, Suwon 443-734, Republic of Korea
[c] College of Biomedical Science, Kangwon National University, Chuncheon 200-701, Republic of Korea

ARTICLE INFO

Article history:
Received 30 June 2010
Revised 4 September 2010
Accepted 8 September 2010
Available online 21 September 2010

Keywords:
Kojyl thioether
Derivatives
Tyrosinase
Anti-inflammatory property

ABSTRACT

This study was conducted to examine the tyrosinase inhibitory and anti-inflammatory activities of kojic acid derivatives. A series of kojic acid derivatives containing thioether, sulfoxide, and sulfone linkages were synthesized. In the tyrosinase assay, kojyl thioether derivatives containing appropriate lipophilic alkyl chains (pentane, hexane, and cyclohexane) showed potent inhibitory activity. However, sulfoxides and sulfones exhibited decreased activity. Similar experimental results were obtained with inhibitory activities of NO production being induced by LPS. The presence of thioether linkage and appropriated lipophilic acid moiety was critical for the tyrosinase inhibitory and anti-inflammatory activities.

It is well known that 4-pyrones and their derivatives are present in many natural materials and are biologically active.[1] Kojic acid, 5-hydroxy-2-(hydroxymethyl)-4*H*-pyran-4-one (**1**), which is found in various traditional Japanese foods, is produced from carbohydrate sources in an aerobic process by a variety of microorganisms. Kojic acid has chelating activity[2] and inhibits tyrosinase,[3] and polyphenoloxidase (PPO).[4] Based on these effects, kojic acid has been used as a depigmenting agent for cosmetics and as a food additive to prevent enzymatic browning due to PPO. Kojic acid has also been shown to scavenge free radicals and to prevent photodamage.[5] However, only a few studies on kojic acid and its derivatives as NO inhibitors have been conducted. The biological activities of kojic acid are due to its γ-pyranone structure having an enolic hydroxyl group. Thus, various kojic acid derivatives that, are modified at the 2-position, have been developed to enhance biological activities.[6] Recently, we synthesized dimeric kojic acid derivatives containing various chemical linkages including ester, amide, and thioether. Among them, dithioether derivative (**2**) exhibited the most potent inhibitory activities of tyrosinase[7] and NO production in LPS activated macrophages[8] (Fig. 1).

We further investigated the structure–activity relationship of kojic acid derivatives[9] possessing sulfur linkages on the inhibition of tyrosinase[10] and NO production.[11] In the present study, we synthesized kojyl thioethers, sulfoxides, and sulfones and analyzed their structural importance for two biological activities. The synthetic pathways are shown in Schemes 1 and 2.

Kojic acid derivatives (**4a**–**h**) were synthesized by the condensation of kojyl chloride **3** with potassium salts of thiols. Kojic acid **1** was reacted with thionyl chloride to afford kojyl chloride **3**. Kojyl chloride **3** was reacted with potassium salts of thiols in DMF to afford the corresponding thioether derivatives (**4a**–**h**). The thioether derivatives (**4d**, **4e**, and **4h**) were then reacted with MCPBA (*m*-chloroperbenzoic acid) in methylene chloride to produce sulfoxide derivatives (**5d**, **5e**, and **5h**). Finally, sulfone derivatives (**6d**, **6e**, and **6h**) were obtained by the treatment of thioether derivatives (**4d**, **4e**, and **4h**) with oxone in a mixture of MeOH/H_2O.

The inhibitory activities of kojic acid derivatives against mushroom tyrosinase were initially investigated and compared with that of kojic acid. The results are shown in Table 1.

Compound **4a**, which contains an ethyl group, showed good inhibitory activity (IC_{50} = 2.60 μM). Increases in the activities were detected along with increasing chain length to compounds such as *n*-propyl, *n*-butyl, and *n*-pentyl (Table 1, **4b**–**d**). Compound **4d**, 2-(pentylthiomethyl)-5-hydroxy-4*H*-pyran-4-one, showed potent inhibitory activity (IC_{50} = 0.097 μM). However, compounds with greater chain lengths such as *n*-hexyl, *n*-heptyl, and *n*-octyl groups had negative influence on inhibitory activities. The activity of compound **4e** containing a *n*-hexyl group (IC_{50} = 0.19 μM) was lower than that of compound **4d** containing a *n*-pentyl group. Among the tested compounds, compound **4h**, 2-(cyclohexylthiomethyl)-5-hydroxy-4*H*-pyran-4-one, showed the most potent activity (IC_{50} = 0.087 μM); however, its IC_{50} was about 1/1100 that of kojic acid (IC_{50} = 97.38 μM). To identify the structural importance

* Corresponding authors. Tel.: +82 31 280 5805; fax: +82 31 281 8397 (H.S.R.).
E-mail addresses: thiocarbon@freechal.com (H.S. Rho), jaecho@kangwon.ac.kr (J.Y. Cho).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.09.042

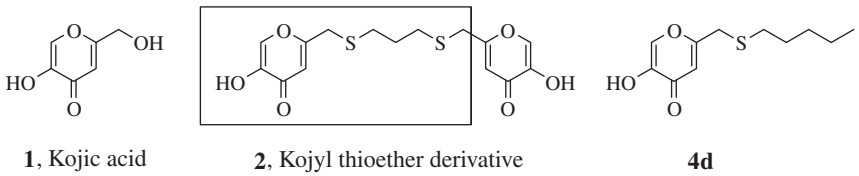
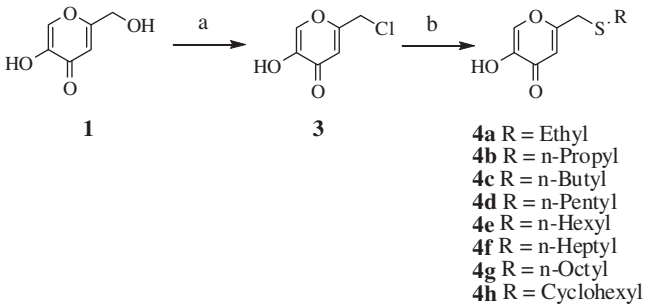
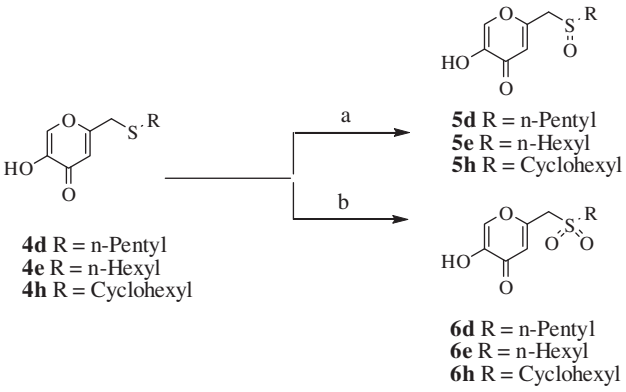


Figure 1. Structures of kojic acid and thioether derivatives.



Scheme 1. Reagents and conditions: (a) $SOCl_2$, DMF, rt; (b) potassium salt of thiols, DMF, rt.



Scheme 2. Reagents and conditions: (a) MCPBA, methylene chloride, rt; (b) oxone, MeOH/H_2O, rt.

Table 1
Inhibitory activities of kojic acid derivatives on mushroom tyrosinase

Compounds	IC_{50}[a] (μM)	Compounds	IC_{50}[a] (μM)
4a	2.60	**5d**	79.87
4b	1.93	**5e**	73.75
4c	1.48	**5h**	79.03
4d	0.097	**6d**	76.60
4e	0.19	**6e**	49.15
4f	2.65	**6h**	69.01
4g	46.18	Kojic acid	87.38
4h	0.087		

[a] Values were determined from the logarithmic concentration–inhibition curves and those presented are the mean values of the results of three experiments.

of sulfide linkages, we oxidized the sulfur functional group of the compounds (**4d**, **4e**, and **4h)** and evaluated their inhibitory activities. Interestingly, sulfoxide derivatives (**5d**, **5e**, and **5h**) and sulfone derivatives (**6d**, **6e**, and **6h**) exhibited decreased activity. In tyrosinase assays, the sulfide linkage is a critical factor for inhibition activity. Without the sulfide group, the kojic acid moiety may not bind tightly to the active site or to other essential parts of tyrosinase. The additional oxygen groups in the sulfoxides and sulfones had an effect on conformational changes, which caused decreased inhibitory activities (Fig. 2).

After evaluating the tyrosinase inhibitory activity, we investigated the anti-inflammatory activities of kojic acid derivatives against NO production induced by LPS in macrophages and cytotoxicity.[12] The results are shown in Table 2.

The inhibitory activity of NO production was found to be very similar to inhibitory results of tyrosinase. Specifically, compounds **4d**, **4e**, and **4h** showed inhibitory activities with IC_{50} values of 61.98, 25.75, and 56.97 μM, respectively. However, sulfoxide derivatives (**5d, 5e**, and **5h**) and sulfone derivatives (**6d**, **6e**, and **6h**) showed no inhibitory activity. Taken together these results suggest that sulfide linkage and compounds with appropriate hydrophobic chain lengths such as *n*-pentyl, *n*-heptyl, and cyclohexyl were important for the inhibition of tyrosinase and NO production in the structure of kojic acid derivatives.

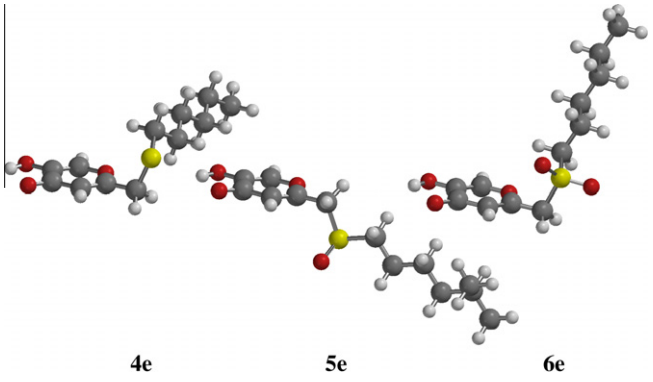


Figure 2. Energy minimized structures of **4e**, **5e**, and **6e**. Calculations were conducted using Spartan '04' for Windows. The global minimum-energy structures were obtained by molecular-mechanics computations and were further refined by DFT computation (B3LYP at the 6-31G* level).

Table 2
NO inhibitory activities of kojic acid derivatives

Compounds	Inhibitory activity [IC_{50}[a] (μM)]	
	NO	Cytotoxicity
4a	>100	>100
4b	>100	>100
4c	>100	>100
4d	61.98	>100
4e	25.75	>100
4f	>100	98.30
4g	>100	>100
4h	56.87	>100
5d	>100	>100
5e	>100	>100
5h	>100	>100
6d	>100	>100
6e	>100	>100
6h	>100	>100
Kojic acid	89.41	>100

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

In conclusion, we synthesized a series of kojyl thioether derivatives (**4a**–**h**), sulfoxide derivatives (**5d**, **5e**, and **5h**), and sulfone derivatives (**6d**, **6e**, and **6h**). Compound **4h**, 2-(cyclohexylthiomethyl)-5-hydroxy-4*H*-pyran-4-one, showed the most potent activity in the tyrosinase assay (IC_{50} = 0.087 μM). Compound **4e**, 2-(hexylthiomethyl)-5-hydroxy-4*H*-pyran-4-one, showed the most potent activity in the NO assay (IC_{50} = 25.75 μM). Analysis of the structure–activity relationship revealed that sulfide linkage and appropriate hydrophobic moieties such as *n*-pentyl, *n*-heptyl, and cyclohexyl are important for higher inhibitory activity in both tyrosinase and NO production assays.

References and notes

1. (a) Gerwick, W. H. *J. Nat. Prod.* **1989**, *52*, 252; (b) Yamamura, S.; Nishiyama, S. *Bull. Chem. Soc. Jpn.* **1997**, *70*, 2025; (c) Rossi, M. H.; Yoshida, M.; Maia, G. S. *Phytochemistry* **1997**, *45*, 1263; (d) Bransova, J.; Brtko, J.; Uher, M.; Novotny, L. *Int. J. Biochem. Cell Biol.* **1995**, *27*, 701.
2. Yuen, V. G.; Caravan, P.; Gelmini, L.; Glover, N.; Mcneill, J. H.; Setyawati, I. A.; Zhou, Y.; Orvig, C. *J. Inorg. Biochem.* **1997**, *73*, 109.
3. Ohyama, Y.; Mishima, Y. *Fragrance J.* **1990**, *6*, 53.
4. Chen, J. S.; Wei, C. I.; Rolle, R. S.; Otwell, W. S.; Balaban, M. O.; Marshall, M. R. *J. Agric. Food Chem.* **1991**, *39*, 1396.
5. Mitani, H.; Koshiishi, I.; Sumita, T.; Imanari, T. *Eur. J. Pharmacol.* **2001**, *411*, 169.
6. (a) Kobayashi, Y.; Kayahara, H.; Tasada, K.; Nakamura, T.; Tanaka, H. *Biosci. Biotechnol. Biochem.* **1995**, *59*, 1745; (b) Kobayashi, Y.; Kayahara, H.; Tadasa, K.; Tanaka, H. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1303; (c) Kadokawa, J.; Nishikura, T.; Muraoka, R.; Tagaya, H.; Terada, Y.; Fukuoka, N. *Synth. Commun.* **2003**, *33*, 1081; (d) Lee, Y. S.; Park, J. H.; Kim, M. H.; Seo, S. H.; Kim, H. J. *Arch. Pharm. Chem. Life Sci.* **2006**, *339*, 111; (e) Rho, H. S.; Baek, H. S.; You, J. W.; Kim, S.; Lee, J. Y.; Kim, D. H.; Chang, I. S. *Bull. Korean Chem. Soc.* **2007**, *28*, 471.
7. Rho, H. S.; Baek, H. S.; Ahn, S. M.; Kim, D. H.; Chang, I. S. *Bull. Korean Chem. Soc.* **2008**, *29*, 1569.
8. Yoo, D. S.; Lee, J.; Choi, S. S.; Rho, H. S.; Cho, D. H.; Shin, W. C.; Cho, J. Y. *Pharmazie* **2010**, *65*, 261.
9. The data of selected compounds: *compound* **4e**: ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.13 (s, 1H), 8.05 (s, 1H), 6.37 (s, 1H), 3.63 (s, 2H), 2.53 (m, 2H), 1.51 (m, 2H), 1.24 (m, 6H), 0.87 (t, 3H, J = 6.3 Hz). ^{13}C NMR (125 MHz, DMSO-d_6): δ 173.6, 164.8, 145.6, 139.6, 112.0, 32.3, 31.2, 30.7, 28.6, 27.7, 21.9, 13.8. FABMS: (*m*/*e*) 243 [M+H]$^+$. *Compound* **4h**: ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.12 (s, 1H), 8.04 (s, 1H), 6.39 (s, 1H), 3.67 (s, 2H), 2.69 (m, 1H), 1.90 (m, 2H), 1.65 (m, 2H), 1.51–0.90 (m, 6H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 173.7, 165.3, 145.6, 139.6, 111.9, 43.1, 32.9, 30.8, 25.31, 25.21. FABMS: (*m*/*e*) 241 [M+H]$^+$. *Compound* **5e**: ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.23 (s, 1H), 8.08 (s, 1H), 6.39 (s, 1H), 4.17 (d, 1H, J = 13.2 Hz), 3.95 (d, 1H, J = 13.2 Hz), 2.83 (m, 2H), 1.65 (m, 2H), 1.38–1.27 (m, 6H), 0.86 (t, 3H, J = 6.3 Hz). ^{13}C NMR (125 MHz, DMSO-d_6): δ 173.2, 158.9, 145.9, 140.1, 115.2, 54.0, 51.1, 30.7, 27.6, 21.89, 21.82, 13.8. FABMS: (*m*/*e*) 259 [M+H]$^+$. *Compound* **5h**: ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.21 (s, 1H), 8.09 (s, 1H), 6.41 (s, 1H), 4.13 (d, 1H, J = 13.2 Hz), 3.96 (d, 1H, J = 13.2 Hz), 2.72 (m, 1H), 1.98–0.72 (m, 10H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 173.2, 159.4, 145.9, 140.1, 115.2, 57.8, 51.7, 26.0, 25.0, 24.8, 24.5, 23.6. FABMS: (*m*/*e*) 257 [M+H]$^+$. *Compound* **6e**: ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.32 (s, 1H), 8.11 (s, 1H), 6.49 (s, 1H), 4.58 (s, 2H), 3.26 (m, 2H), 1.69 (m, 2H), 1.50–1.22 (m, 6H), 0.86 (t, 3H, J = 6.3 Hz). ^{13}C NMR (125 MHz, DMSO-d_6): δ 173.3, 156.1, 146.1, 140.4, 116.2, 55.7, 52.4, 30.6, 27.2, 21.7, 21.0, 13.7. FABMS: (*m*/*e*) 275 [M+H]$^+$. *Compound* **6h**: ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.23 (s, 1H), 8.07 (s, 1H), 6.43 (s, 1H), 4.51 (s, 2H), 3.09 (m, 1H), 2.02 (m, 2H), 1.80 (m, 2H), 1.60–0.95 (m, 6H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 173.3, 156.1, 146.1, 140.4, 116.4, 60.5, 53.0, 24.64, 24.39, 24.27. FABMS: (*m*/*e*) 273 [M+H]$^+$.
10. Measurements of mushroom tyrosinase activity: 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 (2100 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, Richmond, CA, USA) 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}).
11. Measurements of NO production: RAW264.7 cells (1×10^6 cells/ml) were preincubated with kojic acid derivatives for 30 min and continuously activated with LPS (1 μg/ml) for 24 h. Nitrite in culture supernatants was measured by adding 100 μl of Griess reagent (1% sulfanilamide and 0.1% *N*-[1-naphthyl]-ethylenediamine dihydrochloride in 5% phosphoric acid) to 100 μl samples of the medium for 10 min at room temperature. OD at 570 nm (OD_{570}) was measured using a Spectramax 250 microplate reader (Molecular Devices, Sunnyvale, CA, USA). A standard curve of NO was made with sodium nitrite.
12. *MTT assay*: After the preincubation of RAW264.7 cells (1×10^6 cells/ml) for 18 h, kojyl thioether derivatives (0–100 μM) were added to the cells and incubated for 24 h. The cytotoxic effect of kojyl thioether derivatives was then evaluated by a conventional MTT assay. At 3 h prior to culture termination, 10 μl of the MTT solution (5 mg/ml in a phosphate buffered-saline, pH 7.4) were added and the cells were continuously cultured until termination. The incubation was halted by the addition of 15% sodium dodecyl sulfate into each well, solubilizing formazan. The absorbance at 570 nm ($OD_{570-630}$) was measured by a Spectramax 250 microplate reader.