Inhibitory Effects of 1,3-Selenazol-4-one Derivatives on Mushroom Tyrosinase

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This study reports depigmenting potency of 1,3-selenazol-4-one derivatives, which would be based upon the finding of direct inhibition to mushroom tyrosinase. 1,3-Selenazol-4-one derivatives exhibited inhibitory effect on dopa oxidase activity of mushroom tyrosinase. In this study, inhibitory effects of six kinds of 1,3-selenazol-4-one derivatives (A, B, C, D, E and F) on mushroom tyrosinase were investigated. Compounds at a concentration of 500 μM exhibited 33.4—62.1% of inhibition on dopa oxidase activity of mushroom tyrosinase. Their inhibitory effects were higher than that of kojic acid (31.7%), a well known tyrosinase inhibitor. 2-(4-Methylphenyl)-1,3-selenazol-4-one (A) exhibited the strongest inhibitory effect among them dose-dependently and in competitive inhibition manner.

Key words 1,3-selenazol-4-one; tyrosinase; whitening agent

Selenium, an essential biological trace element, is an integral component of several enzymes, and its use as a nutritional supplement has been popularized recently due to its potential role in low concentrations as an antioxidant and in higher concentrations as an anticancer agent.[1,2] Some selenium-containing heterocyclic compounds have been reported to possess biological efficacy.[3—6] Recently, we have developed a preparation of 1,3-selenazol-4-one derivatives by the reaction of primary selenoamides with α-haloacyl halides in the presence of pyridine.[7]

We report inhibitory effects of 1,3-selenazol-4-one derivatives on mushroom tyrosinase. Tyrosinase is the key enzyme in undesirable browning of fruits and vegetables, and coloring of skin, hair and eyes in animals.[8—10] This enzyme plays a role in oxidation from tyrosine to L-dopa and from the dopa to dopaquinone.[11] 2-(4-Methylphenyl)-1,3-selenazol-4-one (**A**) showed the strongest tyrosinase inhibitory activity among six kinds of 1,3-selenazol-4-one derivatives. Furthermore, **A** was identified as a competitive inhibitor on tyrosinase. In this work, structure–activity-relationship of 1,3-selenazol-4-one derivatives on inhibition activity of tyrosinase was determined.

Experimental

Materials Kojic acid (5-hydroxy-2-(hydroxymethyl)-4*H*-pyran-4-one), mushroom tyrosinase and L-dopa (3-(3,4-dihydroxyphenyl)-L-alanine) were purchased from Aldrich Chemical, Inc. (U.S.A.). All other chemicals and solvents were analytical grade and used without further purification. 1,3-Selenazol-4-one derivatives were prepared according to a procedure previously reported.[7] For example, 2-(4-methylphenyl)-1,3-selenazol-4-one (**A**) was synthesized as follows: chloroacetyl chloride (0.12 g, 1.0 mmol) in dry dichloromethane (5 ml) was added dropwise to stirred solution of 4-methylbenzeneselenoamide (0.20 g, 1.0 mmol) in dry dichloromethane (5 ml) at 0 °C under an argon atmosphere. The reaction mixture was stirred for 1 h at room temperature. Then dry pyridine (0.16 g, 2.0 mmol) in dry dichloromethane (5 ml) was added dropwise into the mixture at 0 °C. The reaction mixture was stirred for 2 h at 0 °C. The mixture was extracted with dichloromethane (100 ml) and washed with water (30 ml). The organic layer was dried over sodium sulfate and evaporated to dryness. The residue was purified by flash chromatography on silica gel with dichloromethane to give **A** (0.19 g, Yield: 80%). mp: 109.5—111.5 °C. IR (KBr): 1707, 1609 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 2.45 (s, 3H, CH_3Ar), 4.26 (s, 2H, CH_2), 7.32 (d, J=7.6 Hz, 2H, Ar), 7.96 (d, J=7.6 Hz, 2H, Ar). ^{13}C-NMR ($CDCl_3$): δ 21.9, 34.8, 129.2, 129.8, 131.7, 146.6, 193.1, 195.9. ^{77}Se-NMR ($CDCl_3$): δ 458.1. MS (CI): m/z=240 [M^++1]. *Anal.* Calcd for $C_{10}H_9NOSe$: C, 50.43; H, 3.81; N, 5.88. Found: C, 50.23; H, 3.92; N, 5.88.

B (Yield: 47%). mp: 69.5—71.0 °C. IR (KBr): 1705, 1608 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 1.10 (t, J=7.2 Hz, 3H, CH_3), 2.04 (m, 1H, CH_2), 2.37 (m, 1H, CH_2), 2.44 (s, 3H, CH_3Ar). 4.64 (dd, J=4.0, 8.8 Hz, 1H, CH), 7.31 (d, J=8.4 Hz, 2H, Ar), 7.96 (d, J=8.4 Hz, 2H, Ar) ^{13}C-NMR ($CDCl_3$): δ 13.4, 21.9, 26.9, 56.9, 129.2, 132.0, 146.4, 195.1. ^{77}Se-NMR ($CDCl_3$): δ 521.5. MS (CI): m/z=268 [M^++1]. HR-MS: m/z Calcd for $C_{12}H_{13}NOSe$: 267.0162. Found: 267.0142.

C (Yield: 41%). mp: 83.0—85.0 °C. IR (KBr): 1702, 1610 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 1.86 (s, 6H, $C(CH_3)_2$), 2.44 (s, 3H, CH_3Ar), 7.30 (d, J=8.0 Hz, 2H, Ar), 7.95 (d, J=8.0 Hz, 2H, Ar). ^{13}C-NMR ($CDCl_3$): δ 21.8, 28.6, 60.6, 129.1, 129.7, 132.1, 146.2, 193.7, 197.6. ^{77}Se-NMR ($CDCl_3$): δ 658.7. MS (CI): m/z=268 [M^++1]. HR-MS: m/z Calcd for $C_{12}H_{13}NOSe$: 267.0162. Found: 267.0164.

D (Yield: 76%). mp: 80.0—81.0 °C. IR (KBr): 1711, 1595 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 4.27 (s, 2H, CH_2), 7.51 (t, J=8.0 Hz, 2H, Ar) 7.67 (t, J=8.0 Hz, 1H, Ar), 8.05 (d, J=8.0 Hz, 2H, Ar). ^{13}C-NMR ($CDCl_3$): δ 34.9, 128.99, 129.0, 134.2, 134.9, 192.8, 196.1. ^{77}Se-NMR ($CDCl_3$): δ 464.5. MS (CI): m/z=226 [M^++1]. HR-MS: m/z Calcd for C_9H_7NOSe: 224.9692. Found: 224.9684.

E (Yield: 62%). mp: 147.5—150.0 °C. IR (KBr): 1702, 1591 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 4.30 (s, 2H, CH_2), 7.49 (d, J=8.8 Hz, 2H, Ar) 7.99 (d, J=8.8 Hz, 2H, Ar). ^{13}C-NMR ($CDCl_3$): δ 35.3, 129.4, 130.2, 132.7, 141.5, 192.6, 194.5. ^{77}Se-NMR ($CDCl_3$): δ 465.8. MS (CI): m/z=260 [M^++1]. HR-MS: m/z Calcd for $C_9H_6NOSeCl$: 258.9303. Found: 258.9289.

F (Yield: 58%). mp: 131.0—133.0 °C. IR (KBr): 1695, 1605 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 3.90 (s, 3H, CH_3), 4.24 (s, 2H, CH_2), 6.98 (d, J=8.8 Hz, 2H, Ar). 8.01 (d, J=8.8 Hz, 2H, Ar). ^{13}C-NMR ($CDCl_3$): δ 34.8, 55.6, 114.3, 129.6, 131.4, 165.3, 192.9, 194.5. ^{77}Se-NMR ($CDCl_3$): δ 450.6. MS (CI): m/z=256 [M^++1]. HR-MS: m/z Calcd for $C_{10}H_9NO_2Se$: 254.9798. Found: 254.9776.

Assay of Tyrosinase Activity Each concentration (1 mM, 500 μM, 100 μM and 10 μM) of test substance was dissolved in MeOH. 120 μl of L-dopa (8.3 mM, dissolved in 67 mM phosphate buffer, pH 6.8) and 40 μl of each 1,3-selenazol-4-one derivative solution were added to a 96-well microplate, and then 40 μl of mushroom tyrosinase (125 U) was mixed. After incubation at 37 °C for 30 min, the amount of dopachrome in the reaction mixture was determined. UV spectra were obtained with the Molecular Devices E09090 microplate reader. Based on the optical density at 490 nm (OD_{490}), the inhibitory activity of the sample indicated to be the concentration which inhibits 50% of the enzyme activity (IC_{50}). Kojic acid was used as a reference. Inhibition type of test substance was determined by Lineweaver-Burk's plot using various concentrations of L-dopa.[11]

Statistical Analysis Data were presented as mean±S.E. from three in-

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dependent experiments. Statistical comparison between different treatments was done by Student's *t*-test.

RESULTS

Inhibitory Effects on Tyrosinase Activity of Compounds Six compounds of 1,3-selenazol-4-one derivatives and kojic acid were examined for the tyrosinase inhibitory activity (Table 1). Inhibitory effects of all the 1,3-selenazol-4-one derivatives on tyrosinase were stronger than kojic acid. Among them, **A** revealed the highest inhibitory effects with IC_{50} value of 333.2 μM. Inhibitory effects on dopa oxidase activity of tyrosinase by 1,3-selenazol-4-one derivatives were evaluated in order to examine the relationship between structure and activity. Although compounds **A**, **D**, **E** and **F** bear the same 4-selenazolone skeleton, R_1 group is different. As compared with each data among them, **A** bearing methyl group of the phenyl ring at the 4′ position indicated to be stronger than others such as hydrogen, chloride and methoxy groups. Compounds **B** and **C** bearing ethyl or two methyl groups at the 5 position of the 4-selenazolone skeleton showed weaker activity than **A** (Table 1).

Dose-Dependent Inhibition on Mushroom Tyrosinase of 2-(4-Methylphenyl)-1,3-selenazol-4-one (A) and Kojic Acid L-dopa, each 1,3-selenazol-4-one derivative solution and mushroom tyrosinase were incubated at 37 °C for 30 min. After determination of amount of dopachrome in the reaction mixture, the inhibitory effect of **A** was dose-dependent. The inhibition rates of **A** at 50, 100, 200 and 500 μM were 20±2.0%, 33±3.3%, 44±1.3% and 62±2.1%, respectively. IC_{50} was 333.2 μM. On the other hand, kojic acid indicated only 32±2.9% at 500 μM (Fig. 1).

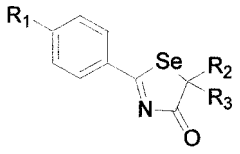
In this study of kinetics and mechanism for the inhibition on tyrosinase, **A** was confirmed to be a competitive inhibitor. When various concentrations (1 mM, 0.5 mM, 0.25 mM, 0.125 mM) of L-dopa being used as substrates, Fig. 2 shows a set of double-reciprocal plots obtained in the presence of the inhibitor and with two different concentrations of a competitive inhibitor. K_m value of compound **A** was decreased, but V_{max} value of compound was not changed. Since the intercept on the V_o axis is equal to $1/V_{max}$, we can see that V_{max} is unchanged by the presence of a **A** compound.

DISCUSSION

We examined the inhibitory effect of 1,3-selenazol-4-one derivatives on melanogenesis using mushroom tyrosinase. Based on such inhibitory effects *in vitro*, they speculated that these compound would be applicable to hyperpigmentory disorders as a depigmenting agent. These compounds appear to be new chemical types as tyrosinase inhibitor, there are few of similar chemicals in structure reported to be capable of inhibiting tyrosinases *in vitro*. Thus, in our study, there is something new in just exhibiting *in vitro* tyrosinase inhibitory activity.

1,3-Selenazol-4-one derivatives exhibited higher inhibitory effect on mushroom tyrosinase as compared with kojic acid. Among the six compounds (**A**, **B**, **C**, **D**, **E**, **F**) tested, **A** was found to be the most potent tyrosinase inhibitor. This result suggest that the presence of methyl group on site 4′ plays a role in enzyme inhibitory effects. Also, the presence of functional group at 5 position of the 4-selenazolone might be the cause of decreased activity. Therefore, the type of functional group of R_1 in compound seems to play a critical role in exerting the inhibitory effect on dopa oxidase activity of tyrosinase, and poor inhibitory effect of compound **C** might be ascribed to a steric hindrance by the dimethyl moiety, which would not allow it to reach the target site of the enzyme. In the present study, we first demonstrated that 2-(4-methylphenyl)-1,3-selenazol-4-one structure indicated the inhibitory effect against mushroom tyrosinase. Thus, the present study would provide a useful basis for the development of potential tyrosinase inhibitor agents using 1,3-selenazol-4-one derivatives. The development of the more effective agents based on **A** as leading compounds need further studies. To focus on *in vitro* effects, we should address the effect on cultured melanocytes at the level of tyrosinase protein and gene. Furthermore, we should include *in vivo* inhibitory effect on hyperpigmented skin tissue.

Table 1. Inhibitory Effects of 1,3-Selenazol-4-one Derivatives and Kojic Acid against Mushroom Tyrosinase



Compound	Substituent			Inhibition at 500 μM[a] (%)	IC_{50}[b] (μM)
	R_1	R_2	R_3		
A	CH_3	H	H	62.1±2.1	333.2
B	CH_3	CH_2CH_3	H	54.3±1.5	384.3
C	CH_3	CH_3	CH_3	33.4±2.6	>500
D	H	H	H	51.5±0.3	478.1
E	Cl	H	H	50.2±1.7	498.0
F	OCH_3	H	H	43.6±5.3	>500
Kojic acid				31.7±2.9	934.3

a) Tyrosinase was preincubated with test substances at 25 °C for 10 min prior to incubation with dopa for 30 min, and the absorbance was read at 490 nm. Each value represents the mean±S.E. of three experiments. *b*) 50% inhibitory concentration.

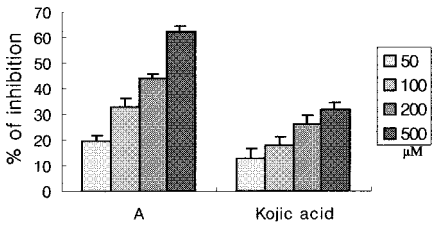


Fig. 1. Inhibitory Effect of 2-(4-Methylphenyl)-1,3-selenazol-4-one (**A**) and Kojic Acid against Mushroom Tyrosinase at Several Concentrations

Each value represents the mean±standard error in triplicate.

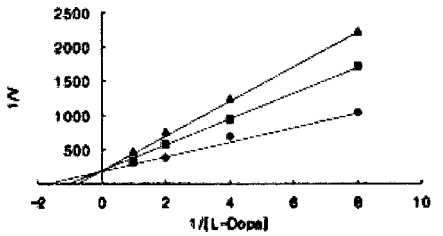


Fig. 2. Kinetics of Mushroom Tyrosinase by 2-(4-Methylphenyl)-1,3-selenazol-4-one (**A**)

200 μM of **A** (triangle), 100 μM of **A** (rectangle) and blank (diamond).

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