Antioxidant Properties of a New Type of Polyene, Falconensone A and Its Derivatives

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Falconensones A and B are new type of yellow compound extracted from ascomycetous fungi, *Emericella falconensis*. Falconensone A *p*-bromophenylhydrazone and falconensone A dioxime are derivatives of falconensone A which have also been synthesized recently. The structural similarity of the falconensones to α -tocopherol (vitamin E) led us to investigate whether falconensones exhibit antioxidant activity. These studies found that falconensone A *p*-bromophenylhydrazone and falconensone A dioxime scavenge α,α -diphenyl- β -picrylhydrazyl (DPPH) radical in a 1 : 1 ratio in contrast to vitamin E, where a 1 : 2 ratio relative to DPPH radicals was observed. In addition, linoleic acid peroxidation initiated by hydroxyl radicals was diminished by falconensone A *p*-bromophenylhydrazone to a greater extent than by vitamin E, and lipid peroxidation in rat liver microsome was reduced by falconensone A dioxime and falconensone A *p*-bromophenylhydrazone. In contrast, falconensone A and falconensone B, the 4'-nor-methyl derivative of falconensone A, showed much lower activity or were inactive in scavenging radicals. These results suggest that falconensone A *p*-bromophenylhydrazone and falconensone A dioxime, may be useful new antioxidant agents, wherein the bromophenyl and hydroxy residues of falconensone A may be important for antioxidant activity. Based on these results, derivatives of falconensone A appear to be effective antioxidants that may have clinical utility for diseases treated with vitamin E.

Key words falconensone, antioxidant; vitamin E; lipid peroxidation; polyene; retinoic acid; α -tocopherol

Two new cyclopentenone derivatives, falconensones A and B, were isolated from the mycelial extract of ascomycetous fungi, *Emericella falconensis*.¹⁾ Structurally, these compounds may be characterized as cyclopent-2-enone derivatives connected to an extensively conjugated methyl ketone located at the third methylene (C-3) (Fig. 1).¹⁾ The absolute configuration of falconensone A has been established as (*R*)-(3*E*,5*E*,7*E*,9*E*,11*E*)-3,11-dimethyl-12-(2,4-dimethyl-3-oxocyclopent-1-enyl)dodeca-3,5,7,9,11-pentaen-2-one (Fig. 1), with falconensone B being the 4'-nor-methyl derivative of

falconensone A (Fig. 1). We synthesized derivatives of falconensone A, consisting of falconensone A *p*-bromophenylhydrazone and falconensone A dioxime. During biological evaluation of these compounds, it became apparent that their structures are related to α -tocopherol (vitamin E) and retinoic acid (RA) as shown in Fig. 1.

The most well known antioxidant is vitamin E, which is considered to be the main natural inhibitor of lipid peroxidation.²⁾ Dietary vitamin E has been shown to inhibit mouse colon cancer³⁾ and hamster buccal pouch tumorigenesis.⁴⁾ Al-

Fig. 1. Chemical Structures of α -Tocopherol, Falconensone A, Falconensone B, Falconensone A *p*-Bromophenylhydrazone, Falconensone A Dioxime, and Retinoic Acid

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though epidemiological studies^{5–7)} have shown that vitamin E has anticancer effects, animal studies have yielded conflicting results.^{3,8,9)} In addition, vitamin E reduces lipid peroxidation products in rats fed cod liver oil and ethanol¹⁰⁾ and exerts a protective effect against oxidative-related disease,¹¹⁾ nitrosamine-induced liver damage,¹²⁾ and coronary heart disease.^{13–15)} Recently, it has been reported that vitamin E exhibits therapeutic effect against Alzheimer's disease¹⁶⁾ and enhances immune response.¹⁷⁾

Various compounds induce the differentiation of HL60 human acute myeloid leukemia cells into granulocyte-like or monocyte/macrophage-like cells along with the inhibition of cell growth. We have shown that falconensones induce differentiation of HL60 cells into monocyte/macrophage-like cells and that falconensone A enhances differentiation induced by RA in a synergistic manner.¹⁸⁾ RA is well known as an inducer of terminal differentiation of HL60 cells to cells having many of the functional and morphological characteristics of mature granulocytes.¹⁹⁾ Thus, these results indicate that falconensone A influences actions of RA as well as exhibiting antioxidant activity.^{20–23)} These findings considered aforementioned structural similarities, led us to examine free radical scavenging activities of the falconensones.

MATERIALS AND METHODS

Chemicals Falconensones A and B were isolated from the mycelium of *Emericella farconensis*. Their structures have been determined by spectroscopic and X-ray analysis.¹⁾ Falconensone A *p*-bromophenylhydrazide and falconensone A dioxime were synthesized as described previously.^{1,18,24)} Linoleic acid, α -tocopherol (vitamin E), α,α -diphenyl- β -picrylhydrazyl (DPPH), adenosine 5'-diphosphate (ADP) and dimethylsulfoxide (DMSO) were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Lubrol PX, dithiothreitol (DTT) and trichloroacetic acid (TCA) was purchased from Nacalai tesque, Inc. (Kyoto, Japan). Ascorbic acid and 2-thiobarbituric acid (TBA) were purchased from Wako Pure Chemicals Industries, Ltd. (Osaka, Japan).

DPPH Radical Analysis DPPH radical analysis was performed as described previously.^{25–27)} Ethanol (2 ml) and 500 μ M DPPH in ethanol solution (1 ml) were added to 0.1 M acetic acid buffer, pH 5.5 (2 ml). To this mixture (5 ml) were added 100 μ l of a 1 mM solution of vitamin E or falconensones dissolved in DMSO and cysteine dissolved in 0.1 M acetic acid buffer, pH 5.5. The final concentration of each compound was 20 μ M. After incubation at room temperature for 30 min, absorbance was measured spectrophotometrically at 517 nm (Shimadzu UV-1600, Shimadzu, Kyoto, Japan). Acetic acid buffer or DMSO (100 μ l) were used in place of compounds as blank controls.

Linoleic Acid Peroxidation Assay Lipid peroxidation was assayed as described previously.²⁸⁾ Stock solutions of linoleic acid micelles (10 mM, 1 ml) were prepared daily by adding 2.8 mg/ml linoleic acid to 30 mM NaCl, pH 7.0. Equal volumes of 0.8% Lubrol PX and 10 mM linoleic acid micelles were mixed along with sufficient 30 mM NaCl, pH 7.0 to achieve a concentration of Lubrol PX of 0.08%. The resulting opaque micellar suspension was rendered as an optically clear dispersion by sonication (Branson Ultrasonic, Danbury, CT, U.S.A.). Reaction mixtures containing 1 mM

linoleic acid, 30 μ M FeSO₄, 0.1 mM H₂O₂, and various concentrations of vitamin E or falconensones dissolved in DMSO, were incubated at 37 °C, and conjugated diene formation was monitored spectrophotometrically at 234 nm. DMSO was added as a blank.

Preparation of Rat Liver Microsomes Eight week-old male Sprague-Dawley rats (Slc. SD, 280–300 g) were obtained from Tokyo Laboratory Animals Science Co., Ltd. (Tokyo, Japan). All fractionation and sonication steps were performed at 0–5 °C. Livers (5–8 g) were homogenized in 5 volumes (w/v) of cold 0.25 M sucrose containing 100 mM Tris-HCl (pH 7.4), 5 mM DTT, and 10 mM MgCl₂. The homogenate was fractionized by the usual differential centrifugation techniques. Homogenates were centrifuged at 400 $\times g$ (10 min), then supernatant was centrifuged at 10000 $\times g$ (10 min), and the resulting supernatant was then centrifuged at 105000 $\times g$ (1 h). Pellets were resuspended in 100 mM Tris-HCl (pH 7.4) containing 0.25 M sucrose, 5 mM DTT and 10 mM MgCl₂, and stored at –80 °C. Protein concentrations were determined by the method of Bradford.²⁹⁾

Measurement of Microsomal Lipid Peroxidation Lipid peroxidation in rat liver microsomes was quantitated by measurement of malondialdehyde (MDA) using ADP-chelated ion and ascorbate as described previously.²³⁾ Reaction mixtures consisting of microsomes (0.5 mg protein/ml) and compounds (vitamin E or falconensones dissolved in DMSO) in 100 mM Tris-HCl (pH 7.5) containing 15 μ M FeCl₃, and 4 mM ADP, were preincubated at 37 °C for 1 min. Reaction mixtures in which ascorbic acid was added at a final concentration of 1 mM, were incubated at 37 °C for 20 min, then equal volumes of TBA reagent (0.375% TBA and 15% TCA in 0.25 N hydrochloride) were introduced. Mixtures were heated in boiling water for 15 min, and centrifuged at 1000 $\times g$ (10 min). Supernatant absorbance was measured spectrophotometrically at 535 nm (ϵ = 156000 cm^{–1} M^{–1}).

Measurement of Superoxide Scavenging Activity The superoxide scavenging activities of the compounds were determined by monitoring their competition with cytochrome c for superoxide anion generated by xanthine/xanthine oxidase system.³⁰⁾ Cytochrome c (0.5 mg/ml), Na₂-EDTA (0.3 mM), xanthine (100 μ M), and compounds at the concentration of 10 μ M, were incubated in NaH₂PO₄/Na₂HPO₄ buffer (50 mM; pH 7.4) for 1 min, and then 0.02 U ml^{–1} xanthine oxidase was added. Reduction of cytochrome c was measured spectrophotometrically at 550 nm at 25 °C, using the extinction coefficient for the reduction of ferricytochrome c as given by Massey,³¹⁾ $\Delta\epsilon$ (550 nm) = 21000 M^{–1} cm^{–1}. The first-minute rate of superoxide-induced cytochrome c reduction was determined by the subtraction of that of cytochrome c reduction in the presence of 100 U ml^{–1} superoxide dismutase (SOD). SOD was used as a positive control.

Presentation of Results Each experiment was performed at least 3 times, and most experiments were repeated at least 4 times with consistent results.

RESULTS

Scavenging of DPPH Radicals by Falconensones Previous reports have shown the stoichiometry of the reaction of DPPH radicals and antioxidants.^{25–27)} One molecule of cys-

Fig. 2. Effects of Vitamin E, Cysteine, Falconensone A, Falconensone B, Falconensone A *p*-Bromophenylhydrazine, and Falconensone A Dioxime on DPPH Radicals

Ethanol (2 ml) and a 500 μM solution of DPPH radicals in ethanol (1 ml) were added to 0.1 M acetic acid buffer, pH 5.5 (2 ml). Vitamin E, falconensones and cysteine were added to final concentrations of 20 μM , and mixtures were incubated at room temperature for 30 min as described under "Materials and Methods." Absorbance was measured spectrophotometrically at 517 nm using acetic acid buffer as a blank. (A) Absorbance at 517 nm in the presence of vitamin E, falconensones and cysteine, and DPPH radical (OD_{517}) expressed as % of control. (B) Antioxidant activity expressed as % of vitamin E calculated according to the following formula: (control absorbance-absorbance in the presence of compound)/(control absorbance-absorbance in the presence of vitamin E) $\times$ 100.

teine reacts with one DPPH radical quenching it to the non-radical DPPH.²⁵⁾ In contrast, vitamin E reacts with two equivalents of DPPH radicals as compared to cysteine, such that each molecule of vitamin E scavenges two DPPH radicals.²⁷⁾ The stable DPPH free radical was incubated with falconensones at a concentration of 20 μM , and absorbance was measured spectrophotometrically at 517 nm. Vitamin E and cysteine were used as internal standards for scavenging DPPH radicals, with DMSO being added as a control. As shown in Fig. 2A, the absorbance of control was approximately 0.82. In the presence of vitamin E, cysteine, falconensone A, falconensone B, falconensone A *p*-bromophenylhydrazine and falconensone A dioxime at concentrations of 20 μM , absorbances at 517 nm were approximately 0.33, 0.59, 0.84, 0.82, 0.57, and 0.58, respectively. Vitamin E showed an approximate 60% decrease in the absorbance of DPPH radicals at 517 nm ($\Delta\text{OD}_{517}=0.49$) relative to control. In contrast, cysteine, falconensone A *p*-bromophenylhydrazine and falconensone A dioxime reduced absorbance approximately 29% relative to control ($\Delta\text{OD}_{517}=0.24$), with falconensone A and falconensone B being the same as control. As shown in Fig. 2B, falconensone A *p*-bromophenylhydrazine, falconensone A dioxime and cysteine exhibited approximately 50% of the antioxidant activity relative to vitamin E. In addition, falconensone A *p*-bromophenylhydrazine, falconensone A dioxime, cysteine, and vitamin E each exhibited antioxidant activity (ΔOD_{517}) in a dose-dependent manner in the range of 0–40 μM (Fig. 3), and the level of antioxidant activity of falconensone A *p*-bromophenylhydrazine, falconensone A dioxime or cysteine was approximately half that of vitamin E at all concentrations (Fig. 3). These results indicate that falconensone A *p*-bromophenylhydrazine and falconensone A dioxime are antioxidants as potent as cysteine, such that one molecule of falconensone A *p*-bromophenylhydrazine or falconensone A dioxime scavenges

Fig. 3. Dose Dependency of Antioxidant Activity of Vitamin E, Cysteine and Falconensones

Ethanol (2 ml) and a 500 μM solution of DPPH radicals in ethanol (1 ml) were added to 0.1 M acetic acid buffer, pH 5.5 (2 ml). Vitamin E (■), cysteine (◇), falconensone A (○), falconensone B (△), falconensone A *p*-bromophenylhydrazine (□), and falconensone A dioxime (●) were added to various concentrations, and mixtures were incubated at room temperature for 30 min as described under "Materials and Methods." Absorbance was measured spectrophotometrically at 517 nm using acetic acid buffer as a blank. Antioxidant activity of compounds expressed as ΔOD_{517} calculated by the following formula: (OD_{517} of control)–(OD_{517} of compound).

one DPPH radical, and that the *p*-bromophenylhydrazine or dioxime residue may be critical for the antioxidant activity of DPPH radicals.

Effects of Falconensones on Linoleic Acid Peroxidation Initiated by Hydroxyl Radicals Free ferrous iron reacts with H_2O_2 to produce hydroxyl radicals by the Fenton reaction. Formation of hydroxyl radicals causes the rapid oxidation of linoleic acid as determined by the extent of MDA

Fig. 4. Effects of Vitamin E, Falconensone A, Falconensone B, Falconensone A *p*-Bromophenylhydrazone, and Falconensone A Dioxime on Linoleic Acid Peroxidation Initiated by Hydroxyl Radicals

(A) Reaction mixtures containing 30 mM NaCl, pH 7.0, 1 mM linoleic acid, 0.08% Lubrol PX, $100 \mu\text{M}$ H_2O_2 , $30 \mu\text{M}$ FeSO_4 , and various concentrations of vitamin E ($\square$), falconensone A ($\diamond$), falconensone B ($\circ$), falconensone A *p*-bromophenylhydrazone ($\triangle$), or falconensone A dioxime ($\blacksquare$), were incubated at 37°C , and absorbance was measured at 234 nm as described in "Materials and Methods." Antioxidant activity was calculated by the following formula: $(OD_{234}/\text{min of control}) - (OD_{234}/\text{min of compound}) = \Delta OD_{234}/\text{min}$. (B) % antioxidant activity relative to vitamin E for compounds at $10 \mu\text{M}$ concentration was based on the antioxidant activity of vitamin E at $10 \mu\text{M}$ concentration being defined as 100%. The means $\pm$ S.D. of at least four measurements are shown. Experiments were repeated at least four times.

generation, which results in a rapid increase in absorbance at 234 nm. The initial rate of diene formation (OD_{234}/min) was affected by all falconensones and vitamin E. High concentrations ($100 \mu\text{M}$) produced an immediate decrease in the rate of conjugated diene formation (data not shown). Antioxidant activity ($\Delta OD_{234}/\text{min}$) that inhibited diene formation was calculated according to the following formula: $(OD_{234}/\text{min of control}) - (OD_{234}/\text{min of compound})$. All falconensones and vitamin E exhibited antioxidant activity in dose-dependent manners (Fig. 4A). Linear dependencies (Fig. 4A) were seen in the concentration range of 5– $15 \mu\text{M}$. Antioxidant activity by all compounds began to show saturation at $25 \mu\text{M}$ concentration (data not shown). Of the five, falconensone A *p*-bromophenylhydrazone was the strongest antioxidant at all concentrations (Fig. 4A). In order to compare the antioxidant activity of falconensones and vitamin E both at $10 \mu\text{M}$ concentrations, the antioxidant activity of vitamin E was set at 100% and other values adjusted accordingly. In Fig. 4B, falconensone A, falconensone B, falconensone A *p*-bromophenylhydrazone and falconensone A dioxime showed approximately 113%, 68%, 177%, and 65% antioxidant activity respectively, relative to vitamin E. The difference between vitamin E and falconensone A *p*-bromophenylhydrazone was significant ($p < 0.01$). These results indicate that falconensone A *p*-bromophenylhydrazone is the most potent antioxidant in suppressing conjugated diene formation caused by hydroxyl radicals.

Inhibition of Liver Microsomal Lipid Peroxidation by Falconensones

In order to determine ascorbate-dependent lipid peroxidation, microsomes were incubated with falconensone A, falconensone B, falconensone A *p*-bromophenylhydrazone, falconensone A dioxime or vitamin E at $10 \mu\text{M}$ concentration. As shown in Fig. 5, falconensone A *p*-bromophenylhydrazone, falconensone A dioxime or vitamin E,

Fig. 5. Effects of Ascorbate-Dependent Lipid Peroxidation by Vitamin E, Falconensone A, Falconensone B, Falconensone A *p*-Bromophenylhydrazone, and Falconensone A Dioxime

Microsomes (0.5 mg protein/ml) and compounds at concentrations of $10 \mu\text{M}$ in 100 mM Tris-HCl (pH 7.5) containing $15 \mu\text{M}$ FeCl_3 and 4 mM ADP were preincubated at 37°C for 1 min. To reaction mixtures was added 1 mM ascorbic acid and incubation was continued at 37°C for 20 min. TBA reagent was added, the mixtures were heated in a boiling waterbath for 15 min, and centrifuged as described in "Materials and Methods." Supernatant absorbance was measured at 535 nm ($\epsilon = 156000 \text{ cm}^{-1} \text{ M}^{-1}$). % antioxidant activity relative to vitamin E for compounds at $10 \mu\text{M}$ concentration was based on the MDA of control being defined as 100%. The means $\pm$ S.D. of at least four measurements are shown. Experiments were repeated at least four times.

decreased the extent of MDA formation resulting from the breakdown of polyunsaturated fatty acids. MDA has been identified as a product of lipid peroxidation that reacts with TBA giving a red species absorbing at 535 nm. A reaction mixture containing the vehicle DMSO, was identical to a

Fig. 6. Dose Dependency of Inhibition of Ascorbate-Dependent Lipid Peroxidation by Vitamin E, Falconensone A *p*-Bromophenylhydrazone, and Falconensone A Dioxime

Microsomes (0.5 mg protein/ml) and various concentrations of vitamin E (□), falconensone A *p*-bromophenylhydrazone (◇), or falconensone A dioxime (○) in 100 mM Tris-HCl (pH 7.5) containing 15 μM FeCl₃, and 4 mM ADP, were preincubated at 37 °C for 1 min. To reaction mixtures was added 1 mM ascorbic acid and incubation was continued at 37 °C for 20 min. TBA reagent was added, then the mixtures were heated in a boiling waterbath for 15 min, and centrifuged as described in "Materials and Methods." Supernatant absorbance was measured at 535 nm ($\epsilon = 156000 \text{ cm}^{-1} \text{ M}^{-1}$). The means $\pm$ S.D. of at least four measurements are shown. Experiments were repeated at least five times.

control mixture in the absence of compounds. MDA formation was inhibited by falconensone A *p*-bromophenylhydrazone, falconensone A dioxime and vitamin E, but not by DMSO, falconensone A, and falconensone B (Fig. 5). As shown in Fig. 6, falconensone A *p*-bromophenylhydrazone, falconensone A dioxime, and vitamin E inhibited lipid peroxidation in dose dependent manners in the range of 0–5 μM, with falconensone A *p*-bromophenylhydrazone and falconensone A dioxime exhibiting an IC₅₀ value of 2.5 μM and 5 μM, respectively. At concentrations higher than 5 μM, vitamin E significantly increased MDA formation, while 10 μM falconensone A *p*-bromophenylhydrazone or falconensone A dioxime inhibited lipid peroxidation completely (Fig. 6). These results indicate that falconensone A *p*-bromophenylhydrazone and falconensone A dioxime decrease microsomal lipid peroxidation more efficiently than dose vitamin E.

Effects on Superoxide Anion by Falconensones Superoxide generated by xanthine/xanthine oxidase reduces cytochrome c. The absorbance at 550 nm (OD₅₅₀) of control was set at 100% and adjusted accordingly. SOD (100 U ml⁻¹) and 10 μM vitamin C scavenged approximately 90% and 40% of superoxide anion, respectively. However, all falconensones and vitamin E at the concentration of 10 μM, did not significantly change OD₅₅₀ relative to control (data not shown).

DISCUSSION

In this study, falconensone A *p*-bromophenylhydrazone and falconensone A dioxime, which are synthetic derivatives of falconensone A identified recently (Fig. 1)¹⁾ scavenged DPPH radicals, inhibited linoleic acid peroxidation initiated by hydroxyl radicals and reduced lipid peroxidation in rat liver microsomes to the same extent or greater than did vita-

min E.

Falconensone A is a yellow powder possessing a unique structure, consisting of a novel cyclopent-2-en-1-one skeleton with a methyl ketone residue connected through a conjugated pentaene moiety (Fig. 1). Such a structure is related to retinoic acid (RA), which possess a monocyclic parent nuclei containing five ethylene units with additional functionality at the terminus of the acyclic portion. Previous reports have shown that RA and retinoids act as antioxidants in quenching DPPH radicals.³²⁾ While all retinoids fail to exhibit a quenching effect on DPPH radicals at concentrations below 500 μM,^{32,33)} 13-*cis* RA and a furyl analog of RA, scavenge 19.3% of DPPH radicals at 2.5 mM concentrations.³²⁾ Falconensone A *p*-bromophenylhydrazone and falconensone A dioxime at 20 μM concentration, scavenge approximately 29% of DPPH radicals (Figs. 2 and 3). In contrast, falconensone A and B at the same concentration, do not quench DPPH radicals (Figs. 2 and 3). Thus, falconensone A *p*-bromophenylhydrazone and falconensone A dioxime quench DPPH radicals to a much greater extent than do retinoids.

Linoleic acid peroxidation was inhibited by all falconensones (Fig. 4), while only falconensone A *p*-bromophenylhydrazone and falconensone A dioxime quenched DPPH radicals (Figs. 2 and 3) and suppressed microsomal lipid peroxidation (Figs. 5 and 6). Linoleic acid is peroxidized by Fenton's reagent, generating hydroxy radical as follows³⁴⁾: (1) $\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \cdot\text{OH} + \text{OH}^-$ (2) $\cdot\text{OH} + \text{LH} \rightarrow \text{L}\cdot + \text{OH}^-$ (3) $\text{L}\cdot + \text{O}_2 \rightarrow \text{LOO}\cdot$ (4) $\text{LOO}\cdot + \text{LH} \rightarrow \text{LOOH} + \text{L}\cdot$. This is a chain reaction producing various radicals, including $\cdot\text{OH}$, $\text{LOO}\cdot$, and $\text{L}\cdot$. Vitamin E scavenges these three radicals as well as Fe^{3+} , and inhibits O₂ production.³⁴⁾ One reason for the low structural specificity of the falconensones, may be the presence of many kinds of radicals produced by this reaction. It would be interesting to determine whether each falconensone differentially effects different radicals.

The extent of lipid peroxidation *in vitro* was measured by MDA production derived from lipids. In Fig. 5, falconensone A *p*-bromophenylhydrazone and falconensone A dioxime at a concentration of 10 μM, inhibited 94% and 90% of microsomal lipid peroxidation, respectively. The IC₅₀ values were 2.5 μM for falconensone A *p*-bromophenylhydrazone and 5 μM for falconensone A dioxime (Fig. 6). The level of MDA generation was not markedly altered by vitamin E at between 1 μM and 10 μM concentrations. MDA formation in rat liver microsomes induced by ascorbic acid has been reported to be suppressed 97% by 25 μM 13-*cis*-RA, which reacts with peroxy radicals at an IC₅₀ value of 10 μM²³⁾ and to be inhibited completely by 10 μM all-*trans* RA exhibiting an IC₅₀ value of between 2 μM to 3 μM.³³⁾ These are similar to both falconensone A *p*-bromophenylhydrazone (IC₅₀ = 2.5 μM) and falconensone A dioxime (IC₅₀ = 5 μM). These results indicate that falconensone A *p*-bromophenylhydrazone and falconensone A dioxime are effective antioxidants.

Previously, our studies have shown that falconensone A *p*-bromophenylhydrazone and falconensone A dioxime suppress growth, induce apoptosis, and produce intracellular reactive oxygen species (ROS) in HL60 cells.^{18,35)} The mechanism(s) by which ROS are produced by falconensone A *p*-bromophenylhydrazone and falconensone A dioxime are not known. In the current study, falconensone A *p*-bromophenylhydrazone and falconensone A dioxime, while being stimula-

tors of ROS production in cells, are also potent antioxidants. Similar effects have been reported with curcumin, gallic acid and RA.^{36–38)} It was of interest to investigate how these two different effects are regulated in cells.

Many diseases including cardiovascular and cerebrovascular diseases, certain forms of cancer and ischemia/reperfusion injuries, are considered to involve free radicals, with antioxidants such as vitamins, and flavonoids, *etc.*, preventing these diseases.^{39–42)} Antioxidant properties of falconensone A *p*-bromophenylhydrazone and falconensone A dioxime may affect many physiological processes, including immunostimulation, enhancement of cell communication, and inhibition of metabolic activation. It is possible that falconensone A *p*-bromophenylhydrazone and falconensone A dioxime may be clinically useful for various diseases, which are currently treated by antioxidants.

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