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Antioxidant and anticancer activities of novel *p*-alkylaminophenols and *p*-acylaminophenols (aminophenol analogues)

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Abstract—Novel compounds were designed based on fenretinide, *N*-(4-hydroxyphenyl)retinamide (**2**), which is a synthetic amide of all-*trans*-retinoic acid (**1**) that is a potent antioxidant and anticancer agent. Our recent findings indicated that antioxidant and anticancer activities were due to *p*-methylaminophenol moiety (**8**) in **2**, and that *p*-octylaminophenol (**7**), which has an elongated alkyl chain, was more potent than **8**. This finding lets us to investigate whether compounds containing alkyl or acyl chains linked to an aminophenol residue as long as **2** and **1**, would show activities greater than **2**. For this purpose, we prepared *p*-dodecanoylaminophenol (**3**), *p*-decanoylaminophenol (**4**), *p*-dodecylaminophenol (**5**), and *p*-decylaminophenol (**6**). The *p*-alkylaminophenols, **5** and **6**, exhibited superoxide scavenging activities, but not *p*-acylaminophenols, **3** and **4**. Elongation of the alkyl chain length reduced superoxide trapping capability (**8** > **7** > **6** > **5**). In contrast, lipid peroxidation in rat liver microsomes was reduced by **5** and **6** in dose-dependent manner. Compounds **3** and **4** were poor lipid peroxidation inhibitors, being approximately 400- to 1300-fold lower than **5** and **6**. In addition, all compounds inhibited cell growth of human leukemia cell lines, HL60 and HL60R, in dose-dependent manners (**5** > **6** > **3** = **4**). The HL60R cell line is resistant against **1**. Growth of both cell lines was suppressed by **5** and **6** in a fashion dependent on the length of the aminophenol alkyl chain, but not by **3** and **4**. These results indicate that **5**, a potent anticancer agent greater than **2**, may potentially have clinical utility, and that its anticancer activity is correlated with inhibitory potency against lipid peroxidation, but not with superoxide scavenging activity.

1. Introduction

N-(4-Hydroxyphenyl)retinamide (**2**, fenretinide), a synthetic amide of all-*trans*-retinoic acid (**1**, Fig. 1), is an effective chemopreventive and antiproliferative agent,[1–4] which is used against a wide variety of tumor types. Compound **2** currently is in clinical trials for the treatment of breast, bladder, renal, and neuroblastoma malignancies.[4–10] It induces apoptosis in HL60 and NB4 human leukemia cell lines, as well as in C33A, a human cervical carcinoma cell line, meningioma, and neuroblastoma.[8,11–17]

Previous studies have shown that **2** exhibits antioxidant activities that include scavenging α,α-diphenyl-β-picrylhydrazyl radicals, inhibiting linoleic acid peroxidation initiated by hydroxyl radicals, and reducing lipid peroxidation in rat liver microsomes to the same extent as or greater than vitamin E.[18,19] The *p*-methylaminophenol moiety (**8**, Fig. 1) contributes most significantly to the inhibition of lipid peroxidation of **2** as compared with the 4-aminophenol and *p*-aminoacetophen moieties.[18,20] This is derived from the side-chain amido portion of **2** that distinguishes it from **1**, which itself is an antioxidant. In addition, **7**, which has an elongated methylene chain in **8**, increases the inhibitory activity of lipid peroxidation as compared with **8**,[21] while **7** decreases the superoxide scavenging activity (Fig. 1).

Abbreviations: **1**, retinoic acid; **2**, *N*-(4-hydroxyphenyl)retinamide, fenretinide; **3**, *p*-dodecanoylaminophenol, 4′-hydroxydodecanilide, *N*-(*p*-hydroxyphenyl)dodecananamide, *N*-(4-hydroxyphenyl) dodecananamide; **4**, *p*-decanoylaminophenol, 4′-hydroxydecanilide, *N*-(*p*-hydroxyphenyl) decananamide, *N*-(4-hydroxyphenyl)decananamide; **5**, *p*-dodecylaminophenol, 4-(dodecylamino)phenol; **6**, *p*-decylaminophenol, 4-(decylamino)phenol; **7**, *p*-octylaminophenol, 4-(octylamino)phenol; **8**, *p*-methylaminophenol, 4-(methylamino)phenol; 4-HBR, 4-hydroxybenzylretinone; PBS, phosphate-buffered saline (1.5 mM KH_2PO_4, 8.1 mM Na_2HPO_4, and 136.9 mM NaCl, pH 7.2); RAR, retinoic acid nuclear receptor; EDTA, ethylenediaminetetraacetic acid; ROS, reactive oxygen species.

Keywords: Aminophenol; Antioxidant; Anticancer; Retinoid.
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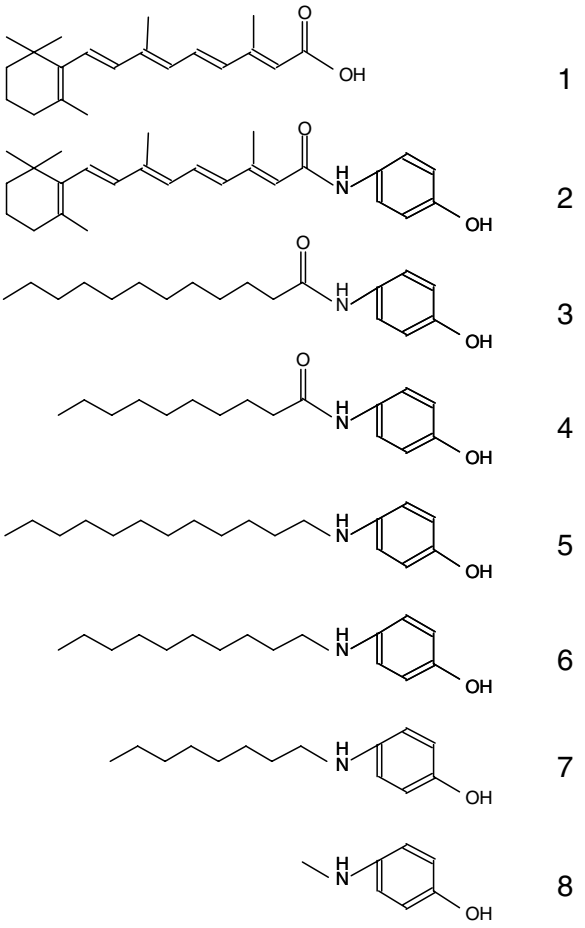

Figure 1. Chemical structures of retinoids (**1** and **2**), *p*-acylaminophenols (**3** and **4**), and *p*-alkylaminophenols (**5**, **6**, **7**, and **8**).

On the other hand, growth of various cancer cell lines including human leukemia cell lines, HL60 and HL60R, which is resistant against **1**, was suppressed by **7**, *p*-hexylaminophenol, *p*-butylaminophenol, and **8** in fashions dependent on the alkyl chain length linked to aminophenol, with **7** being the most potent inhibitor of cell growth in HL60R cells among these *p*-alkylaminophenols tested, including **2**.[21]

In the present study, we synthesized novel four compounds, *p*-dodecanoylaminophenol (**3**), *p*-decanoylaminophenol (**4**), *p*-dodecylaminophenol (**5**), and *p*-decylaminophenol (**6**) (Fig. 1), which bear carbon side-chain lengths similar to those of **1** and **2**, and we examined their antioxidant and anticancer properties.

2. Results

2.1. Scavenging of superoxides by *p*-alkylaminophenols, *p*-acylaminophenols, and retinoids

The superoxide scavenging activities of compounds were evaluated by measuring the absorbance of blue formazan produced from the yellow dye NBT^{2+}. Blue formazan is formed by the reaction of remaining superoxide produced by a hypoxanthine/xanthine oxidase system. The absorbance of control was approximately 0.8. At 10 μM concentration, *p*-alkylaminophenols, **6** (C_{10}) and **5** (C_{12}), scavenged superoxide to the extent of approximately 82% and 70%, respectively (Fig. 2A). This indicated little effect on xanthine oxidase activity (Fig. 2B). Compound **6**, which has shorter alkyl chain than **5**, was a more potent superoxide scavenger. In con-

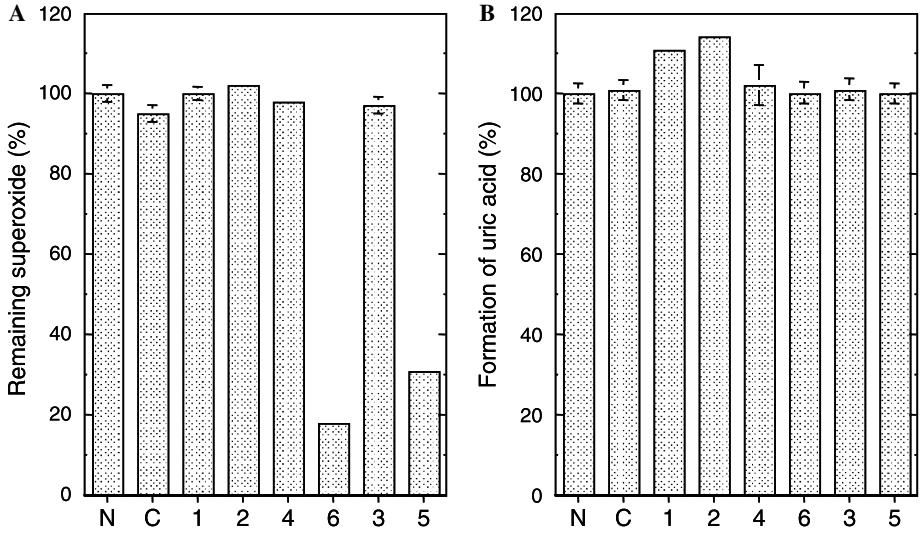

Figure 2. Superoxide scavenging activities and effects of the formation of uric acid by retinoids, *p*-acylaminophenols, and *p*-alkylaminophenols. (A) Reaction mixtures containing compounds (**1**, **2**, **3**, **4**, **5**, and **6**) at the concentration of 10 μM or DMSO (C) were mixed, and then xanthine oxidase was added as described under Section 4. After the incubation at 25 °C for 20 min, $CuCl_2$ was added to the reaction mixture. The subsequent extent of NBT reduction was determined at 560 nm by spectrophotometer. The SD of each data point was ≤8% of the mean. N: none. (B) Reaction mixtures containing compounds (**1**, **2**, **3**, **4**, **5**, and **6**) at the concentration of 10 μM or DMSO (C) without NBT solution were mixed, and then xanthine oxidase was added as described under Section 4. After the incubation at 25 °C for 20 min, $CuCl_2$ was added to the reaction mixture. Absorbances of uric acid were determined at 290 nm by spectrophotometer. The SD of each data point was ≤8% of the mean. Experiments were repeated at least four times. N: none.

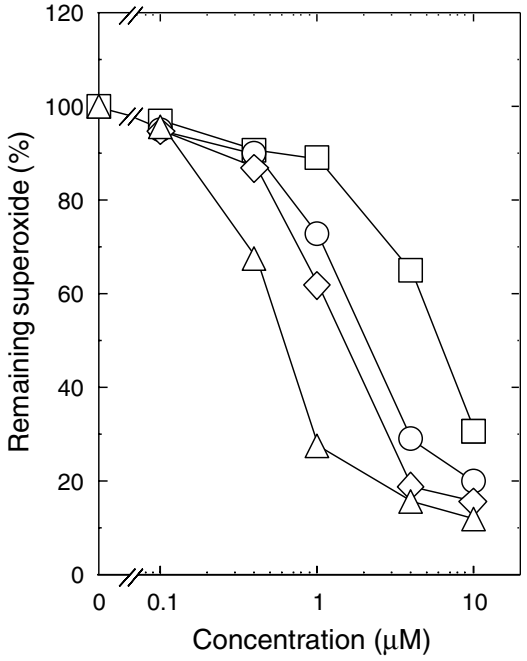

Figure 3. Superoxide scavenging activity by various concentrations of *p*-alkylaminophenols (**5**, **6**, **7**, and **8**). Reaction mixture containing various concentrations of compounds (**5** (□), **6** (○), **7** (◇), and **8** (△)) was mixed, and then xanthine oxidase was added as described under Section 4. After the incubation at 25 °C for 20 min, $CuCl_2$ was added to the reaction mixture. The subsequent extent of NBT reduction was determined at 560 nm by spectrophotometer. The SD of each data point was ≤8% of the mean. Experiments were repeated at least three times.

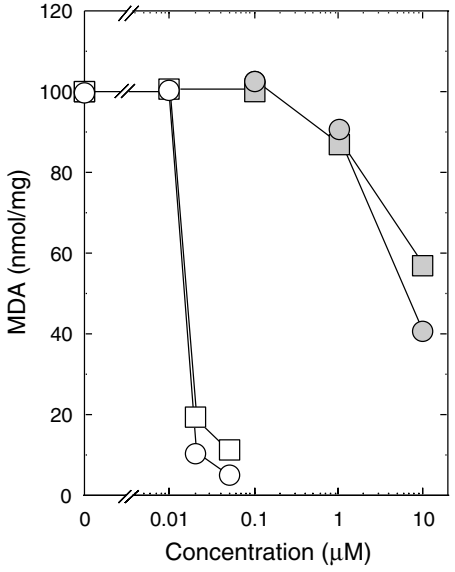

Figure 4. Inhibition of ascorbate-dependent lipid peroxidation by *p*-acylaminophenols and *p*-alkylaminophenols. Microsomes (0.5 mg protein/ml) and various concentrations (0.01–10 μM) of **3** (■), **4** (●), **5** (□), and **6** (○) in 100 mM Tris–HCl (pH 7.5) containing 15 μM $FeCl_3$, and 4 mM ADP, 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, then the mixtures were heated in a boiling water bath for 15 min and centrifuged as described in Section 4. Supernatant absorbance was measured at 535 nm ($\varepsilon = 156{,}000\ cm^{-1}M^{-1}$). The SD of each data point was ≤8% of the mean. Experiments were repeated at least three times.

trast, retinoids (**1** and **2**) and *p*-acylaminophenols (**3** and **4**) were inactive on both scavenging of superoxides and production of superoxides by the xanthine oxidase system (Fig. 2). In addition, *p*-alkylaminophenols, **8** (C_1), **7** (C_8), **6** (C_{10}), and **5** (C_{12}), eliminated superoxide in dose-dependent manners with IC_{50} values of approximately 0.6 μM for **8**, 1.5 μM for **7**, 2 μM for **6**, and 6 μM for **5** (Fig. 3). These results indicate that *p*-alkylaminophenols, **6** and **5**, exhibit superoxide scavenging activity, and that elongation of the alkyl chain, which increases hydrophobicity, reduces the capability to trap superoxide. Compounds **4** and **3**, which inserted carbonyl moieties into **6** and **5**, were inactive.

2.2. Inhibition of liver microsomal lipid peroxidation by *p*-alkylaminophenols and *p*-acylaminophenols

The extent of lipid peroxidation in vitro was measured by lipid-derived malondialdehyde (MDA) production. A reaction mixture containing the vehicle DMSO was identical to a control mixture in the absence of compounds. As shown in Figure 4, analogues **3**, **4**, **5**, and **6** inhibited lipid peroxidation in dose-dependent fashions in the range of 0.01–10 μM. The *p*-alkylaminophenols, **5** and **6** showed marked inhibition of lipid peroxidation and were more efficient than the *p*-acylaminophenols, **3** and **4**. Estimation of IC_{50} values from Figure 4, indicated values of approximately 0.015 μM for **6** (C_{10}) and 0.0155 μM for **5** (C_{12}), while IC_{50} values for **4** and **3** were approximately 6.1 and 20 μM, respectively (Table 1). Thus, antioxidant activities of **5** and **6** were approximately 400- and 1300-fold higher than those of **3** and **4**, respectively (Fig. 4, Table 1). Compounds **5** and **6** inhibited lipid peroxidation to a similar extent as **7** (IC_{50} value approximately 0.014 μM), but not to the same extent as **8** (IC_{50} value approximately 4.6 μM). These results indicate that the insertion of carbonyl moieties into *p*-alkylaminophenols affected their ability to inhibit lipid peroxidation greater than did differences in alkyl chain length.

Table 1. Comparison of IC_{50} values of *p*-acylaminophenols and *p*-alkylaminophenols on MDA production derived from lipids

Compound	IC_{50} (μM)	Fold
6	0.015	1
5	0.0155	1.03
4	6.1	407
3	20	1333

IC_{50} values were obtained from Figure 4. Fold (ratio) of the IC_{50} values for **3**, **4**, **5**, and **6** were calculated according to the following formula: (IC_{50} of **3**, **4**, **5**, and **6**)/(IC_{50} of **6**).

2.3. Growth inhibition of HL60 and HL60R cells by *p*-alkylaminophenols, *p*-acylaminophenols, and 2

HL60 and HL60R cells, which were resistant to **1**, were grown in medium containing 10% FBS in the presence of the new aminophenols (Fig. 5). The parent compound **2** was used as an internal standard for measuring

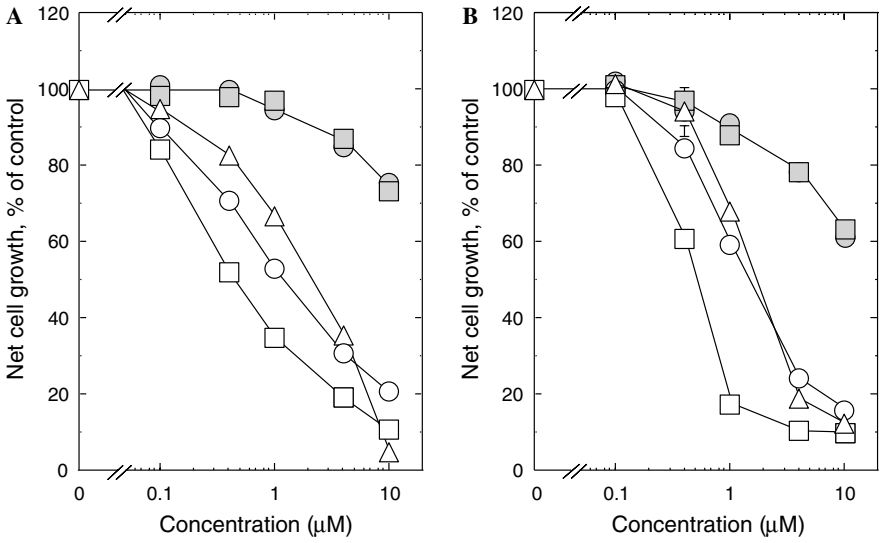

Figure 5. Growth of HL60 and HL60R cells in the presence of **2**, *p*-acylaminophenols, and *p*-alkylaminophenols. Cells (1×10^5 cells/ml) were grown with various concentrations of **2** (△), **3** (■), **4** (●), 5 (□), and 6 (○) in RPMI medium containing 10% FBS. Growth was measured at 94 h for HL60 (A) or 65 h for HL60R (B) as described under Section 4. Net growth % of control is shown for each compound. Each point is the mean of at least four measurements. The SD of each point was ≤8% of the mean.

inhibition of cell growth. Shown in Figure 5 is the percent net cell growth in the presence of the aminophenol analogues with values adjusted by subtracting the initial cell concentrations of experimental cultures from the initial concentrations of control cultures, which were defined as 100% at each time period. Growth of HL60 and HL60R cells was inhibited by various concentrations of *p*-alkylaminophenols (**5** and **6**), *p*-acylaminophenols (**3** and **4**), and **2** in dose-dependent manners (Fig. 5). In HL60 cells, at low concentrations in the range of 0.1–1 μM, **5** and **6** inhibited cell growth more potently than **2** (Fig. 5A). In contrast, **3** and **4** were poor inhibitors of cell growth as compared to **2**, **5**, and **6**. On the other hand, growth of HL60R cells was inhibited more strongly in the order **5**, **6**, **2**, than by **3** or **4** (Fig. 5B). Cell growth in the presence of aminophenol analogues at a concentration of 1 μM indicated that **5** (approximately 65% inhibition) was more potent than **6** (approximately 47% inhibition), **2** (approximately 33% inhibition), **3** and **4** (approximately 3% inhibition) in HL60 cells. In contrast, **5** (approximately 82% inhibition) was more potent than **6** (approximately 42% inhibition), **2** (approximately 32% inhibition), **3** and **4** (approximately 10% inhibition) in HL60R cells (Fig. 5). In both HL60 and HL60R cells, elongation of the alkyl chain attached to the aminophenol residue (**5** and **6**) increased antiproliferative activities, while elongation of the acyl chain attached to the aminophenol residue (**3** and **4**) had no effect. Inhibition of cell growth by *p*-alkylaminophenols was approximately 10- to 50-fold greater than by *p*-acylaminophenols, having acyl residues instead of alkyl chains. These results indicate that the inhibition of cell growth by these compounds cannot be attributed to cell necrosis, and that leukemia cell growth is suppressed by **5** more strongly than by other aminophenols and retinoids. Compound **5** was the most potent inhibitor among the seven *p*-alkylaminophenols examined in previous study, including **7** and **8**. These results may indicate that the hydrophobic properties of an dodecanyl residue linked to the aminophenol may be significant for antitumor potency.

3. Discussion

Analogue **5** exhibited the most potent anticancer and lipid peroxidation inhibitory activity as compared to **2**, a cancer chemopreventive and antiproliferative agent, and a potent inducer of apoptosis. In the current study, a series of aminophenols (**3**, **4**, **5**, and **6**) having *p*-acyl and *p*-alkyl residues of similar lengths to **1** and **2** were prepared and examined for two kinds of antioxidant activities and anticancer efficacy. Compound **5** suppressed growth of both HL60 and HL60R cells to an extent greater than **2** and the other aminophenols examined. Cell growth inhibition by the *p*-alkylaminophenols was elevated according to the elongation of the alkyl chains. The extent of lipid peroxidation reduction in rat liver microsomes by *p*-alkylaminophenols (**5**, **6**, **7**, and **8**) depended on the length of the alkyl chain appended to the aminophenol residue with positive correlation. Compounds **6** and **5** were potent inhibitors of lipid peroxidation, being of approximately 400- and 1300-fold higher potency than **4** and **3**, respectively. In addition, **6** and **5** exhibited superoxide scavenging ability dependent on the length of alkyl chain with negative correlation. The *p*-acylaminophenols, **3** and **4**, were either inactive or weakly active. Thus, the presence of carbonyl moieties in *p*-alkylaminophenols diminished their capabilities as anticancer agents and antioxidants.

Previous reports have shown that alkylaminophenols containing **7** and **8** inhibited lipid peroxidation (MDA formation).[20,22] Compound **8** showed lipid peroxidation inhibitory activity in dose-dependent fashion in the range of 1–5 μM, and **7**, *p*-hexylaminophenol, and

p-butylaminophenol showed marked inhibition of lipid peroxidation. At concentrations higher than 5 μM, **2** significantly increased MDA formation, while 10 μM of **1** inhibited lipid peroxidation with an IC_{50} value of approximately 3.2 μM. IC_{50} values were approximately 4.6 μM for **8** (C_1), 0.3 μM for *p*-butylaminophenol (C_4), 0.033 μM for *p*-hexylaminophenol (C_6), and 0.014 μM for 7 (C_8). Thus, the elongation of the *p*-alkylaminophenol chains affects ability to inhibit lipid peroxidation. The longer the alkyl chains of the *p*-alkylaminophenols, the higher was the antioxidant potency. In the current study, IC_{50} values for **6** (C_{10}) and **5** (C_{12}) were approximately 0.015 and 0.0155 μM, respectively. These values were not significantly different from the 0.014 μM, IC_{50} value of **7** (C_8). The extent of inhibitory activity against lipid peroxidation incurred by elongation of the *p*-alkylaminophenol chains increased until compound **7** and remained at this maximum value with compounds **6** and **5**. These results suggest that novel compounds **6** and **5** as well as **7** are extremely effective antioxidants.

Mitochondrial lipid peroxidation was inhibited by retinoids (retinol, retinol acetate, **1**, retinol palmitate, and retinal) only at high concentrations ($0.1 \sim 10$ mM).[23] In contrast, 13-*cis*-retinoic acid and **1** suppressed microsomal lipid peroxidation with IC_{50} values of 10 μM[24] and 3.2 μM, respectively.[20,22] It was found that **2** was less effective than **8** and that elongation of the methyl residue of **8** to an octyl residue increased the efficacy of lipid peroxidation inhibition.[20] In the current study, **3** and **4**, *p*-acylaminophenols containing carbonyl residues, which exhibited structures similar to **2** and other retinoids, showed much less activity than the *p*-alkylaminophenols **5** and **6**. These results indicate that the addition of oxygen into the alkyl residue may be critical for lipid peroxidation inhibitory activity. The *p*-alkylaminophenols are excellent antioxidants as compared with *p*-acylaminophenol and retinoids.

It has been reported previously that *p*-alkylaminophenols (**8**, *p*-butylaminophenol, *p*-hexylaminophenol, and **7**) exhibit superoxide radical scavenging activity, which is another antioxidant activity different from lipid peroxidation inhibitory activity.[21] Compound **8** was the most potent scavenger of superoxide radicals (**8** > *p*-butylaminophenol > *p*-hexylaminophenol > **7**). In the current study, compound **6** showed superoxide radical scavenging activity greater than **5**, but less than **7** and **8**. In contrast, the *p*-acylaminophenols, **3** and **4** were inactive. The hydrophobic nature of the compounds was not related to superoxide trapping ability. Elongation of the alkyl chain of *p*-alkylaminophenols correlated negatively with superoxide scavenging activity and positively with inhibitory activity against lipid peroxidation.

Even though **2** is a potent anticancer drug, clinical studies have shown that treatment of patients with **2** is accompanied by a side effect of night blindness due to decreasing of serum retinol levels.[25,26] It appears that this side effect is due to the displacement of retinol from serum retinol binding protein, resulting in reduced delivery of retinol to eye.[27–29] It is generally accepted that **2** may act via pathways which are independent of the nuclear retinoid receptors, RARs and RXRs, since **2** exhibits extremely poor binding to the nuclear retinoid receptors and shows anticancer activity against cells resistant to **1**. Therefore, **2** may act on cells directly rather than through hydrolysis to free **1**. Recently, in order to overcome side effects of **2**, several non-hydrolyzable compounds were designed and evaluated. 4-Hydroxybenzylretinone (4-HBR), a stable C-linked analogue of **2** that cannot be hydrolyzed to **1**, was synthesized and shown to exhibit antitumor effects similar to **2**, without night blindness side effects.[25,30] In addition, **2**-C-glucuronide (**2**CG), a C-linked analog of **2**-glucuronide that cannot be hydrolyzed to **2**, is an effective chemopreventive agent with almost no side-effects (e.g., reduction of retinol level and elevation of triglyceride in blood, etc.).[31,32] However, as both 4-HBR and **2**CG contain the same aromatic ring as retinol and **1** and **2**, they may affect endogenous retinol levels in the body, because retinol binding protein recognizes and binds the aromatic ring of retinol. In the current study, we designed compounds without aromatic rings (**3**, **4**, **5**, and **6**) that have markedly different structures from retinol and **1** and **2**. Compound **5** was the most potent antiproliferative agent among these compounds including **2**. Compound **5** inhibited cell growth gradually from low to high concentrations as compared to **2**, which was toxic at high concentrations. Compound **5** was more potent than **2** by approximately 5-fold in HL60 cells and approximately 3-fold in HL60R cells. Therefore, it is possible that **5** may be an effective anticancer drug without the side effects observed with **2**. It would be interesting to further examine anticancer activities of **5** against various cancer cell lines.

In the current study, **5** inhibited cell growth more potently than **2** against HL60 and HL60R cells, which are resistant to **1** (Fig. 5). In cytodifferentiation therapy of promyeloid leukemia, a high percentage of patients in complete remission induced by **1** alone relapse within a few months.[33–35] Most relapsed patients are resistant to further treatment with **1**.[35–37] The clinical outcome of patients treated with **1** may be modified by administration of **5**. Studies are presently underway to determine the toxicity of **5** in animals and to measure the effectiveness of **5** on tumors growing in animals.

Free radicals can cause certain forms of cancer, cardiovascular and cerebrovascular diseases and ischemia/reperfusion injuries. Antioxidants, including vitamins and flavonoids, can prevent these diseases.[38–41] Our study showed that novel **5** and **6** exhibited antioxidative and anticancer activities. Superoxide scavenging activity may be due to the phenolic hydroxyl hydroxyl· In contrast, inhibition of lipid peroxidation and anticancer action may be due to the three-dimensional structure formed by the phenolic hydroxyl and the alkyl residues. The alkyl residue may be particularly significant for associations to membranes in cells, showing inhibition of lipid peroxidation or anticancer action. Compounds **5** and **6** may be effective in diseases concerned with hydroxy radicals and may affect diseases involving super-

oxide. The antioxidant and antiproliferative activities of **5** and **6** may influence various physiological processes, including immunostimulation, enhancement of cell communication, and inhibition of metabolic activation.

4. Experimental

4.1. General

Adenosine 5′-diphosphate (ADP), **1**, nitroblue tetrazolium (NBT), ethylenediaminetetraacetic acid (EDTA), bovine serum albumin (BSA, fraction V), and dimethylsulfoxide (DMSO) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). **2** was provided by Dr. R. C. Moon, University of Illinois, Chicago, IL, USA. Dithiothreitol (DTT), trichloroacetic acid (TCA), and **8** were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). 4-Aminophenol, decanoic anhydride, dodecanoic anhydride, tetrahydrofuran, and $NaBH_4$ were obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). Ascorbic acid and 2-thiobarbituric acid (TBA) were purchased from Wako Pure Chemicals Industries, Co. Ltd (Osaka, Japan).

4.2. Synthesis of compounds

4.2.1. Chemistry. The synthesis of **3**, **4**, **5**, and **6** began by treating commercially available **4**-aminophenol with the corresponding acyl anhydride. The N-acylation provided the desired amide (**3**, **4**) in excellent yield.[42] Reduction of the amide (**3**, **4**) using $NaBH_4$ and I_2 in refluxing tetrahydrofuran (THF) gave *p*-alkylaminophenol (**5**, **6**).[43]

4.2.2. Analysis. Melting points were measured with a Yanagimoto micro melting point apparatus (Yanagimoto Manufacturing Co., Tokyo, Japan) without correction. IR spectra were recorded on a JASCO FT/IR- 200 and major absorption was listed in cm^{-1}. ^1H and ^{13}C NMR spectra were obtained on a JEOL GSX 270 instrument (Tokyo, Japan), and chemical shifts were reported in ppm on the δ-scale from internal Me_4Si. MS spectra were measured with a JEOL JMS 600 spectrometer by using the electron impact (EI) methods. Elemental analyses were performed on a Perkin-Elmer 240-B instrument (Perkin-Elmer Inc., MA, USA). Column chromatography was performed on silica gel (45–75 μm, Wakogel C-300). TLC was carried out on glass plates coated with silica gel F_{254} (Merk, Germany). Spot detection was performed with UV 254 nm or iodine vapor. THF was distilled over potassium metal.

4.2.3. *p*-Decanoylaminophenol (4). Decanoic anhydride (10.78 g, 33 mmol) was added portionwise to 4-aminophenol (3.27 g, 30 mmol) in dry THF (200 ml) at 0 °C. After being stirred at room temperature for 16 h, the reaction mixture was evaporated under reduced pressure. The residue was added $CHCl_3$ (200 ml), stirred for another 1 h, and filtered to give **4** (7.18 g, 91%) as a colorless solid which was stored under N_2 and could be used directly without further purification: mp 129–130 °C. MS *m*/*z* EI, 263 (M^+). ^1H NMR (DMSO) 0.84 (3H, t, J = 6.3 Hz), 1.24 (12H, m), 1.54 (2H, m), 2.21 (2H, t, J = 7.3 Hz), 6.65 (2H, d, J = 8.9 Hz), 7.33 (2H, d, J = 8.9 Hz), 9.11 (1H, s), 9.56 (1H, s). ^{13}C NMR (DMSO) 13.9, 22.0, 25.1, 28.6, 28.6, 28.7, 28.8, 31.2, 36.1, 114.8, 120.7, 130.9, 152.9, 170.4. IR (KBr) 3310, 1650, 1550, 1520, 1250, 830 cm^{-1}. Anal. Calcd for $C_{16}H_{25}NO_2$: C, 72.96; H, 9.57; N, 5.32. Found: C, 73.02; H, 9.30; N, 5.26.

4.2.4. *p*-Dodecanoylaminophenol (3). 4-Aminophenol (3.27 g, 30 mmol) converted to the title compound by the procedure described for amide **4** using dodecanoic anhydride. Compound **3** (86% yield) as a colorless solid was stored under N_2 and could be used directly without further purification: mp 133–134 °C. MS *m*/*z* EI, 291 (M^+). ^1H NMR (DMSO) 0.85 (3H, t, J = 6.4 Hz), 1.24 (16H, m), 1.55 (2H, m), 2.22 (2H, t, J = 7.4 Hz), 6.66 (2H, d, J = 8.9 Hz), 7.34 (2H, d, J = 8.9 Hz), 9.12 (1H, s), 9.57 (1H, s). ^{13}C NMR (DMSO) 14.0, 22.1, 25.2, 28.7, 28.7, 28.8, 28.9, 29.0, 29.0, 31.3, 36.2, 114.9, 120.8, 131.0, 153.0, 170.5. IR (KBr) 3310, 1650, 1550, 1520, 1250, 830 cm^{-1}. HRMS Calcd for $C_{18}H_{29}NO_2$: 291.2198. Found: 291.2204.

4.2.5. *p*-Decylaminophenol (6). $NaBH_4$ (0.946 g, 25 mmol) was added to **4** (2.63 g, 10 mmol) in dry THF (30 ml). To the suspension treated dropwise iodine (2.54 g, 10 mmol) in dry THF (20 ml) under N_2 at 0 °C for 1 h. After the reaction mixture was refluxed for 3 h, it was cooled to room temperature, treated by careful addition of 3 M HCl (6 ml), neutralized by 1 M NaOH, and extracted twice with ethylacetate. The combined organic extract was washed with H_2O and brine, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (ether/hexane = 1:2) to give **6** (2.43 g, 98%); mp 82–83 °C. MS *m*/*z* EI, 249 (M^+). ^1H NMR ($CHCl_3$) 0.88 (3H, t, J = 6.9 Hz), 1.26 (14H, m), 1.58 (2H, m), 3.03 (2H, t, J = 6.9 Hz), 4.08 (2H, br), 6.53 (2H, d, J = 8.6 Hz), 6.66 (2H, d, J = 8.6 Hz). ^{13}C NMR ($CHCl_3$) 14.1, 22.6, 27.1, 29.3, 29.4, 29.5, 29.6, 29.6, 31.9, 45.5, 114.8, 116.2, 142.3, 148.1. IR (KBr) 3280, 1520, 1250, 820 cm^{-1}. Anal. Calcd for $C_{16}H_{27}NO$: C, 77.06; H, 10.91; N, 5.62. Found: C, 76.90; H, 10.67; N, 5.70.

4.2.6. *p*-Dodecylaminophenol (5). Compound **3** (2.91 g, 10 mmol) converted to the title compound by the procedure described for aminophenol **6**. Purification by column chromatography on silica gel (ether/hexane = 1:1) afforded the product (87% yield) as a colorless needle; mp 86–88 °C. MS *m*/*z* EI, 277 (M^+). ^1H NMR ($CHCl_3$) 0.88 (3H, t, J = 6.7 Hz), 1.26 (18H, m), 1.59 (2H, m), 3.04 (2H, t, J = 7.4 Hz), 6.53 (2H, d, J = 8.7 Hz), 6.69 (2H, d, J = 8.7 Hz). ^{13}C NMR ($CHCl_3$) 14.1, 22.6, 27.1, 29.3, 29.4, 29.5, 29.6, 29.6, 31.9, 45.5, 114.8, 116.2, 142.3, 148.1. IR (KBr) 3370, 1520, 1240, 820 $cm^{-}1$. HRMS Calcd for $C_{18}H_{31}NO$: 277.2406. Found: 277.2396.

4.3. 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.[24] Rat liver microsomes were prepared by the method as described previously.[20] Reaction mixtures

consisting of microsomes (0.5 mg protein/ml) and compound (**1**, **2**, **3**, **4**, **5**, **6**, **7** or **8** dissolved in DMSO) in 100 mM Tris–HCl (pH 7.5) containing 15 μM $FeCl_3$, 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*g* (10 min). Supernatant absorbance was measured spectrophotometrically at 535 nm ($\varepsilon = 156{,}000\ cm^{-1}\ M^{-1}$).

4.4. Measurement of superoxide anion scavenging activity

The superoxide radicals were generated in vitro by the hypoxanthine/xanthine oxidase system. The scavenging activities of compounds were determined by the NBT reduction method. In this method, $O_2{\cdot}^-$ reduces the yellow dye (NBT^{2+}) to produce the blue formazan, which is measured spectrophotometrically at 560 nm. Antioxidants are able to inhibit the purple NBT formation.

The capacity of compounds to scavenge the superoxide radical was measured by a partial modified method of previous assay as described.[44] Briefly, a reaction mixture was prepared with a final volume of 2.6 ml/tube. Sodium carbonate buffer (50 mM, pH 10.2)(2.0 ml), 3 mM EDTA (0.1 ml), 1.5 mg/ml BSA (0.1 ml), 0.75 mM NBT (0.1 ml), 3 mM xanthine (0.1 ml), and 2.4 μl compound (**1**, **2**, **3**, **4**, **5**, **6**, **7** or **8** dissolved in DMSO) were mixed, and then xanthine oxidase (0.1 ml, one unit) was added. After the incubation at 25 °C for 20 min, 6 mM $CuCl_2$ (0.1 ml) was added to the reaction mixture. The subsequent extent of NBT reduction was determined at 560 nm by spectrophotometer.

In order to determine effects on xanthine oxidase by compounds, the formation of uric acid was measured. Reaction mixtures without NBT solution above were incubated at 25 °C for 20 min, and 6 mM $CuCl_2$ (0.1 ml) was added to the reaction mixture. Absorbances of uric acid were determined at 290 nm by spectrophotometer.

The solutions were prepared daily and kept from light. The results were expressed as the percentage inhibition of the NBT reduction with respect to the reaction mixture without sample (buffer only).

4.5. Cell growth

Early passage (<30) human myeloid leukemia cell lines, HL60 and HL60R, a mutant subclone of HL60 that exhibits relative resistance to **1** and that harbors RARs with markedly reduced affinity for **1**, were maintained in RPMI 1640 medium (GIBCO, Grand Island, NY) supplemented with 10 mM 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid (HEPES), pH 7.3, and 10% fetal bovine serum (FBS) (GIBCO).[45–47]

HL60 and HL60R cells (1×10^5/ml) were grown in RPMI 1640 medium containing 10% FBS and various concentrations of compounds. Cell number was estimated by an electric particle counter (Coulter Electronics, Hialeah, FL) and viability by Trypan blue dye exclusion. The percentage of net growth is shown with values adjusted by subtracting the initial cell concentration of experimental cultures from the initial concentrations of control cultures which were defined as 100%. Values for percent net growth were calculated with the following formula: [(cell concentration of experimental culture) − (initial cell concentration)/(cell concentration of control culture) − (initial cell concentration)] × 100.

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