Synthesis and Biological Activity of Sulfur-Containing Aryl-aldehyde Schiff Bases

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A series of chemically modified aryl-aldehyde Schiff bases has been synthesized and tested for their antioxidant activity and radiation protection. We observed that disulfide-containing aryl-aldehyde Schiff base 6c exhibited potent free radical scavenging, antioxidation, and radioprotection activities.

Key words aryl-aldehyde Schiff base; antioxidant; radioprotection

The interaction of ionizing radiation with the human body, arising either from external sources outside the body or from internal contamination of the body by radioactive substances, leads to biological effects which may later show up as clinical symptoms. In general, these symptoms result from damage to the blood forming organs, digestive system, or central nervous system, depending on the radiation dose.[1—3] Therefore, protecting the body from the toxic effects of radiation has been a major concern in public health. Patt *et al.*[4] reported that the administration of the sulfurhydryl amino acid cysteine, given to rats before an 8-Gy dose of whole-body X-irradiation, significantly increased the animals' resistance to the cytotoxic effects of the exposure. Since this initial finding, new agents have been developed for cytoprotection. As reviewed[5,6] by Foye, these agents include mercaptans, di- and trisulfides, phosphorothioates, acid hydrazides, imidazoles, and amine oxides. Although these compounds are too toxic to be used in the clinic, the need still exists for compounds that are more effective and less toxic for both military and emergency use. Recently, the fact that amifostine (WR2721; NH_2–$(CH_2)_3$–NH–$(CH_2)_2$–SPO_3H_2) has been used to protect[7] cancer patients against the cytotoxicity of ionizing radiation in the clinic has gained much more attention in the search for new cytoprotective agents. Substituted anilines[8] were reported to possess significant radioprotective activity, but some of them are toxic. Blickenstaff *et al.*[9] reported the Schiff base products of substituted anilines and aryl-aldehyde could reduce the toxicity of substituted anilines but preserve the radioprotective activity. Since the radical scavenging ability of mercaptans and disulfides is well-known, we speculated that the introduction of these important functional groups into the Schiff base products might provide potent antioxidative and radioprotective activity. In this work, the sulfur-containing Schiff bases of substituted anilines and benzaldehyde have been synthesized and their free radical scavenging, antioxidation, and radioprotection effects were also investigated.

Chemistry

The synthetic routes to the target compounds (**2a**—**c**), (**4a**, **b**) and (**6a**, **c**) are outlined in Charts 1—3. Initially, the key intermediates, 2-(phenylthio)benzaldehyde (**1**), 2,2′-dibenzaldehyde sulfide (**3**), and 2,2′-dithiodibenzaldehyde (**5**) were prepared from thiophenol, 2-mercaptobenzoic acid, and (2-mercapto-phenyl)-methanol, respectively. Thiophenol was reacted with 2-chlorobenzaldehyde in hexamethylphosphoramide (HMPA) in the presence of sodium carbonate at 85 °C for 5 h producing the aldehyde **1** in a good yield. Reduction of 2-mercaptobenzoic acid with lithium aluminum hydride gave (2-mercapto-phenyl)-methanol which was used for the subsequent preparation of **3** and **5**. Treatment of (2-mercapto-phenyl)-methanol with 2-chlorobenzaldehyde in HMPA in the presence of sodium carbonate at 100 °C for 5 h

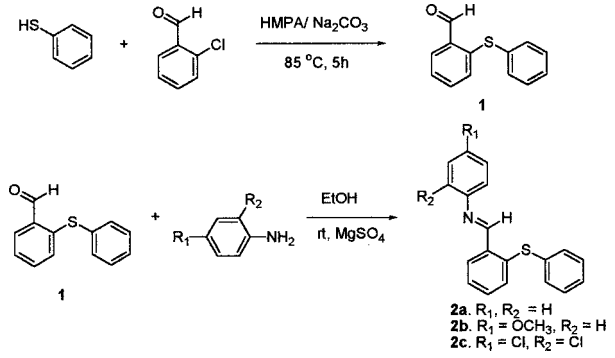

Chart 1

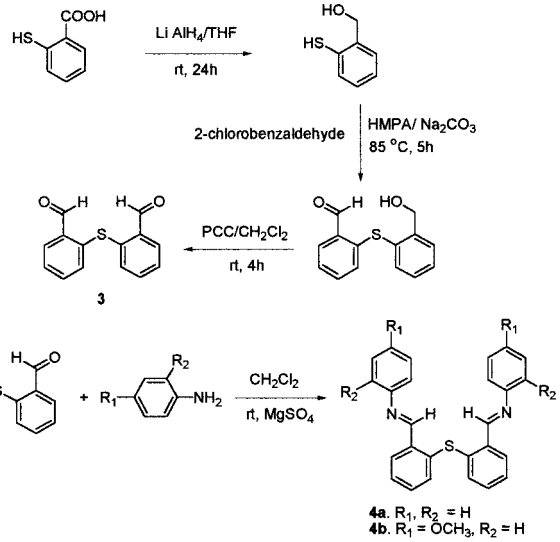

Chart 2

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followed by oxidation with pyridine chlorochromate (PCC) yielded **3**. (2-Mercapto-phenyl)-methanol could also be oxidized with PCC to produce **5**. Seven Schiff bases **2a**—**c**, **4a**, **b** and **6a**, **c** were obtained using mono- and bis-benzaldehyde (**1**, **3**, **5**) with aniline, *p*-anisidin, and 2,4-dichloraniline, respectively, in the presence of magnesium sulfate at reflux temperature.

Results and Discussion

The model of scavenging DPPH free radical[10] is a simple method to evaluate the antioxidative activity of antioxidants. As shown in Table 1, these compounds displayed various degrees of free radical scavenging activity, with decreasing activity in the following order: **6a**>**6c**>**2b**>**4b**>**4a**>**2c**>**2a**. Among them, compounds **6a** and **6c**, containing disulfide structures, were the most potent, having antiradical effects comparable to that of trolox.

The *in vitro* model using AAPH-induced lipid peroxidation of Tween-emulsified linoleic acid is a common method[11] used to measure the antioxidative activity of synthetic antioxidants. In this assay, the oxidation is carried out under conditions relatively similar to *in vivo* biological systems. The inhibitory effects on lipid peroxidation or the antioxidative activity of these compounds are listed in Table 2. We defined the antioxidative activity of these compounds as the relative rate of lipid peroxidation initiated by AAPH radical. The stronger the antioxidative activity, the smaller the rate of lipid peroxidation. The inhibitory activity of these compounds in decreasing order was **6c**>**4a**>**2a**>**6a**. Compound **6c** exhibited antioxidative activity that was comparable to that of trolox.

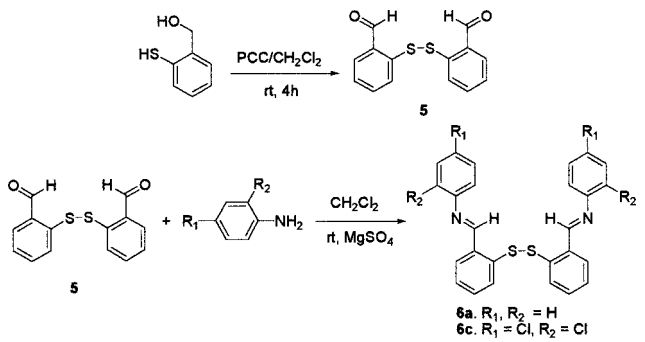

Chart 3

The radiation protection activities of these test compounds were assessed using cultured human HeLa tumor cells. The cells were irradiated in the absence or presence of 10^{-4} M test compounds and the percent of cell survival after 7 d, summarized in Table 3, was in the following the order of activity: **6c**>**4a**>**2a**=**6a**. These results indicated that all of the test compounds exhibited a good radioprotective effect compared to the untreated control cells. The radioprotection was most

Table 1. Free Radical Scavenging Activity of Compounds for DPPH Radical

Compound name	IC_{50}[a] (μM)	Inhibition (%)[b]
Trolox	34.5	62.9
WR2721	106.5	24.3
Glutathione	96.5	38.6
2a	880	5.8
2b	116	20.7
2c	185	12.8
4a	155	14.4
4b	128	17.9
6a	44.4	49.8
6c	35.5	72.0

a) IC_{50}: The concentration of test compounds needed to reduce DPPH absorption by 50% at 517 nm. The values were calculated from the slope equation of the dose–response curves. Values are means of three independent determinations. *b*) Inhibition (%) indicates the percent inhibition at 50 μM of antioxidant.

Table 2. Antioxidant Activity of the Compounds on 2,2′-Azobis(2-aminopropane)dihydrochloride (AAPH)-Induced Lipid Peroxidation of a Tween-Emulsified Linoleic Acid System

Compound name	Rate of peroxidation (ΔA_{500}/min)	Inhibition of peroxidation (%)[a]
Control	4.30×10^{-3}	0
Trolox	7.54×10^{-4}	82.5
WR2721	2.48×10^{-3}	13.9
Glutathione	1.75×10^{-2}	59.3
2a	1.92×10^{-3}	55.3
4a	1.25×10^{-3}	70.9
6a	2.99×10^{-3}	30.5
6c	7.67×10^{-4}	82.2

a) Inhibition of peroxidation (%)=(1−rate of test compound/rate of control)×100%. Peroxidation was initiated by the addition of AAPH (0.1 M) solution to the Tween-emulsified linoleic acid mixture. Degree of peroxidation was estimated by measuring absorption at 500 nm for the formation of complex $[Fe(SCN)]^{2+}$. Trolox and glutathione (GSH) served as reference compounds. All tests were performed in triplicate. Data shown here represent the slope of the time course-absorption curves of each compound analyzed. The control for the assay was carried out identically but in the absence of the test compound and the slope set as 100%.

Table 3. Radiation Protection of Compounds in Human Cells

Treatment		Cell survival (%)				
		0 Gy	2 Gy	5 Gy	10 Gy	Fold of protection at 10 Gy
Untreated	0 M	100.0	51.4	29.0	16.6	1.0
Trolox	10^{-4} M	100.0	92.0	57.5	56.2	3.38
WR2721	10^{-4} M	100.0	48.6	27.0	16.8	1.01
2a	10^{-4} M	100.0	71.3	52.1	28.0	1.69
4a	10^{-4} M	100.0	76.8	57.1	41.0	2.47
6a	10^{-4} M	100.0	73.5	40.5	27.9	1.68
6c	10^{-4} M	100.0	91.8	71.2	69.2	4.17

HeLa cells were treated in the absence or presence of indicated synthetic or standard compounds for 30 min before being irradiated with various doses of ^{137}Cs. The treated cells were then split and seeded in identical cell numbers into appropriate cultured vessels. After seven days, the number of viable cells was assessed. Results shown here represent the average of two independent experiments.

prominent with compound **6c**, which is greater than that of the reference compound trolox. The lack of a radioprotective effect for WR2721 may be due to the lack of alkaline phosphatase in the medium that is required to convert WR2721 to its thiol active form. It should be noted that the efficacy of the test compounds in terms of radioprotective activity correlates well with the antioxidative activity as shown in Table 2. These results demonstrate the Tween-emulsified lipid peroxidation system used in our study could reflect the *in vivo* cell system.

In summary, we have synthesized sulfur-containing aryl-aldehyde Schiff bases in the present study. The results have demonstrated that these sulfur-containing aryl-aldehyde Schiff bases exhibit evident antioxidative activity that enables them to be potential antioxidative, anti-inflammatory, or radioprotective agents. Further experiments evaluating the radioprotective effects of **6c** in normal cell or mice are currently in progress.

Experimental

General Melting points (mp) were taken on a BUCHI 530 apparatus and are uncorrected. Merck Art No. 105554 plates precoated with Silica gel 60 containing fluorescent indicator were used for thin-layer chromatography, and Silica gel 60 (Merck Art No 109385, 230—400 mesh) was employed for column chromatography. Evaporations were carried out at $<50\,^{\circ}C$ using a rotary evaporator at reduced pressure (water aspirator). ^{1}H- and ^{13}C-NMR spectra were obtained with a Varian 300 NMR spectrometer at 300 and 75 MHz, respectively. Where necessary, deuterium exchange experiments were used to obtained proton shift assignments. Mass spectra were recorded on a JEOL J.M.S-300 spectrophotometer. Analytical samples were dried under reduced pressure at 78 °C in the presence of P_2O_5 for at least 12 h unless otherwise specified. Elemental analyses were obtained using a Perkin-Elmer 2400 Elemental Analyzer.

2-(Phenylthio)benzaldehyde (1) The reaction mixture of 2-chlorobenzaldehyde (15 g, 107 mmol), thiophenol (15 g, 136 mmol), sodium carbonate (20 g), and hexamethylphosphoramide (31 g, 173 mmol) was heated at 85 °C for 5 h. After cooling, the mixture was poured into water (100 ml). After extraction with ether and drying with magnesium sulfate, the mixture was concentrated under reduced pressure to a residue. The residue was crystallized with *n*-hexane to give pure **1** (18.5 g, 80%), mp: 47—48 °C (lit.[12] 50—51 °C).

(2-Mercapto-phenyl)-methanol The suspension of $LiAlH_4$ (2 g, 52 mmol) in dry tetrahydrofuran (THF) (80 ml) was added dropwise to a solution of 2-mercaptobenzoic acid (4.36 g, 28.4 mmol) in dry THF (50 ml). After stirring at room temperature for 24 h, 10% H_2SO_4 (40 ml) and ethyl acetate (40 ml) were added carefully and the reaction mixture was filtered. The residue was washed with ethyl acetate (3×10 ml). The filtrate and washings were combined, dried with magnesium sulfate, and concentrated under reduced pressure to give an oily product (2 g, 50%), which became solid after cooling. Pure compound was obtained by crystallization with a mixture of *n*-hexane and ethyl acetate, mp: 31—32 °C (lit.[13] 32—32 °C).

2-(2-Hydroxymethylphenylthio)benzaldehyde The reaction mixture of 2-chlorobenzaldehyde (6.6 g, 47 mmol), (2-mercapto-phenyl)-methanol (6.8 g, 49 mmol), 63% NaOH (3 ml), and hexamethylphosphoramide (12 g, 69 mmol) was heated at 100 °C for 5 h. After cooling, the mixture was poured into water (100 ml). After extraction with benzene and drying with magnesium sulfate, the mixture was concentrated under reduced pressure to a residue (5.4 g, 80%). Pure compound was obtained by chromatography (silica gel, *n*-hexane/ethyl acetate=3/1) as an oily product. *Rf* 0.27 (*n*-hexane/ethyl acetate=3/1). ^{1}H-NMR ($CDCl_3$) δ: 3.31 (s, 1H, OH), 4.69 (d, 2H, J=18 Hz, CH_2), 6.77—7.76 (m, 8H, ArH), 10.19 (s, 1H, CHO).

2,2′-Dibenzaldehyde Sulfide (3) A mixture of pyridine chlorochromate (10 g, 46 mmol) in dry dichloromethane (60 ml) under nitrogen atmosphere was added dropwise to a mixture of 2-(2-hydroxymethylphenylthio)benzaldehyde (3.37 g, 24 mmol) in dry dichloromethane (50 ml). After stirring at room temperayure for 4 h, the reaction mixture was filtered. The residue was washed with dichloromethane (3×30 ml) and ether (30 ml). The filtrate and washings were combined, and then concentrated to give the solid product (3.5 g, 88%). Pure compound was obtained by crystallization with ethanol, mp: 96—97 °C. *Rf* 0.42 (*n*-hexane/ethyl acetate=3/1). ^{1}H-NMR ($CDCl_3$) δ: 7.15 (d, 2H, J=6.9 Hz, ArH), 7.42—7.51 (m, 4H, ArH), 7.94 (dd, 2H, J=7.35, 1.6 Hz, ArH), 10.33 (s, 2H, CHO). ^{13}C-NMR ($CDCl_3$) δ: 128.47, 132.32, 133.15, 135.21, 135.62, 139.16, 191.99. IR (KBr) cm^{-1}: 1674 (C=O). MS *m/z*: 243 $(M+H)^+$. *Anal*. Calcd for $C_{14}H_{10}O_2S_2$: C, 69.40; H, 4.16. Found: C, 69.20; H, 4.25.

2,2′-Dithiodibenzaldehyde A mixture of pyridine chlorochromate (13 g, 60 mmol) in dry dichloromethane (60 ml) under nitrogen atmosphere was added dropwise to the mixture of (2-mercapto-phenyl)-methanol (3.37 g, 24 mmol) in dry dichloromethane (50 ml). After stirring at room temperature for 4 h, the reaction mixture was filtered. The residue was washed with dichloromethane (3×30 ml) and ether (30 ml). The filtrate and washings were combined, and then concentrated to give the solid product. Pure compound was obtained by crystallization with ethanol (3.1 g, 47%), mp: 145—148 °C (lit[14] mp 145 °C).

Schiff Base Formation, General Procedure The substituted anilines and anhydrous magnesium sulfate were added under a nitrogen atmosphere to a stirred solution of sulfur-containing aryl-aldehyde in absolute ethanol. The reaction mixture was heated under reflux for 4 or 14 h and then filtered. The residue was washed with absolute ethanol. The combined solution of filtrate and washings were combined, concentrated and then purified by chromatography (silica gel, *n*-hexane/ethyl acetate=15/1 or 5/1) to give the corresponding products.

[*N*-2-(Phenylthio)benzylidene]aniline (**2a**): Yield: 40%. *Rf* 0.5 (*n*-hexane/ethyl acetate=7/1). ^{1}H-NMR ($CDCl_3$) δ: 7.24—7.53 (m, 13H, ArH), 8.39 (d, 1H, J=7.4 Hz, ArH), 9.16 (s, 1H, CNH). ^{13}C-NMR ($CDCl_3$) δ: 121.80, 126.85, 127.62, 128.78, 128.92, 129.26, 129.42, 130.10, 130.42, 130.53, 130.74, 131.01, 132.30, 134.43, 137.01, 137.35, 152,68, 159.27. IR (KBr) cm^{-1}: 1618 (C=N). UV λ_{max} (EtOH) nm (log ε): 289 (3.90). FAB-MS *m/z*: 290 $(MH)^+$. *Anal.* Calcd for $C_{19}H_{15}NS$: C, 78.86; H, 5.22; N, 4.84. Found: C, 78.60; H, 5.37; N, 4.82.

4-Methoxyl-[*N*-(2-phenylthio)benylidene]aniline (**2b**): Yield: 38%. *Rf* 0.47 (*n*-hexane/ethyl acetate=7/1). ^{1}H-NMR ($CDCl_3$) δ: 3.82 (s, 3H, CH_3), 6.93—6.97 (q, 2H, ArH), 7.20—7.47 (m, 10H, ArH), 8.33 (d, 1H, J=7.4 Hz, ArH), 9.10 (s, 1H, CHN), ^{13}C-NMR ($CDCl_3$) δ: 56.07, 115.02, 123.09, 127.45, 128.88, 129.07, 123.0, 130.52, 131.89, 134.45, 136.81, 157.09. IR (KBr) cm^{-1}: 1618 (C=N). UV λ_{max} (EtOH) nm (log ε): 289 (3.91). FAB-MS *m/z*: 320 $(MH)^+$. *Anal.* Calcd for $C_{20}H_{17}NOS$: C, 75.20; H, 5.36; N, 4.39. Found: C, 75.06; H, 5.46; N, 4.28.

2,4-Dichloro-[*N*-(2-phenylthio)benylidene]aniline (**2c**): Yield: 42%. *Rf* 0.37 (*n*-hexane/ethyl acetate=13/1). ^{1}H-NMR ($CDCl_3$) δ: 6.69 (d, 2H, J=8.5 Hz, ArH), 7.12 (dd, 2H, J=8.4, 2.2 Hz, ArH), 7.28—7.38 (m, 4H, ArH), 7.44—7.46 (m, 3H, ArH), 8.35 (dd, 1H, J=6.2, 2.4 Hz, ArH), 8.98 (s, 1H, CHN). ^{13}C-NMR ($CDCl_3$) δ: 121.33, 127.66, 128.36, 128.95, 129.33, 129.86, 130.10, 130.27, 130.68, 131.79, 132.82, 134.35, 136.70, 136.86, 137.68, 148.60, 161.23. IR (KBr) cm^{-1}: 1618 (C=N). UV λ_{max} (EtOH) nm (log ε): 289 (3.82). FAB-MS *m/z*: 358 (M^+). *Anal.* Calcd for $C_{19}H_{13}NCl_2S$: C, 63.69; H, 3.66; N, 3.91. Found: C, 63.39; H, 3.51; N, 3.73.

2,2′-Di(phenylaminobenzylidene)sulfide (**4a**): Yield: 21%. *Rf* 0.4 (*n*-hexane/ethyl acetate=15/1). ^{1}H-NMR ($CDCl_3$) δ: 7.24—7.35 (m, 10H, ArH), 7.40—7.46 (m, 6H, ArH), 8.29 (dd, 2H, J=9.5, 2.5 Hz, ArH), 9.08 (s, 2H, CNH). ^{13}C-NMR ($CDCl_3$) δ: 121.71, 126.89, 128.51, 129.76, 129.81, 132.39, 136.68, 137.87, 152.48, 158.71. IR (KBr) cm^{-1}: 1618 (C=N). UV λ_{max} (EtOH) nm (log ε): 321 (3.91), 289 (3.94). MS: FAB-MS *m/z*: 393 $(MH)^+$. *Anal.* Calcd for $C_{19}H_{13}NCl_2S$: C, 79.56; H, 5.14; N, 7.14. Found: C, 79.43; H, 5.22; N, 7.01.

2,2′-Di(4-methoxylphenylaminobenzylidene)sulfide (**4b**): Yield: 35%. *Rf* 0.4 (*n*-hexane/ethyl acetate=15/1). mp: 91—92 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.82 (s, 6H, CH_3), 6.89 (d, 4H, J=8.9 Hz, ArH), 7.12—7.27 (m, 6H, ArH), 7.30—7.41 (m, 4H, ArH), 8.19 (dd, 2H, J=7.2, 1.9 Hz, ArH), 9.02 (s, 2H, CHN). ^{13}C-NMR ($CDCl_3$) δ: 56.05, 114.0, 122.88, 122.99, 128.20, 128.35, 129.28, 131.87, 131.92, 133.90, 133.25, 136.84, 137.49, 156.54. IR (KBr) cm^{-1}: 1618 (C=N). UV λ_{max} (EtOH) nm (log ε): 337 (4.28). MS: FAB-MS *m/z*: 453 $(MH)^+$. *Anal.* Calcd for $C_{28}H_{24}N_2S$: C, 74.31; H, 5.34; N, 6.19. Found: C, 74.07; H, 5.36; N, 6.12.

2,2′-Di-phenylaminobenzylidene Disulfide (**6a**): Yield: 50%. *Rf* 0.35 (*n*-hexane/ethyl acetate=5/1). ^{1}H-NMR ($CDCl_3$) δ: 7.24—7.44 (m, 7H, ArH), 7.83 (d, 2H, J=7.26 Hz, ArH), 7.96 (d, 2H, J=7.35 Hz, ArH), 8.86 (s, 2H, CHN). ^{13}C-NMR ($CDCl_3$) δ: 121.65, 121.74, 126.88, 127.91, 129.72, 130.29, 130.36, 130.78, 130.92, 131.76, 158.74. IR (KBr) cm^{-1}: 1625 (C=N). UV λ_{max} (EtOH) nm (log ε): 297 (3.78), 289 (3.78). MS: FAB-MS *m/z*: 423 (M^+). *Anal.* Calcd for $C_{28}H_{24}N_2S$: C, 73.55; H, 4.75; N, 6.60. Found: C, 73.44; H, 4.89; N, 6.43.

2,2′-Di(2,4-dichlorophenylaminobenzylidene)disulfide (**6c**): Yield: 52%. *Rf* 0.35 (*n*-hexane/ethyl acetate=3/1). mp: 132—134 °C. ^{1}H-NMR ($CDCl_3$)

δ: 6.95 (d, 3H, J=8.46 Hz, ArH), 7.21—7.47 (d, 3H, J=8.46 Hz, ArH), 7.78 (d, 2H, J=7.68 Hz, ArH), 7.95 (dd, 2H, J=7.41, 1.29 Hz, ArH), 8.73 (s, 1H, CHN), ^{13}C-NMR ($CDCl_3$) δ: 121.16, 128.21, 128.28, 130.33, 131.33, 132.37, 160.83. IR (KBr) cm^{-1}: 1624 (C=N). UV λ_{max} (EtOH) nm (log ε): 296 (4.00). MS: FAB-MS *m/z*: 560.9 (M+). *Anal.* Calcd for $C_{28}H_{24}N_2S$: C, 55.53; H, 2.87; N, 4.98. Found: C, 55.59; H, 3.03; N, 4.87.

Determination of the Scavenging Effect on 1,1-Diphenyl-2-pycryl-hydrazyl radical (DPPH·) The reactivity of title compounds with DPPH was determined from the change in absorbance at 517 nm, according to the method of Ohnishi *et al*.[10] For radical scavenging measurements, 2 ml of the compound solution under study was added to an ethanolic solution of DPPH· and then the change in absorbance was measured after 30 min. All tests were performed in triplicate.

Determination of Antioxidative Activity The antioxidative activity was evaluated by using 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH)-induced lipid peroxidation of a Tween-emulsified linoleic acid system and measured by the ferric thiocyanate assay as described.[10] Briefly, 0.2 ml of distilled water, 0.5 ml of 0.2 M phosphate buffer (pH 7.0), and 0.5 ml of 0.25% Tween-20 (in buffer solution) were mixed with 0.5 ml of 2.5% (w/v) linoleic acid in ethanol. The mixture was then stirred for 1 min. The peroxidation was initiated by the addition of 50 μl of AAPH solution (0.1 M). The stock solution of antioxidant or test compounds in DMSO (final concentrations for the test compounds and DMSO are 10^{-4} M and 0.1%, respectively) was then added, and the reaction was carried out at 37 °C for 375 min in the dark. The degree of inhibition of oxidation was measured by the ferric thiocyanate method for each interval of 75, 150, 225, 300, and 375 min. To 0.1 ml of peroxidation reaction mixture at each interval, 0.1 ml of 30% ammonium thiocyanate and 0.1 ml of 2×10^{-2} M freshly prepared $FeCl_2$ (in 3.5% aqueous HCl) were added. Precisely 3 min after addition, the absorbance of the red complex $[Fe(SCN)]^{2+}$ was measured at 500 nm. The control for the assay was prepared in the same manner by mixing all of the chemicals and reagents except the test compound. All tests were performed in triplicate.

Cell Culture Human cervical carcinoma (HeLa) cells were cultured at 37 °C in DMEM medium supplemented with 15 mM Hepes, 26 mM sodium bicarbonate, 2 mM L-glutamine, 100 μg/ml streptomycin, 100 unit/ml penicillin, and 10% fetal bovine serum. Cell number was determined by counting the viable cells by trypan blue dye exclusion using a hemocytometer. Cells in the logarithmic growth phases were used in this study.

Irradiation Conditions and Radiation Protection Assays Cells were irradiated at room temperature with a ^{137}Cs source at a dose rate of 300 cGy/min. Radiation doses ranged from 0 to 10 Gy. Radiation protection assays were carried out by the evaluation of cell survival after irradiation. Human HeLa cells were trypsinized from exponentially growing monolayers of stock cultures and cell counts were determined using a hemocytometer. Cells were washed with PBS and adjusted to achieve a concentration of 1×10^6 cells/ml of medium. Cells exposed to 0.1 mM of test compounds were treated at 37 °C for 30 min prior to irradiation. Immediately following irradiation, the cells were washed free of drug with PBS, and along with the untreated control groups were counted diluted, and seeded in triplicate into 60-mm-diameter dishes containing 3 ml of standard growth medium. The cells were allowed to grow for 7 d and cell survival and proliferation were analyzed using a Cell Counting Kit-8 (CCK-8, Dojin East, Tokyo, Japan). Cell survival was expressed as the ratio of absorbance of the drug-treated cells to that of the untreated cells. Trolox and amifostine (WR2721) were used as standard compounds for assessing the efficacy of the synthetic test compounds. All assays were performed in triplicate.

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