Original Article

Preparation and antioxidant/pro-oxidant activities of 3-monosubstituted 5-hydroxyoxindole derivatives

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Antioxidant treatments have been expected to be a novel therapeutics for various oxidative stress-mediated disorders. Our previous study revealed that 5-hydroxyoxindole and its 3-phenacyl-3-hydroxy derivatives showed excellent antioxidant activities such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and lipid-peroxidation inhibitory activity. However, the DPPH radical scavenging activity of the 3,3-disubstituted derivatives was lower than that of the original 5-hydroxyoxindole. In the present study, we synthesized novel 3-monosubstituted 5-hydroxyoxindole derivatives that exhibited stronger DPPH radical scavenging activities and lipid peroxidation-inhibitory activities than the 3,3-disubstituted 5-hydroxyoxindoles. Moreover, the 3-monosubstituted 5-hydroxyoxindole derivatives showed neither an iron-mediated pro-oxidant effect nor a remarkable cytotoxicity against HL-60 cell lines except some of the highly lipophilic compounds. These results indicate that 3-monosubstituted 5-hydroxyoxindoles can be used as a promising antioxidant scaffold for drug discovery.

Key Words: **antioxidant, radical scavenging activity, lipid peroxidation, pro-oxidant effect, 5-hydroxyoxindole**

Reactive oxygen species (ROS) including superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\bullet}$), singlet oxygen ($^1O_2$), peroxyl radicals ($ROO^{\bullet}$) and peroxynitrite ($ONOO^-$) are constantly formed in the environment and in living organism from the abundant triplet oxygen molecule ($^3O_2$). ROS are necessary for maintaining biological homeostasis, whereas excessive ROS causes oxidative stress and subsequent unfavorable biological events. Oxidative stress that is mediated by ROS and free radicals is involved in various intractable diseases such as cancer,[1] Parkinson's disease,[2] Alzheimer's disease,[3] amyotrophic lateral sclerosis (ALS),[4] multiple sclerosis[5] and diabetes mellitus.[6] A number of naturally occurring or synthetic antioxidants are considered to be novel therapeutic candidates for such diseases.[7,8] Indeed, recently, edaravone (5-methyl-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one), a clinically approved free radical scavenger for brain ischemic stroke, was recently approved in Japan as an antioxidative drug for the progression of motor dysfunction in ALS.[9] However, there are no other examples of success as an antioxidative medicine.[10] To accelerate the development of therapeutics for neurodegenerative diseases and other intractable diseases, identification of a lead-compound for clinically relevant antioxidant is greatly needed.

Uric acid is an endogenous antioxidant and has been suggested as a neuroprotective agent.[11–13] However, uric acid has poor solubility in serum, and therefore, provokes gouty arthritis, which makes it difficult to apply to therapeutic agents. In our previous study, 5-hydroxyoxindole (**1**, Fig. 1) was designed as a structural analog for uric acid for its antioxidant activity.[14] Compound **1** exhibited a good antioxidant profile, such as a stronger 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and a stronger lipid peroxidation-inhibitory activity than uric acid.[14] Compound **1** showed greater solubility in phosphate-buffered saline than uric acid,[14] which makes it a good candidate as a lead compound for drug discovery. Furthermore, 3,5-dihydroxy-3-phenacyl-2-oxindole (**2a**, Fig. 1), 3,5-dihydroxy-3-(4'-methylphenacyl)-2-oxindole (**2b**, Fig. 1), and the **2b** analogs that have OCH_3, CF_3, F, Cl, Br and I on the 4'-position of the phenacyl ring exhibited more potent lipid peroxidation-inhibitory activity than **1**.[15] The inhibitory effects of these derivatives tended to correlate with their own lipophilicity. Because the 5-hydroxyoxindole skeleton had a low molecular weight, good cell tolerability,[14,15] suitable solubility[14] and one can introduce

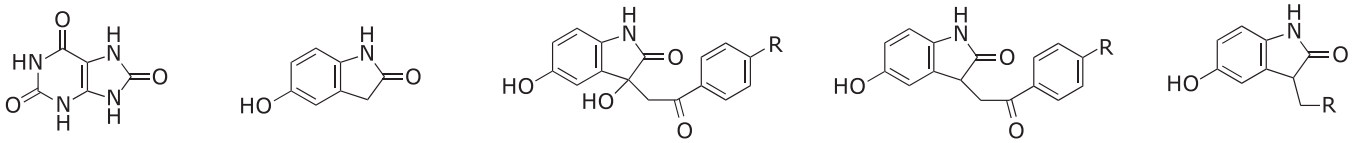

Fig. 1. Structures of uric acid, 5-hydroxyoxindole (**1**) and its 3-substituted derivatives.

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substituents to the C-3 position easily, **1** may be an excellent scaffold for a novel antioxidants with drug-like characteristics. However, because the derivatives **2a** and **2b** showed lower DPPH scavenging activity than the original **1**,[15] a modification that maintains the fundamental radical scavenging activity of **1** is desired. In our previous study, because 3-substituted-3-hydroxyoxindole is a common structure for naturally occurring compounds,[16] and because the synthetic route of 3-hydroxy-3-phenacyloxindole has been established,[17–19] 3,3-disubstituted derivatives were the focused. However, 3,3-disbsutitution disables to tautomerize between the C-2 and C-3 position of the oxindole, which changes the chemical characteristics from those of **1**. Furthermore, the 3-hydroxy group of oxindoles seemed to be unnecessary to increase both of the antioxidant activity and lipophilicity.

In the present study, to evaluate the effect of the 3-hydroxy substituent on the antioxidant activities and to adjust the lipophilicity using simpler substituents, 3-(4'-alkylphenacyl)-5-hydroxyoxindole derivatives **3a**–**e** and 3-alkyl-5-hydroxyoxindole derivatives **4a**–**c** were newly designed and synthesized. Additionally, for comparison, 3-(4'-alkylphenacyl)-3,5-dihydroxyoxindole derivatives **2c**–**e** were also newly synthesized. The antioxidant activities of all the compounds were evaluated compared with uric acid, **1**, **2a** and **2b**.

Materials and Methods

Generals. ^{1}H NMR spectra (500 MHz) were measured on a Varian 500 FT-NMR (Varian Medical Systems, Palo Alto, CA) with tetramethylsilane as an internal standard ($\delta = 0.00$) in CD_3OD or DMSO-d_6. ^{13}C NMR spectra (125 MHz) were obtained on the same spectrometer and the chemical shifts were referenced to the signals of CD_3OD ($\delta = 49.0$) or DMSO-d_6 ($\delta = 39.5$). ^{1}H NMR spectra (600 MHz) were measured on a JEOL JNM-ECP600 FT-NMR (JEOL, Tokyo, Japan) with tetramethylsilane as an internal standard ($\delta = 0.00$) in CD_3OD or DMSO-d_6. ^{13}C NMR spectra (150 MHz) were obtained on the same spectrometer and the chemical shifts were referenced to the signals of CD_3OD ($\delta = 49.0$) or DMSO-d_6 ($\delta = 39.5$). Mass spectra were recorded on a JEOL JMS-700 mass spectrometer (FAB-MS) or JEOL JMS-T100LP AccuTOF LC-plus 4G mass spectrometer (ESI-MS). Open column chromatography was performed using Merck Silica gel 60 (Darmstadt, Germany). Medium pressure preparative liquid chromatography (MPLC) was performed with a Yamazen AI-580S equipped with a Universal Column (26 × 124 mm, silica gel, 40 μm, 60 Å) (Yamazen, Tokyo, Japan). 5-Hydroxyoxindole (**1**) was purchased from Apin Chemical Co. (Oxon, UK). Ascorbic acid, hydrobromic acid, 5-methoxyisatin, diethylamine, 1,1-diphenyl-2-picrylhydrazyl, 3,5-di(*tert*-butyl)-4-hydroxytoluene and uric acid were purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan) Ethanol was purchased from Wako Pure Chem. Ind., Ltd. (Osaka, Japan). Heat-inactivated fatal bovine serum was purchased from Thermo Fisher Scientific Inc. (Waltham, MA) Dimethyl sulfoxide (DMSO), 5-methyl-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one (edaravone), 4-morpholino-ethanesulfonic acid, penicilline-streptomycin solution and RPMI-1640 medium were purchased from Sigma-Aldrich Co. (Milwaukee, WI). Iron(III) chloride was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan).

Chemistry.

Synthesis of 3,5-dihydroxy-3-phenacyl-2-oxindoles, general procedure. 5-Hydroxyisatin (200 mg, 1.22 mmol) was dissolved into methanol (20 ml) followed by addition of corresponding *para*-substituted acetophenone (5.0 equiv.) and diethylamine (446 mg, 5.2 equiv.). The reaction mixture was stirred at room temperature for 1 h. Then methanol and diethylamine were evaporated under reduced pressure. The dark-colored crude product was purified with silica-gel column chromatography (*n*-hexane/ethyl acetate = 2:1–2:3).

*3-(4'-Ethylphenacyl)-3,5-dihydroxy-2-oxindole (**2c**).* The light-yellow solid (150 mg, 35% yield) was obtained. Recrystallization from ethyl acetate yielded the yellow plates. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 1.18 (*t*, 3H, $J = 7.6$ Hz, -$CH_2C\underline{H}_3$), 2.66 (*q*, 2H, $J = 7.6$ Hz, -$C\underline{H}_2CH_3$), 3.47 (*d*, 1H, $J = 17.4$ Hz, -CH_2CO-), 3.92 (*d*, 1H, $J = 17.4$ Hz, -CH_2CO-), 5.96 (*s*, 1H, -OH), 6.55 (*dd*, 1H, $J = 8.3$, 2.4 Hz, H-6), 6.60 (*d*, 1H, $J = 8.3$ Hz, H-7), 6.71 (*d*, 1H, $J = 2.4$ Hz, H-4), 7.33 (*d*, 2H, $J = 8.2$ Hz, -$C_6\underline{H}_4$Et), 7.81 (*d*, 2H, $J = 8.2$ Hz, -$C_6\underline{H}_4$Et), 8.85 (*s*, 1H), 9.95 (*s*, 1H). ^{13}C NMR (CD_3OD, 150 MHz) δ 15.11, 28.11, 45.56, 73.42, 109.64, 111.75, 141.54, 128.02, 128.09, 132.77, 134.07, 134.55, 149.72, 152.17, 178.13, 195.84. FAB-HRMS: calcd. for $C_{18}H_{17}NO_4$ 311.1158, found 311.1123.

*3-(4'-Propylphenacyl)-3,5-dihydroxy-2-oxindole (**2d**).* The light-yellow solid (120 mg, 30% yield) was obtained. Recrystallization from ethyl acetate yielded the brown plates. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 0.88 (*d*, 6H, $J = 7.4$ Hz, -$CH_2CH_2C\underline{H}_3$), 1.59 (*tq*, 1H, $J = 7.5$, 7.4 Hz, -$CH_2C\underline{H}_2CH_3$), 2.61 (*t*, 2H, $J = 7.5$ Hz, -$C\underline{H}_2CH_2CH_3$), 3.47 (*d*, 1H, $J = 17.4$ Hz, -CH_2CO-), 3.92 (*d*, 1H, $J = 17.4$ Hz, -CH_2CO-), 5.96 (*s*, 1H, -OH), 6.54 (*dd*, 1H, $J = 8.3$, 2.4 Hz, H-6), 6.59 (*d*, 1H, $J = 8.3$ Hz, H-7), 6.71 (*d*, 1H, $J = 2.4$ Hz, H-4), 7.31 (*d*, 2H, $J = 8.4$ Hz, -$C_6\underline{H}_4$Pr), 7.80 (*d*, 2H, $J = 8.0$ Hz, -$C_6\underline{H}_4$Pr), 8.85 (*s*, 1H, -OH), 9.95 (*s*, 1H, -NH-). ^{13}C NMR (DMSO-d_6, 150 MHz) δ 13.51, 23.64, 37.06, 45.56, 73.41, 109.63, 111.75, 114.54, 127.99, 128.59, 132.78, 134.08, 134.56, 148.14, 152.18, 178.14, 195.84. FAB-HRMS: calcd. for $C_{19}H_{19}NO_4$ 325.1314, found 325.1339.

*3-(4'-Isopropylphenacyl)-3,5-dihydroxy-2-oxindole (**2e**).* The light-yellow solid (119 mg, 30% yield) was obtained. Recrystallization from ethyl acetate yielded the yellow plates. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 1.20 (*d*, 6H, $J = 6.9$ Hz, -$CH(C\underline{H}_3)_2$), 2.94 (*sept*, 1H, $J = 6.9$ Hz, -$C\underline{H}(CH_3)_2$), 3.47 (*d*, 1H, $J = 17.4$ Hz, -CH_2CO-), 3.92 (*d*, 1H, $J = 17.4$ Hz, -CH_2CO-), 5.96 (*s*, 1H), -OH, 6.54 (*dd*, 1H, $J = 8.2$, 2.4 Hz, H-6), 6.59 (*d*, 1H, $J = 8.2$ Hz, H-7), 6.70 (*d*, 1H, $J = 2.4$ Hz, H-4), 7.36 (*d*, 2H, $J = 8.4$ Hz, -$C_6\underline{H}_4$*i*-Pr), 7.82 (*d*, 2H, $J = 8.4$ Hz, -$C_6\underline{H}_4$*i*-Pr), 8.85 (*s*, 1H, -OH), 9.95 (*s*, 1H, -NH-). ^{13}C NMR (DMSO-d_6, 150 MHz) δ 23.43, 33.66, 45.51, 73.42, 109.65, 111.72, 114.55, 126.60, 128.14, 132.78, 134.23, 134.57, 152.18, 154.21, 178.14, 195.84. FAB-HRMS: calcd. for $C_{19}H_{19}NO_4$ 325.1314, found 325.1297.

Synthesis of 3-hydroxy-5-methoxy-3-phenacyl-2-oxindoles, general procedure. 5-Methoxyisatin (1.00 g, 5.65 mmol) was dissolved into methanol (50 ml) followed by addition of corresponding acetophenone (2.4 equiv.) and diethylamine (5.4 equiv.). The reaction mixture was stirred at room temperature for 48 h. Then methanol and diethylamine were evaporated under reduced pressure. The dark-colored crude product was purified with silica-gel column chromatography (*n*-hexane/ethyl acetate = 1:2–2:3).

*3-Hydroxy-5-methoxy-3-phenacyl-2-oxindole (**6a**).* The yellow-brown solid (993 mg, 60% yield) was obtained. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 3.53 (*d*, 1H, $J = 17.4$ Hz, -CH_2CO-), 3.74 (*s*, 3H, -$OC\underline{H}_3$), 3.80 (*d*, 1H, $J = 17.4$ Hz, -CH_2CO-), 6.78 (*dd*, 1H, $J = 8.4$, 2.4 Hz, H-6), 6.81 (*d*, 1H, $J = 8.4$ Hz, H-7), 7.01 (*d*, 1H, $J = 2.4$ Hz, H-4), 7.45 (*dd*, 1H, $J = 8.1$, 7.5 Hz, -C_6H_5) 7.58 (*dd*, 1H, $J = 7.5$, 7.4 Hz, -C_6H_5), 7.80 (*brs*, 1H), 7.91 (*d*, 2H, $J = 7.4$ Hz, -C_6H_5). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 44.54, 55.53, 75.55, 110.98, 111.63, 114.85, 128.38, 128.90, 131.74, 133.92, 134.09, 136.50, 156.33, 177.98, 198.61. FAB-HRMS: calcd. for $C_{17}H_{15}NO_4$ 297.1001, found 297.0984.

*3-Hydroxy-5-methoxy-3-(4'-methylphenacyl)-2-oxindole (**6b**).* The yellow-brown solid (1.26 g, 72% yield) was obtained. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.39 (*s*, 3H, -$C_6H_4C\underline{H}_3$), 3.48 (*d*, 1H, $J = 17.3$ Hz, -CH_2CO-), 3.72 (*s*, 3H, -OCH_3), 3.76 (*d*, 1H, $J = 17.3$ Hz, -CH_2CO-), 4.75 (*brs*, 1H, -OH), 6.77 (*dd*, 1H, $J = 8.5$, 2.5 Hz, H-6), 6.80 (*d*, 1H, $J = 8.5$ Hz, H-7), 7.06 (*d*, 1H, $J = 2.5$ Hz, H-4), 7.24 (*d*, 2H, $J = 7.9$ Hz, -$C_6\underline{H}_4CH_3$), 7.80 (*d*, 2H, $J = 8.2$ Hz, -$C_6\underline{H}_4CH_3$), 8.05 (*brs*, 1H, -NH-). ^{13}C NMR ($CDCl_3$,

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125 MHz) δ 21.86, 44.25, 55.92, 75.45, 111.01, 111.60, 114.79, 128.51, 129.57, 131.89, 133.94, 134.08, 145.12, 156.29, 178.22, 198.36. FAB-HRMS: calcd. for $C_{18}H_{17}NO_4$ 311.1158, found 311.1134.

*3-(4'-Ethylphenacyl)-3-hydroxy-5-methoxy-2-oxindole (**6c**).* The yellow-brown solid (1.03 g, 57% yield) was obtained. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 1.17 (*t*, 3H, *J* = 7.6 Hz, -$CH_2C\underline{H}_3$), 2.65 (*q*, 2H, *J* = 7.6 Hz, -$C\underline{H}_2CH_3$), 3.53 (*d*, 1H, *J* = 17.4 Hz, -CH_2CO-), 3.63 (*s*, 3H, -OCH_3), 4.02 (*d*, 1H, *J* = 17.4 Hz, -CH_2CO-), 6.03 (*s*, 1H, -OH), 6.71–6.72 (*m*, 2H, H-6 and H-7), 6.94 (*d*, 1H, *J* = 2.3 Hz, H-4), 7.33 (*d*, 2H, *J* = 8.3 Hz, -$C_6\underline{H}_4$Et), 7.81 (*d*, 2H, *J* = 8.3 Hz, -$C_6\underline{H}_4$Et), 10.07 (*s*, 1H, -NH-). ^{13}C NMR (CD_3OD, 150 MHz) δ 15.12, 28.11, 45.89, 55.33, 73.41, 109.56, 110.90, 113.24, 128.01, 128.10, 132.97, 134.03, 136.09, 149.75, 154.52, 178.22, 195.89. FAB-HRMS: calcd. for $C_{19}H_{19}NO_4$ 325.1314, found 325.1313.

*3-Hydroxy-5-methoxy-3-(4'-propylphenacyl)-2-oxindole (**6d**).* The yellow-brown solid (1.03 g, 52% yield) was obtained. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 0.87 (*t*, 3H, *J* = 7.4 Hz, -$CH_2CH_2C\underline{H}_3$), 1.59 (*tq*, 2H, *J* = 7.5, 7.4 Hz, -$CH_2C\underline{H}_2CH_3$), 2.60 (*t*, 2H, *J* = 7.4 Hz, -$C\underline{H}_2CH_2CH_3$), 3.53 (*d*, 1H, *J* = 17.4 Hz, -CH_2CO-), 3.63 (*s*, 3H, -OCH_3), 4.03 (*d*, 1H, *J* = 17.4 Hz, -CH_2CO-), 6.03 (*s*, 1H, -OH), 6.69–6.71 (*m*, 2H, H-6 and H-7), 6.94 (*d*, 1H, *J* = 2.1 Hz, H-4), 7.31 (*d*, 2H, *J* = 8.3 Hz, -$C_6\underline{H}_4$Pr), 7.80 (*d*, 2H, *J* = 8.3 Hz, -$C_6\underline{H}_4$Pr), 10.06 (*s*, 1H, -NH-). ^{13}C NMR (DMSO-d_6, 150 MHz) δ 13.49, 23.64, 37.01, 45.58, 55.33, 73.41, 109.55, 110.90, 113.23, 128.00, 128.58, 132.96, 134.04, 136.09, 148.15, 154.51, 178.22, 195.89. FAB-HRMS: calcd. for $C_{20}H_{21}NO_4$ 339.1471, found 339.1448.

*3-Hydroxy-3-(4'-isopropylphenacyl)-5-methoxy-2-oxindole (**6e**).* The yellow-brown solid (1.03 g, 54% yield) was obtained. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 1.20 (*d*, 6H, *J* = 6.9 Hz, -$CH(C\underline{H}_3)_2$), 2.94 (*sept*, 1H, *J* = 6.9 Hz, -$C\underline{H}(CH_3)_2$), 3.53 (*d*, 1H, *J* = 17.4 Hz, -CH_2CO-), 3.63 (*s*, 3H, -OCH_3), 4.03 (*d*, 1H, *J* = 17.4 Hz, -CH_2CO-), 6.03 (*s*, 1H, -OH), 6.70–6.73 (*m*, 2H, H-6 and H-7), 6.94 (*d*, 1H, *J* = 1.8 Hz, H-4), 7.36 (*d*, 2H, *J* = 8.4 Hz, -$C_6\underline{H}_4$*i*-Pr), 7.82 (*d*, 2H, *J* = 8.4 Hz, -$C_6\underline{H}_4$*i*-Pr), 10.07 (*s*, 1H, -NH-). ^{13}C NMR (DMSO-d_6, 150 MHz) δ 23.41, 33.45, 45.59, 55.33, 73.42, 109.57, 110.90, 113.25, 126.58, 128.14, 132.97, 134.19, 136.11, 154.23, 154.53, 178.23, 195.89. FAB-HRMS: calcd. for $C_{20}H_{21}NO_4$ 339.1471, found 339.1454.

Synthesis of 5-methoxy-3-phenacylidene-2-oxindoles, general procedure. 3-Hydroxy-5-methoxy-3-phenacyl-2-oxindoles (**6a**–**e**, 600 mg) was dissolved into a mixture of glacial acetic acid (34 ml) and conc. hydrochloric acid (1 ml) and then stirred at 95°C for 30 min. Then cooled down to room temperature, the reaction mixture diluted with water (70 ml) and extracted with ethyl acetate (×2). The combined organic layer was washed with brine (×2) and dried with sodium sulfate. The solvent was evaporated under reduced pressure.

*5-Methoxy-3-phenacylidene-2-oxindole (**7a**).* The dark-purple solid (564 mg, quantitative yield) was obtained. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 3.80 (*s*, 3H, -OCH_3), 6.79 (*d*, 1H, *J* = 8.4 Hz, H-7), 6.90 (*dd*, 1H, *J* = 8.4, 2.6 Hz, H-6), 7.53 (*dd*, 2H, *J* = 7.9. 7.6 Hz, -CO-Ph), 7.63 (*dd*, 1H, *J* = 7.4 Hz, -CO-Ph), 7.86 (*s*, 1H, -C=C$\underline{H}$-), 8.00 (*d*, 1H, *J* = 2.6 Hz, H-4), 8.11 (*dd*, 2H, *J* = 7.2 Hz, -CO-Ph), 8.25 (*s*, 1H, -NH-). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 56.03, 110.75, 113.45, 119.41, 121.50, 126.59, 128.96, 129.07, 133.97, 137.31, 137.52, 137.84, 155.82, 169.70, 191.20. ESI(–)-HRMS: calcd. for $C_{17}H_{12}NO_3$ 278.0817, found 278.0846.

*5-Methoxy-3-(4'-methylphenacylidene)-2-oxindole (**7b**).* The dark-purple solid (565 mg, quantitative yield) was obtained. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.44 (*s*, 3H, -$C_6H_4C\underline{H}_3$), 3.81 (*s*, 3H, -OCH_3), 6.74 (*d*, 1H, *J* = 8.5 Hz, H-7), 6.89 (*dd*, 1H, *J* = 8.5, 2.8 Hz, H-6), 7.33 (*d*, 2H, *J* = 8.3 Hz, -$C_6\underline{H}_4CH_3$), 7.85 (*s*, 1H, -C=C$\underline{H}$-), 7.99 (*d*, 1H, *J* = 2.8 Hz, H-4), 8.01 (*d*, 2H, *J* = 8.3 Hz, -$C_6\underline{H}_4CH_3$). ^{13}C NMR ($CDCl_3$, 600 MHz) δ 21.96, 56.05, 110.55, 113.42, 119.25, 121.60, 127.06, 129.13, 129.78, 135.39, 136.96, 137.00, 145.10, 155.82, 169.37, 190.81. ESI(–)-HRMS: calcd. for $C_{18}H_{14}NO_3$ 292.0974, found 292.1020.

*3-(4'-Ethylphenacylidene)-5-methoxy-2-oxindole (**7c**).* The dark-purple solid (566 mg, quantitative yield) was obtained. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.28 (*t*, 3H, *J* = 7.6 Hz, -$CH_2C\underline{H}_3$), 2.74 (*q*, 2H, *J* = 7.6 Hz, -$C\underline{H}_2CH_3$), 3.79 (*s*, 3H, -OCH_3), 6.79 (*d*, 1H, *J* = 8.4 Hz, H-7), 6.88 (*dd*, 2H, *J* = 8.4, 2.6 Hz, H-6), 7.35 (*d*, 2H, *J* = 8.4 Hz, -$C_6\underline{H}_4$Et), 7.85 (*s*, 1H, -C=C$\underline{H}$-), 7.96 (*d*, 1H, *J* = 2.6 Hz, H-4), 8.04 (*d*, 2H, *J* = 8.4 Hz, -$C_6\underline{H}_4$Et), 8.51 (*s*, 1H, -NH-). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 15.27, 29.21, 56.01, 110.78, 113.35, 119.19, 121.53, 127.06, 128.58, 129.23, 135.57, 137.17, 137.30, 151.23, 155.77, 169.96, 190.84. ESI(–)-HRMS: calcd. for $C_{19}H_{16}NO_3$ 306.1130, found 306.1202.

*5-Methoxy-3-(4'-propylphenacylidene)-2-oxindole (**7d**).* The dark-purple solid (557 mg, 98% yield) was obtained. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 0.96 (*t*, 3H, *J* = 7.4 Hz, -$CH_2C\underline{H}_3$), 1.68 (*tq*, 2H, *J* = 7.5, 7.4 Hz, -$C\underline{H}_2CH_3$), 2.67 (*t*, 2H, *J* = 7.5 Hz -$C\underline{H}_2CH_2CH_3$), 3.78 (*s*, 3H, -OCH_3), 6.79 (*d*, 1H, *J* = 8.5 Hz, H-7), 6.87, (*dd*, 1H, *J* = 8.5, 2.7 Hz, H-6), 7.32 (*d*, 2H, *J* = 8.3 Hz, -$C_6\underline{H}_4CH_3$), 7.85 (*s*, 1H, -C=C$\underline{H}$-) 7.96 (*d*, 1H, *J* = 2.7 Hz, H-4), 8.03 (*d*, 2H, *J* = 8.3 Hz, -$C_6\underline{H}_4CH_3$), 8.80 (*s*, 1H, -NH-). ^{13}C NMR ($CDCl_3$, 600 MHz) δ 13.88, 24.31, 38.26, 55.98, 110.84, 113.33, 119.16, 121.50, 127.00, 129.12, 129.15, 135.58, 137.25, 137.40, 149.72, 155.74, 170.13, 190.83. ESI(–)-HRMS: calcd. for $C_{20}H_{18}NO_3$ 320.1287, found 320.1381.

*3-(4'-Isopropylphenacylidene)-5-methoxy-2-oxindole (**7e**).* The dark-purple solid (532 mg, 94% yield) was obtained. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.29 (*d*, 6H, *J* = 7.0 Hz, -$CH(C\underline{H}_3)_2$), 3.00 (*sept*, 1H, *J* = 7.0 Hz, -$C\underline{H}(CH_3)_2$), 3.72 (*s*, 3H, -OCH_3), 6.79 (*d*, 1H, *J* = 8.5 Hz, H-7), 6.88 (*dd*, 1H, *J* = 8.5, 2.4 Hz, H-6), 7.38 (*dd*, 2H, *J* = 6.6, 1.8 Hz, -$C_6\underline{H}_4$*i*-Pr), 7.86 (*s*, 1H, -C=C$\underline{H}$-), 7.95 (*d*, 1H, *J* = 2.4 Hz, H-4), 8.05 (*dd*, 2H, *J* = 6.6, 1.8 Hz, -$C_6\underline{H}_4$*i*-Pr), 8.50 (*brs*, 1H, -NH-). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 23.76, 34.53, 56.01, 110.78, 113.32, 119.20, 121.53, 127.09, 127.18, 129.28, 135.70, 137.15, 137.29, 155.78, 169.96, 190.85. ESI(–)-HRMS: calcd. for $C_{20}H_{18}NO_3$ 320.1287, found 320.1376.

Synthesis of 5-methoxy-3-phenacyl-2-oxindoles, general procedure. 5-Methoxy-3-phenacylidene-2-oxindole (**7a**–**e**, 400 mg) was dissolved into ethanol (20 ml) and refluxed and then 10 w/v% sodium hydrosulfite *aq.* (10 ml) was added. The reaction mixture was stirred at 95°C for 30 min. Then cooled down to room temperature, ethanol was evaporated under reduced pressure. The remained water solution was extracted with ethyl acetate (×2). The combined organic layer was washed with brine (×2) and dried with sodium sulfate. The solvent was evaporated under reduced pressure.

*5-Methoxy-3-phenacyl-2-oxindole (**8a**).* The yellow solid (331 mg, 82% yield) was obtained. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 3.46 (*dd*, 1H, *J* = 18.2, 9.0 Hz, -CH_2CO-), 3.73 (*s*, 3H, -OCH_3), 3.82 (*dd*, 1H, *J* = 18.2, 3.0 Hz, -CH_2CO-), 4.08 (*d*, 1H, *J* = 9.0, 3.0 Hz, H-3), 6.74 (*dd*, 1H, *J* = 8.5, 2.4 Hz, H-6), 6.80 (*d*, 1H, *J* = 8.5 Hz, H-7), 6.86 (*d*, 1H, *J* = 2.4 Hz, H-4), 7.47 (*dd*, 2H, *J* = 8.1, 7.4 Hz, -CO-Ph), 7.59 (*dd*, 1H, *J* = 7.4 Hz, -CO-Ph), 7.84 (*brs*, 1H, -NH-), 7.99 (*d*, 2H, *J* = 8.4 Hz, -CO-Ph). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 40.09, 42.12, 55.93, 109.95, 112.12, 112.83, 128.31, 128.86, 131.19, 133.66, 134.81, 136.44, 155.97, 179.53, 196.96. ESI(–)-HRMS: calcd. for $C_{17}H_{14}NO_3$ 280.0974, found 280.1015.

*5-Methoxy-3-(4'-methylphenacyl)-2-oxindole (**8b**).* The yellow solid (360 mg, 89%) was obtained. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.41 (*s*, 3H, -$C_6H_4C\underline{H}_3$), 3.43 (*dd*, 1H, *J* = 18.1, 9.0 Hz, -CH_2CO-), 3.72 (*s*, 3H, -$OC\underline{H}_3$), 3.89 (*dd*, 1H, *J* = 18.1, 3.0 Hz, -CH_2CO-), 4.07 (*d*, 1H, *J* = 9.0, 3.0 Hz, H-3), 6.73 (*dd*, 1H, *J* = 8.4, 1.9 Hz, H-6), 6.80 (*d*, 1H, *J* = 8.4 Hz, H-7), 6.85 (*d*, 1H, *J* = 1.9 Hz, H-4), 7.27 (*d*, 2H, *J* = 8.2 Hz, -$C_6\underline{H}_4CH_3$), 7.82 (*brs*, 1H, -NH-), 7.88 (*d*, 2H, *J* = 8.2 Hz, -$C_6\underline{H}_4CH_3$).^{13}C NMR (CD_3OD, 125 MHz) δ 21.83, 39.97, 42.16, 55.92, 109.91, 112.11, 112.82, 128.43, 129.53, 131.29, 134.01, 134.79, 144.52, 155.94, 179.63, 196.54. ESI(–)-HRMS: calcd. for $C_{18}H_{16}NO_3$ 294.1130, found 294.1151.

*3-(4'-Ethylphenacyl)-5-methoxy-2-oxindole (**8c**).* The yellow solid (342 mg, 85%) was obtained. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.26 (*d*, 3H, *J* = 7.6 Hz, -$CH_2C\underline{H}_3$), 2.71 (*q*, 2H, *J* = 7.6 Hz, -$C\underline{H}_2CH_3$), 3.43 (*dd*, 1H, *J* = 18.1, 9.0 Hz, -CH_2CO-), 3.71 (*s*, 3H, -OCH_3), 3.80 (*dd*, 1H, *J* = 18.1, 3.0 Hz, -CH_2CO-), 4.08 (*d*, 1H, *J* = 9.0, 3.0 Hz, H-3), 6.73 (*dd*, 1H, *J* = 8.5, 2.5 Hz, H-6), 6.81 (*d*, 1H, *J* = 8.5 Hz, H-7), 6.85 (*d*, 1H, *J* = 2.0 Hz, H-4), 7.29 (*d*, 2H, *J* = 8.2 Hz, -$C_6\underline{H}_4$Et), 7.91 (*d*, 2H, *J* = 8.2 Hz, -$C_6\underline{H}_4$Et), 8.37 (*s*, 1H, -NH-). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 15.31, 29.12, 39.99, 42.24, 55.91, 110.05, 112.04, 112.84, 128.34, 128.54, 131.30, 134.23, 134.96, 150.68, 155.91, 180.04, 196.60. ESI(–)-HRMS: calcd. for $C_{19}H_{18}NO_3$ 308.1287, found 308.1355.

*5-Methoxy-3-(4'-propylphenacyl)-2-oxindole (**8d**).* The yellow solid (356 mg, 88%) was obtained. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 0.95 (*d*, 3H, *J* = 7.3 Hz, -$CH_2C\underline{H}_3$), 1.66 (*tq*, 2H, *J* = 7.5, 7.3 Hz, -$CH_2C\underline{H}_2CH_3$), 2.64 (*t*, 2H, *J* = 7.5 Hz, -$C\underline{H}_2CH_2CH_3$), 3.43 (*dd*, 1H, *J* = 18.2, 9.0 Hz, -CH_2CO-), 3.72 (*s*, 3H, -OCH_3), 3.80 (*dd*, 1H, *J* = 18.2, 3.0 Hz, -CH_2CO-), 4.08 (*d*, 1H, *J* = 9.0, 3.0 Hz, H-3), 6.73 (*dd*, 1H, *J* = 8.5, 2.6 Hz, H-6), 6.80 (*d*, 1H, *J* = 8.5 Hz, H-7), 6.85 (*d*, 1H, *J* = 2.0 Hz, H-4), 7.26 (*d*, 2H, *J* = 8.2 Hz, -$C_6\underline{H}_4$Pr), 7.91 (*d*, 2H, *J* = 8.2 Hz, -$C_6\underline{H}_4$Pr), 8.10 (*s*, 1H, -NH-). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 13.88, 24.33, 38.19, 39.99, 42.21, 55.92, 109.98, 112.07, 112.83, 128.45, 128.94, 131.30, 134.24, 134.88, 149.18, 155.93, 179.84, 196.60. ESI(–)-HRMS: calcd. for $C_{20}H_{20}NO_3$ 322.1443, found 322.1540.

*3-(4'-Isopropylphenacyl)-5-methoxy-2-oxindole (**8e**).* The yellow solid (357 mg, 89%) was obtained. ^{1}H NMR (CD_3OD, 600 MHz) δ 1.26 (*d*, 6H, *J* = 6.9 Hz, -CH($C\underline{H}_3$)$_2$), 2.97 (*sept*, 1H, *J* = 6.9 Hz, -$C\underline{H}$(CH_3)$_2$) 3.51 (*dd*, 1H, *J* = 18.2, 7.7 Hz, -CH_2CO-), 3.68 (*s*, 3H, -OCH_3), 3.82 (*dd*, 1H, *J* = 18.2, 3.6 Hz, -CH_2CO-), 3.91 (*d*, 1H, *J* = 7.7, 3.6 Hz, H-3), 6.75 (*dd*, 1H, *J* = 8.6, 2.4 Hz, H-6), 6.81–6.83 (*m*, 2H, H-4 and H-7), 7.36 (*d*, 2H, *J* = 8.2 Hz, -$C_6\underline{H}_4$*i*-Pr), 7.93 (*d*, 2H, *J* = 8.2 Hz, -$C_6\underline{H}_4$*i*-Pr). ^{13}C NMR (CD_3OD, 125 MHz) δ 24.00, 35.79, 40.11, 43.75, 56.24, 111.11, 112.20, 113.67, 127.86, 129.53, 132.67, 135.75, 137.08, 156.43, 157.23, 182.20, 198.66. ESI(–)-HRMS: calcd. for $C_{20}H_{20}NO_3$ 322.1443, found 322.1491.

Synthesis of 5-hydroxy-3-phenacyl-2-oxindoles, general procedure. 5-Methoxy-3-phenacyl-2-oxindole (**8a**–**e**, 200 mg) was dissolved into dehydrated dichloromethane (20 ml) and stirred at –10~0°C. Boron tribromide (17% solution in dichloromethane, 4 ml) was dropped into the reaction solution and stirred at –10~0°C for 3 h. Then water (20 ml) was added to the reaction solution and stirred at room temperature for 1 h. Organic solvent was concentrated under reduced pressure, and the remained water layer was extracted with ethyl acetate (×2). The combined organic layer was washed with brine (×2) and dried with sodium sulfate. The solvent was evaporated under reduced pressure and the crude product was purified with MPLC (*n*-hexane/ethyl acetate gradient).

*5-Hydroxy-3-phenacyl-2-oxindole (**3a**).* The light-yellow solid (138 mg, 73% yield) was obtained. Recrystallization from toluene yielded the yellow needles. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 3.51 (*dd*, 1H, *J* = 18.4, 7.4 Hz, -$C\underline{H}_2$CO-), 3.74 (*dd*, 1H, *J* = 18.4, 3.8 Hz -$C\underline{H}_2$CO-), 3.78 (*dd*, 1H, *J* = 7.4, 3.8 Hz, H-3), 6.54 (*dd*, 1H, *J* = 8.2, 2.2 Hz, H-6), 6.63–6.64 (*m*, 2H, H-4 and H-7), 7.54 (*dd*, 2H, *J* = 8.0, 7.7 Hz, -CO-Ph), 7.66 (*dd*, 1H, *J* = 7.5, 7.2 Hz, -CO-Ph), 8.00 (*dd*, 2H, *J* = 7.2 Hz, -CO-Ph), 8.84 (*s*, 1H, -NH-), 10.15 (*s*, 1H, -OH). ^{13}C NMR (DMSO-d_6, 150 MHz) δ 38.72, 41.62, 110.01, 111.87, 113.36, 128.00, 128.74, 130.98, 133.42, 134.67, 136.18, 152.21, 178.40, 197.38. ESI(–)-HRMS: calcd. for $C_{16}H_{12}NO_3$ 266.0817, found 266.0822.

*5-Hydroxy-3-(4'-methylphenacyl)-2-oxindole (**3b**).* The light-yellow solid (127 mg, 66% yield) was obtained. Recrystallization from toluene yielded the yellow plates. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 2.38 (*s*, 3H, -CH_3), 3.45 (*dd*, 1H, *J* = 18.1, 7.7 Hz, -$C\underline{H}_2$CO-), 3.69 (*dd*, 1H, *J* = 18.1, 3.5 Hz -$C\underline{H}_2$CO-), 3.77 (*dd*, 1H, *J* = 7.7, 3.5 Hz, H-3), 6.54 (*dd*, 1H, *J* = 8.3, 2.4 Hz, H-6), 6.61–6.63 (*m*, 2H, H-4 and H-7), 7.37 (*d*, 2H, *J* = 8.0. Hz, -$C_6\underline{H}_4CH_3$), 7.90 (*d*, 2H, *J* = 8.3 Hz, -$C_6\underline{H}_4CH_3$), 8.84 (*s*, 1H, -NH-), 10.14 (*s*, 1H, -OH). ^{13}C NMR (DMSO-d_6, 150 MHz) δ 21.13, 38.62, 41.64, 109.39, 111.86, 113.35, 128.12, 129.26, 131.00, 133.76, 134.65, 143.82, 152.21, 178.43, 196.84. ESI(–)-HRMS: calcd. for $C_{17}H_{14}NO_3$ 280.0974, found 280.0996.

*3-(4'-Ethylphenacyl)-5-hydroxy-2-oxindole (**3c**).* The light-yellow solid (147 mg, 77% yield) was obtained. Recrystallization from toluene yielded the yellow plates. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 1.20 (*t*, 3H, *J* = 7.7 Hz, -$CH_2C\underline{H}_3$), 2.68 (*q*, 2H, *J* = 7.7 Hz, -$C\underline{H}_2CH_3$) 3.45 (*dd*, 1H, *J* = 18.1, 7.7 Hz, -$C\underline{H}_2$CO-), 3.69 (*dd*, 1H, *J* = 18.1, 3.9 Hz -$C\underline{H}_2$CO-), 3.77 (*dd*, 1H, *J* = 7.7, 3.9 Hz, H-3), 6.54 (*dd*, 1H, *J* = 8.3, 2.2 Hz, H-6), 6.61–6.63 (*m*, 2H, H-4 and H-7), 7.37 (*d*, 2H, *J* = 8.3. Hz, -$C_6\underline{H}_4$Et), 7.92 (*d*, 2H, *J* = 8.3 Hz, -$C_6\underline{H}_4$Et), 8.83 (*s*, 1H, -NH-), 10.14 (*s*, 1H, -OH). ^{13}C NMR (DMSO-d_6, 150 MHz) δ 15.19, 28.13, 38.63, 41.62, 109.39, 111.84, 113.33, 128.10, 128.21, 131.01, 134.00, 134.63, 149.84, 152.19, 178.41, 196.87. ESI(–)-HRMS: calcd. for $C_{18}H_{16}NO_3$ 294.1130, found 294.1173.

*5-Hydroxy-3-(4'-propylphenacyl)-2-oxindole (**3d**).* The light-yellow solid (142 mg, 74% yield) was obtained. Recrystallization from toluene yielded the yellow plates. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 0.89 (*t*, 3H, *J* = 7.4 Hz, -$CH_2CH_2C\underline{H}_3$), 1.20 (*tq*, 2H, *J* = 7.5, 7.4 Hz, -$CH_2C\underline{H}_2CH_3$), 2.63 (*t*, 2H, *J* = 7.5 Hz, -$C\underline{H}_2CH_2CH_3$) 3.46 (*dd*, 1H, *J* = 18.4, 7.7 Hz, -$C\underline{H}_2$CO-), 3.70 (*dd*, 1H, *J* = 18.4, 3.6 Hz -$C\underline{H}_2$CO-), 3.77 (*dd*, 1H, *J* = 7.7, 3.6 Hz, H-3), 6.54 (*dd*, 1H, *J* = 8.2, 2.2 Hz, H-6), 6.61–6.63 (*m*, 2H, H-4 and H-7), 7.35 (*d*, 2H, *J* = 8.0. Hz, -$C_6\underline{H}_4$Pr), 7.92 (*d*, 2H, *J* = 8.0 Hz, -$C_6\underline{H}_4$Pr), 8.83 (*s*, 1H, -NH-), 10.14 (*s*, 1H, -OH). ^{13}C NMR (DMSO-d_6, 150 MHz) δ 13.54, 23.68, 37.09, 38.69, 41.63, 109.25, 111.82, 113.00, 128.13, 128.68, 129.04, 133.93, 134.02, 148.26, 152.16, 178.42, 196.87. ESI(–)-HRMS: calcd. for $C_{19}H_{18}NO_3$ 308.1287, found 308.1353.

*5-Hydroxy-3-(4'-isopropylphenacyl)-2-oxindole (**3e**).* The light-yellow solid (133 mg, 69% yield) was obtained. Recrystallization from toluene yielded the light-yellow plates. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 1.22 (*d*, 6H, *J* = 6.9 Hz, -CH($C\underline{H}_3$)$_2$), 2.97 (*sept*, 1H, *J* = 6.9 Hz, -$C\underline{H}$(CH_3)$_2$) 3.46 (*dd*, 1H, *J* = 18.4, 7.7 Hz, -$C\underline{H}_2$CO-), 3.70 (*dd*, 1H, *J* = 18.4, 3.5 Hz -$C\underline{H}_2$CO-), 3.78 (*dd*, 1H, *J* = 7.7, 3.5 Hz, H-3), 6.54 (*dd*, 1H, *J* = 8.2, 2.4 Hz, H-6), 6.61–6.64 (*m*, 2H, H-4 and H-7), 7.38 (*d*, 2H, *J* = 8.2 Hz, -$C_6\underline{H}_4$*i*-Pr), 7.93 (*d*, 2H, *J* = 8.2 Hz, -$C_6\underline{H}_4$*i*-Pr), 8.83 (*s*, 1H, -NH-), 10.14 (*s*, 1H, -OH). ^{13}C NMR (DMSO-d_6, 150 MHz) δ 23.46, 33.46, 38.64, 41.65, 109.40, 111.88, 113.33, 126.67, 128.27, 131.04, 134.16, 134.64, 152.16, 154.34, 178.43, 196.89. ESI(–)-HRMS: calcd. for $C_{19}H_{18}NO_3$ 308.1287, found 308.1355.

Synthesis of 3-alkylidene-5-hydroxyoxindoles and 3-benzylidene-5-hydroxyoxindole, general procedure. Commercially available 5-hydroxyoxindole (200 mg, 1.34 mmol) was dissolved into ethanol (10 ml), to which was added corresponding aldehyde (1.1 equiv.) and piperidine (3.8 equiv.). The reaction mixture was refluxed for 4 h. After the solvent was concentrated under reduced pressure, the residue was purified with MPLC (*n*-hexane/ethyl acetate gradient).

*5-Hydroxy-3-propylidene-2-oxindole (**9a**).* The yellow solid (143 mg, 56% yield) was obtained. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 1.16 (*t*, 3H, *J* = 7.5 Hz, -CH_3), 2.58 (*dq*, 3H, *J* = 7.6, 7.5 Hz, -CH_3), 6.62–6.65 (*m*, 1H, H-6 and H-7), 6.79 (*t*, 1H, *J* = 7.6 Hz, -C=C$\underline{H}$-), 7.00 (*d*, 1H, *J* = 1.4 Hz, H-4), 9.01 (*s*, 1H, -NH-), 10.12 (*s*, 1H, -OH). ^{13}C NMR (DMSO-d_6, 150 MHz) δ 12.86, 21.79, 110.04, 111.15, 115.19, 122.72, 128.27, 134.32, 141.67, 152.15, 168.03. ESI(–)-HRMS: calcd. for $C_{19}H_{18}NO_3$ 308.1287, found 308.1355. ESI(–)-HRMS: calcd. for $C_{11}H_{10}NO_2$ 188.0712, found 188.0674.

*3-Heptylidene-5-hydroxy-2-oxindole (**9b**).* The yellow solid (223 mg, 68% yield) was obtained. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 0.87 (*t*, 3H, *J* = 7.2 Hz, -CH_3), 1.29–1.39 (*m*, 6H), 1.56 (*tt*, 2H, *J* = 7.5 Hz, -CH_2-), 2.57 (*dt*, 2H, *J* = 7.7, 7.5 Hz, -C=CH-C$\underline{H}_2$-), 6.62 (*dd*, 1H, *J* = 8.3, 2.1 Hz, H-6), 6.64 (*d*, 1H, *J* = 8.3 Hz, H-7),

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6.72 (*t*, 1H, *J* = 7.7 Hz, -C=C<u>H</u>-), 7.01 (*d*, 1H, *J* = 1.7 Hz, H-4), 9.01 (*s*, 1H, -NH-), 10.11 (*s*, 1H, -OH). ^{13}C NMR (DMSO-d_6, 150 MHz) δ 13.91, 21.94, 28.03, 28.34, 28.60, 31.02, 109.93, 111.17, 115.18, 122.63, 128.65, 134.33, 140.34, 151.97, 168.02. ESI(–)-HRMS: calcd. for $C_{15}H_{18}NO_2$ 244.1338, found 244.1294.

*3-Benzylidene-5-hydroxy-2-oxindole (**9c**).* The yellow solid (262 mg, 82% yield) was obtained. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 6.66 (*dd*, 1H, *J* = 8.4, 2.2 Hz, H-7), 6.69 (*d*, 1H, *J* = 8.4 Hz, H-7), 7.04 (*d*, 1H, *J* = 2.2 Hz, H-4), 7.47–7.55 (*m*, 3H, -CHC$_6$<u>H</u>$_5$), 7.58 (*s*, 1H, -C=C<u>H</u>-), 7.68 (*d*, 2H, *J* = 7.6 Hz, -CHC$_6$<u>H</u>$_5$), 8.99 (*s*, 1H, -NH-), 10.29 (*s*, 1H, -OH). ^{13}C NMR (DMSO-d_6, 150 MHz) δ 109.91, 110.46, 116.74, 121.51, 128.43, 128.71, 129.12, 129.49, 134.46, 135.32, 135.34, 151.78, 168.60. ESI(–)-HRMS: calcd. for $C_{15}H_{10}NO_2$ 236.0712, found 236.0704.

Synthesis of 3-alkyl-5-hydroxyoxindoles and 3-benzyl-5-hydroxyoxindole, general procedure. Compound **9a**–**c** (150 mg) was dissolved into ethanol (10 ml) and palladium carbon (5%, 30 mg) was added into the solution. The reaction mixture was stirred under hydrogen atmosphere at room temperature for 24 h. The catalyst was removed by filtration through a Celite pad and the solvent was concentrated under reduced pressure. The crude product was purified with MPLC (*n*-hexane/ethyl acetate gradient).

*5-Hydroxy-3-propyl-2-oxindole (**4a**).* The yellow solid (84 mg, 56% yield) was obtained. Recrystallization from ethyl acetate yielded the light-yellow plats. ^{1}H NMR (CD_3OD, 600 MHz) δ 0.91 (*t*, 3H, *J* = 7.4 Hz, -CH$_2$CH$_2$C<u>H</u>$_3$), 1.21–1.39 (*m*, 2H, -CH$_2$C<u>H</u>$_2$CH$_3$), 1.80–1.95 (*m*, 2H, -C<u>H</u>$_2$CH$_2$CH$_3$), 3.41 (*t*, 1H, *J* = 5.7 Hz, H-3) 6.61–6.64 (*m*, 1H, H-6), 6.70 (*d*, 1H, *J* = 8.2 Hz, H-7), 6.73–6.75 (*m*, 1H, H-4). ^{13}C NMR (CD_3OD, 150 MHz) δ 14.34, 19.76, 33.53, 47.73, 111.14, 113.03, 114.89, 132.62, 135.86, 154.28, 182.43. ESI(–)-HRMS: calcd. for $C_{11}H_{12}NO_2$ 190.0869, found 190.0878.

*3-Heptyl-5-hydroxy-2-oxindole (**4b**).* The yellow solid (148 mg, 98% yield) was obtained. Recrystallization from ethyl acetate yielded the light-yellow plats. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 0.84 (*t*, 3H, *J* = 7.3 Hz, -CH$_3$), 1.14–1.38 (*m*, 10H, -(CH$_2$)$_5$-), 1.68–1.83 (*m*, 2H, -CH-C<u>H</u>$_2$-), 3.31 (*t*, 1H, *J* = 5.7 Hz, H-3), 6.55 (*dd*, 1H, *J* = 8.2, 2.4 Hz, H-6), 6.60 (*d*, 1H, *J* = 8.2 Hz, H-7), 6.67 (*d*, 1H, *J* = 1.4 Hz, H-4), 8.91 (*s*, 1H, -NH-), 10.02 (*s*, 1H, -OH). ^{13}C NMR (DMSO-d_6, 150 MHz) δ 13.92, 22.02, 25.05, 28.49, 28.94, 29.81, 31.17, 45.45, 109.38, 111.89, 113.35, 130.90, 134.52, 152.28, 178.61. ESI(–)-HRMS: calcd. for $C_{15}H_{20}NO_2$ 246.1494, found 246.1510.

*5-Hydroxy-3-benzyl-2-oxindole (**4c**).* The yellow solid (102 mg, 68% yield) was obtained. Recrystallization from ethyl acetate yielded the light-yellow plats. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 2.88 (*dd*, 1H, *J* = 13.9, 8.0 Hz, -CH$_2$-), 3.27 (*dd*, 1H, *J* = 13.9, 4.9 Hz, -CH$_2$-), 3.71 (*dd*, 1H, *J* = 8.0, 4.9 Hz, H-3), 6.32 (*s*, 1H, H-4), 6.49 (*d*, 1H, *J* = 8.2, 2.2 Hz, H-6), 6.52 (*d*, 1H, *J* = 8.2 Hz, H-7), 7.14–7.18 (*m*, 3H, -CH$_2$C$_6$<u>H</u>$_5$), 7.23 (*dd*, 2H, *J* = 7.3, 7.0 Hz, -CH$_2$C$_6$<u>H</u>$_5$), 8.83 (*s*, 1H, -NH-), 10.02 (*s*, 1H, -OH). ^{13}C NMR (DMSO-d_6, 150 MHz) δ 35.52, 46.97, 109.34, 112.42, 113.49, 126.24, 128.01, 129.17, 130.14, 134.37, 138.19, 151.97, 177.78. ESI(–)-HRMS: calcd. for $C_{15}H_{12}NO_2$ 238.0868, found 238.0853.

Measurement of DPPH radical scavenging activity. DPPH (12.5 μM) was dissolved into the mixed solution of 41.8 mM 4-morpholinoethanesulfonic acid (MES) buffer (pH 7.4) and ethanol (2/3, v/v). The test compounds (0.25, 0.50, 0.75 and 1.00 mM) were dissolved into the mixed solution of MES buffer (pH 7.4) and ethanol (3/2, v/v). The stock solution of the test compounds (200 μl) and DPPH (200 μl) were mixed automatically with a UNISOKU Rapid-Scan Stopped-Flow Spectroscopy System RSP-1000 and the decrease in the optical absorbance of DPPH at 517 nm was measured. The pseudo first-order rate constant was calculated from the obtained curve with a UNISOKU Spectroscopy and Kinetics software. The obtained pseudo-first order rate constants were plotted against the concentration of test compounds and the second-order rate constant was obtained from the slope of regression line.

Evaluation of lipid peroxidation-inhibitory activity. Rat liver microsomes derived from phenobarbital-treated SD male rats purchased from BIOPREDIC international was used. The incubation mixtures contained microsomes and the test compounds in a mixture of 690 μl of 0.1 M sodium phosphate buffer (pH 7.4, 0.1 mM EDTA) and 300 μl of ethanol were cooled in ice-water bath until lipid peroxidation initiation. *tert*-Butyl hydroperoxide (1 mM) in distilled water (10 μl) was added into the incubation mixture to initiate lipid peroxidation and the mixture was incubated at 37°C for 20 min. After incubation, peroxidation reaction was terminated by 100 μl of BHT in ethanol (2%). Two milliliters of trichloroacetic acid (15%) and thiobarbituric acid (0.38%) in 0.25 M hydrochloric acid were added into the incubation mixture and then incubated at 85°C for 20 min. The precipitate was removed by centrifugation (3,000 rpm, 20°C, 20 min) and the difference between the absorbance at 535 nm and 600 nm of supernatant was measured with a TECAN M200 to gain a level of thiobarbituric acid reactive substances (TBARS) formation.[20] Lipid peroxidation-inhibitory activity was expressed as a ratio of the TBARS formation of control group to that of tested group (*n* = 3).

Pro-oxidant effect. The freshly prepared mixture of ammonium acetate (45 g), acetylacetone (0.6 ml), glacial acetic acid (0.3 ml) in distilled water (300 ml) were used as a Nash's regent. The reaction mixture of DMSO (100 μl), antioxidant (100 μM), iron(III) chloride (500 μM) in 0.1 M sodium phosphate buffer (pH 7.40) was shaken at 37°C for 60 min. Next, the Nash's reagent (2 ml) was added to each sample solution and the mixture was shaken at 37°C for 60 min. The difference between the absorbance at 412 nm and 600 nm of the solution was measured with a TECAN M200 to gain a level of formaldehyde. Pro-oxidant effect was expressed as a ratio of the formaldehyde formation of control group to that of tested group (*n* = 3).

Cell culture. HL-60 cells were cultured in the RPMI-1640 medium supplemented with 5% heat-inactivated fatal bovine serum, 100 IU/ml penicillin and 100 μg/ml streptomycin at 37°C in a 5% CO_2 incubator with a humidified atmosphere.

Cytotoxicity assay. HL-60 cells were seeded on twelve-well multi-plates (1.0×10^6 cells/well, 2 ml/well) and the test compounds (50 μM) dissolved in DMSO (final concentration of DMSO was 1 v/v%) were added followed by incubation for 24 h at 37°C under 5% CO_2 and humidified atmosphere. After incubation, the cells were collected to centrifuging tube and centrifuged at 1,000 rpm for 5 min. The medium was replaced by the same volume of PBS(–) and the cells were re-suspended. The suspension was mixed with trypan blue dye and viable cells were counted by trypan blue dye-exclusion method with a Vi-CELL (Beckman Coulter Inc.). The cell viability was expressed relative to the vehicle control group (*n* = 3).

Results and Discussion

Synthesis. Newly designed 3,5-dihydroxy-3-phenacyl-2-oxindole derivatives (**2c**–**e**) were prepared from 5-hydroxyisatin (**5**) and the corresponding *para*-substituted acetophenones according to our previous method (Fig. 2).[15] The 5-hydroxy-3-phenacyl-2-oxindole derivatives (**3a**–**e**) were prepared from commercially available 5-methoxyisatin via 4 steps. Briefly, the aldol reaction of 5-methoxyisatin and the corresponding *para*-substituted acetophenone was followed by the dehydration under acidic conditions[17] gave **7a**–**e**. Then, the alkene moieties were reduced using sodium hydrosulfite.[17] Finally, the demethylation of the 5-methoxy group with boron tribromide afforded **3a**–**e** (Fig. 3). The 3-alkenyl derivatives (**9a**–**c**) were prepared by the aldol condensation between **1** and the corresponding aldehydes[21] and then the hydrogenation of the 3-alkenyl derivatives afforded 3-alkyl derivatives (**4a**–**c**, Fig. 4). All of the synthesized compounds that were used for the biological assays were recrystallized from a suitable solvent.

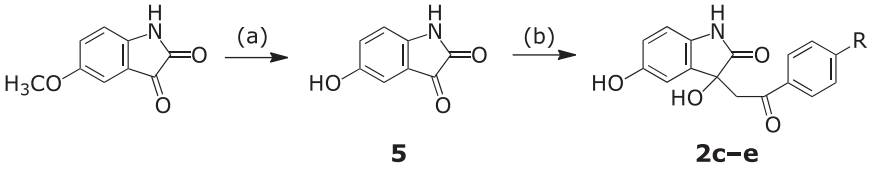

Fig. 2. Synthesis of 2c–e. (a) 47% HBr *aq*., 130°C, 3 h, (b) Corresponding acetophenones, $EtNH_2$, MeOH, rt, 1 h.

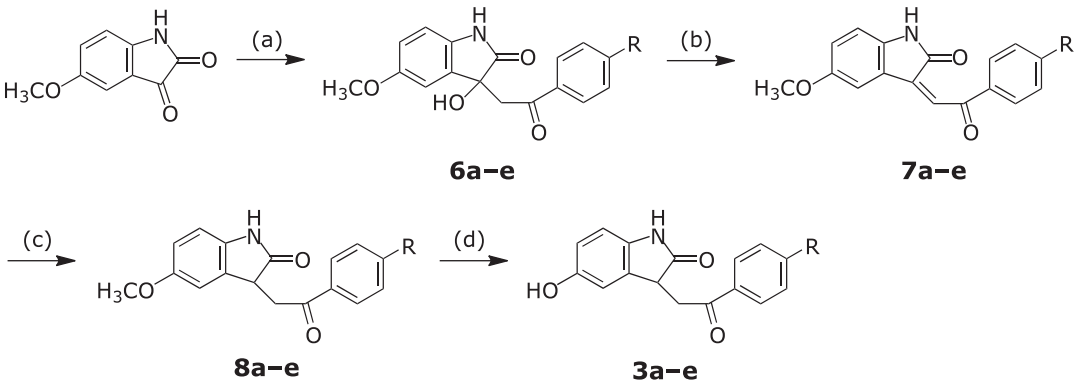

Fig. 3. Synthetic scheme of 3a–e. (a) Corresponding acetophenones, $EtNH_2$, MeOH, rt, 48 h, (b) glacial AcOH/conc. HCl = 34/1 mixture, 95°C, 30 min, (c) 10 w/v% $Na_2S_2O_4$ *aq*, 95°C, 30 min, (d) BBr_3, CH_2Cl_2, –10~0°C, 3 h.

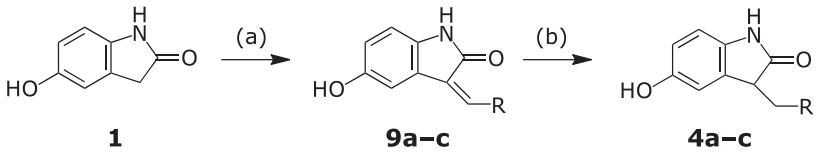

Fig. 4. Synthetic scheme of 4a–c. (a) Corresponding aldehydes, piperidine, EtOH, reflux, 4 h, (b) H_2, Pd-C, EtOH, 24 h.

DPPH radical scavenging activity. DPPH radical scavenging activity is a simple and versatile indicator for the reactivity of antioxidants with various free radicals. Here, the second-order rate constant of the antioxidants toward the DPPH radical under aqueous conditions was measured by stopped-flow techniques to determine the DPPH radical scavenging activity.

The DPPH radical scavenging activity of derivatives **2c**–**e** were stronger than that of uric acid, but they exhibited less than half of the activity of **1**. This result is consistent with our previous data[15] for the DPPH scavenging activity of **2a** and **2b** (Table 1). On the other hand, derivatives **3a**–**e** showed a stronger DPPH radical scavenging activity than **2a**–**e**. The activity of **3a**–**e** was comparable with the activity of the original **1**. The radical scavenging activity of **4a**–**c** was also greater than that of **2a**–**e**, but it was weaker than that of **1** and **3a**–**c**. The oxindole compounds **1**, **3a**–**e** and **4a**–**c** can tautomerize between the keto form and the enol form, whereas **2a**–**e** cannot (Fig. 5). Oxindoles generally exist as the keto form,[22–24] and indeed, the ^{1}H NMR spectra of the **3a**–**e** and **4a**–**c** supported that they entirely exist as the keto form in the solvent (see the experimental section). However, the C-3 position of **1**, **3a**–**e** and **4a**–**c** partially have the characteristics of an sp^2 carbon, and their electrical properties are obviously different from that of **2a**–**e**, which contain only sp^3 features at the C-3 carbon. Thus, it was suggested that the sp^2 characteristics of the C-3 position on 5-hydroxyoxindole was important to exert the radical scavenging activity.

Lipid peroxidation inhibitory activity. Lipids are abundant biological components and the main target of ROS and free radicals attacks. Therefore, the suppressing effect of the oxindole derivatives against lipid peroxidation was evaluated using the rat liver microsome/*tert*-butyl hydroperoxide system, according to our previous method.[15]

All of the tested 5-hydroxyoxindole derivatives showed notable lipid peroxidation-inhibitory effects that are comparable or superior to uric acid and **1** (Fig. 6). Compound **3d** exhibited the most effective inhibitory activity (67% inhibition). Compounds **3a**, **3b**, **3c**, **3e** and **4b** also showed over 50% inhibition. A comparison of the derivatives that have the same fundamental skeleton revealed that the more lipophilic (that have large Clog*P* values) derivatives (**2d** in **2a**–**e**, **3d** in **3a**–**e**, and **4b** in **4a**–**c**) tend to exhibit stronger lipid peroxidation activity (Fig. 6 and Table 1). However, for the overall results of the oxindoles, the order of lipophilicity (Table 1) did not simply correlate with the intensity of the lipid peroxidation-inhibitory activity. The 5-hydroxy-3-phenacyl derivatives **3a**–**e** tended to exhibit stronger lipid peroxidation-inhibitory activity than the 3,5-dihydroxy-3-phenacyl derivatives **2a**–**e**. Moreover, compound **4b**, which is the most lipophilic derivative among the tested compounds, was not the most active compound. The difference of the inhibitory activity between **2a**–**e**, **3a**–**e** and **4a**–**c** seemed to be partially affected by their DPPH radical scavenging activity. This result suggested that not only the high lipophilicity of the molecule but also the selection of a suitable skeleton were

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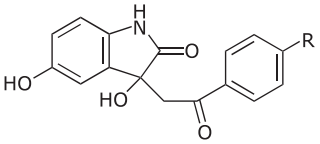
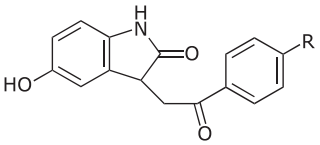
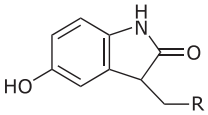
Table 1. DPPH radical scavenging activities and Clog*P* value of the oxindole derivatives

Compound	Skeletone	R	DPPH radical scavenging activity ($M^{-1}\cdot s^{-1}$)†	Clog*P*‡
Uric acid	—		0.63§	—¶
1	—		9.2§	0.04
2a		H	1.9§	0.81
2b		CH_3	1.8§	1.31
2c		CH_2CH_3	3.2	1.84
2d		$CH_2CH_2CH_3$	3.0	2.37
2e		$CH(CH_3)_2$	2.9	2.24
3a		H	7.6	1.52
3b		CH_3	7.8	2.02
3c		CH_2CH_3	8.2	2.55
3d		$CH_2CH_2CH_3$	10.0	3.08
3e		$CH(CH_3)_2$	9.9	2.95
4a		CH_2CH_3	7.2	1.62
4b		$(CH_2)_5CH_3$	6.5	3.73
4c		Ph	5.9	2.13

†The second-order rate constant between the test compound and DPPH in ethanol/MES buffer (pH 7.4) = 3/2 mixture. ‡Caluculated by ChemBioDraw® ultra ver. 13 (PerkinElmer). §Referenced in (15). ¶Uric acid has low lipophilicity and exists as a monobasic anion form at pH 7.4

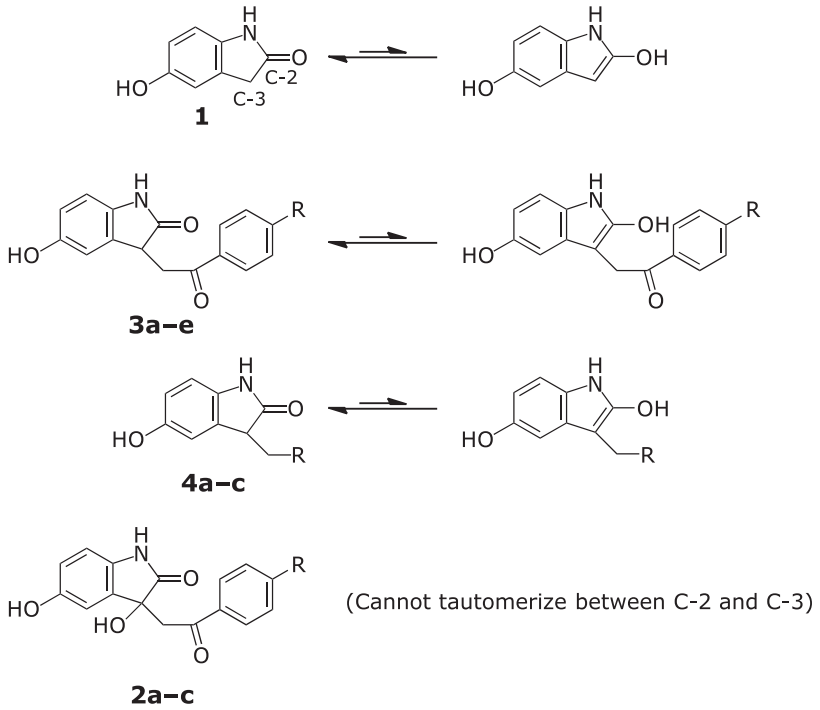


Fig. 5. Tautomerization between the keto and enol form of the 5-hydroxyoxindole derivatives.

important to increase the lipid peroxidation-inhibitory activity. In the present case, the 3-phenacyl substitution and the absence of a 3-hydroxy group were most suitable derivatizations of 5-hydroxyoxindole to increase the lipid peroxidation-inhibitory activity.

Pro-oxidant effect. For safety reasons, it is important to pay attention to the pro-oxidant effect of antioxidants.[25] For example, ascorbic acid and certain polyphenols reduce not only ROS but also transition metal ions such as Fe^{3+} and Cu^{2+}.[26–29] Reduced transition metal ions (Fe^{2+} or Cu^{+}) promote the reduction of triplet oxygen to superoxide, which subsequently transforms to the highly toxic hydroxyl radical. To avoid the expression of unexpected adverse events, novel antioxidative drugs without a pro-oxidant effect are required. Therefore, the iron-induced pro-oxidant activity of selected oxindole derivatives was measured based on the hydroxyl radical-dependent formaldehyde (HCHO) production

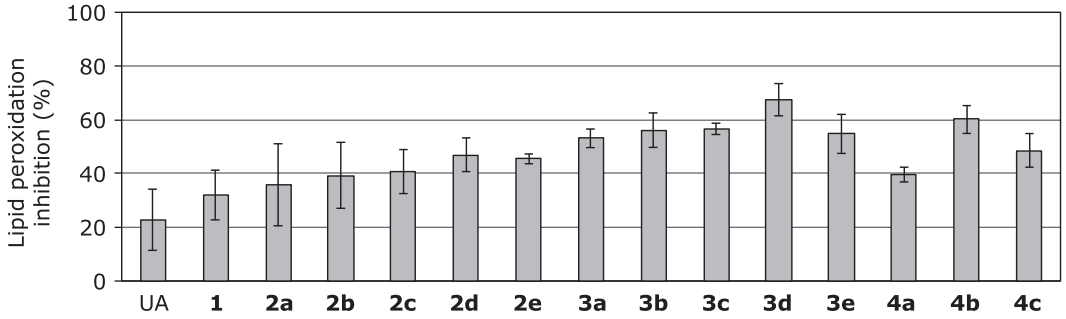


Fig. 6. Lipid peroxidation inhibitory activity. The tested concentration of each compound was 100 μM in ethanol/sodium phosphate buffer (pH 7.4, contains 0.1 mM EDTA).

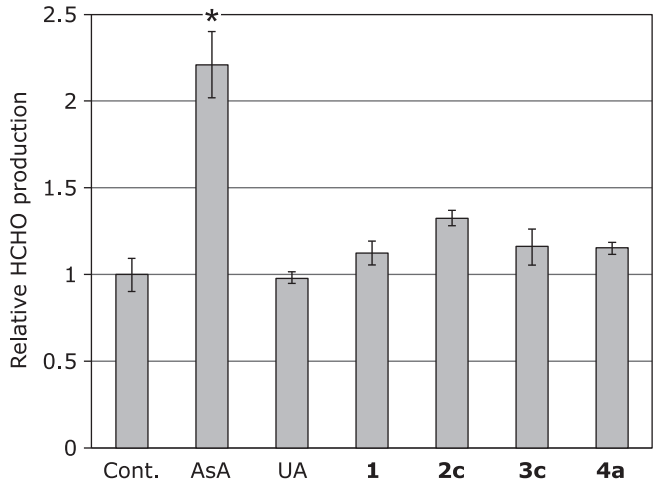


Fig. 7. Fe^{3+}-mediated pro-oxidant effect under aerobic condition. The mean is expressed as the formaldehyde (HCHO) production relative to the conditions without the antioxidant. AsA, ascorbic acid; UA, uric acid. *p>0.01 vs control.

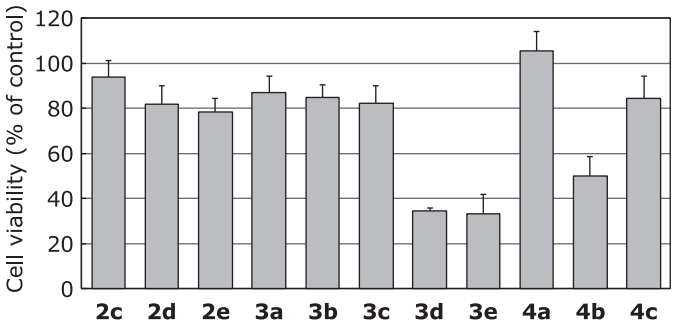


Fig. 8. Cytotoxicity against the HL-60 cells. HL-60 (1.0×10^6 cells/well) was incubated with the test compounds (50 μM) at 37°C for 24 h. The viable cell number was counted with the trypan blue dye-exclusion test. The cell viability was expressed relative to the vehicle (DMSO) control group (n = 3).

from dimethyl sulfoxide (DMSO) under aerobic conditions.[30]

Ascorbic acid significantly increased the HCHO production by 2.2 times compared with the vehicle control. On the other hand, the 5-hydroxyoxindole derivatives showed no significant pro-oxidant effect in the presence of Fe^{3+}, as with uric acid (Fig. 7). Because the other 5-hydroxyoxindole derivatives have a similar skeleton to the tested oxindoles, it is expected that their pro-oxidant effects would also be negligible. The present result suggested that 5-hydroxyoxindole was less potent pro-oxidants and seemed to be a safe scaffold for antioxidants in drug discovery.

Cytotoxicity. Finally, the cytotoxicity of the newly synthetic derivatives (**2c**–**e**, **3a**–**e**, and **4a**–**c**) against human promyelocytic leukemia (HL-60) cells was evaluated. Compounds **2c**, **2d**, **2e**, **3b**, **4a** and **4c** were minimally cytotoxic at a concentration of 50 μM (Fig. 8). On the other hand, **3d**, **3e**, and **4b** showed significant cytotoxicity (Fig. 8). Compound **3d**, **3e** and **4b** had a relatively high Clog$P$ value (Table 1). To avoid the expression of cytotoxicity, the lipophilicity (Clog$P$ value) of the derivatives should be approximately less than **3**.

Conclusions

We designed and synthesized new 5-hydroxyoxindole derivatives, and their antioxidant and pro-oxidant activities were investigated. The 5-hydroxy-3-phenacyl-2-oxindoles (**3a**–**e**) and 5-hydroxy-3-alkyl-2-oxindoles (**4a**–**c**) showed greater DPPH radical scavenging activity than the 3,5-dihydroxy-3-phenacyl-2-oxindoles (**2a**–**e**). The 3-monosubstitution was more preferable to the 3,3-di-substitution to retain DPPH radical scavenging activity of 5-hydroxyoxindole (**1**). The 3-mono-phenacyl substitution of 5-hydroxyoxindole (**3a**–**e**) was also preferable to the 3-hydroxy-3-phenacyl (**2a**–**e**) substitution in exerting the lipid peroxidation-inhibitory activity. The most lipophilic compound **4b** did not show strong lipid peroxidation-inhibitory activity as expected, which suggested that the C-3 mono-phenacyl-substitution was preferable to the C-3 mono-hydrocarbon-substitution. Unlike ascorbic acid, the 5-hydroxyoxindole derivatives showed no significant iron-induced pro-oxidant effect. Except for the extremely lipophilic derivatives, the series of derivatives had a high tolerability against human cell lines.

The present study indicates that the 3-monosubstituted-5-hydroxyoxindole structure can be used as a novel scaffold for antioxidants in drug discovery. Since compounds **3a**, **3b** and **3c** possess a good balance between their antioxidant activities and their cytotoxicity, they could be good lead candidates for novel antioxidant therapeutics.

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Conflict of Interest

No potential conflicts of interest were disclosed.

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