Supporting Information

A new series of anti-prion compounds; pyrazolone derivatives have the potent activity of inhibiting PrP-res accumulation.

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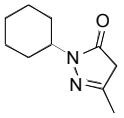
Experimental Section

Synthesis:

Synthesis of compound **1**–**4**, **10**-**17**, **19** was basically performed according to methods in the literature.[1,2]
All the synthesized compounds, except **19**, are already reported as antioxidants or their related compounds.[3]

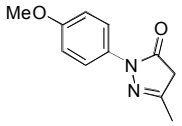
General Methods. Melting points were determined using a Yanagimoto micro melting point apparatus or a Büchi 545 melting point apparatus and were left uncorrected. Proton nuclear magnetic resonance spectra (^{1}H-NMR) were recorded on a JEOL JNM-LA400, JEOL JNM-LA500 spectrometer in solvent as indicated. Chemical shifts (δ) are reported in parts per million relative to the internal standard tetramethylsilane (TMS). Elemental analysis was performed with a Yanaco CHN CORDER NT-5 analyzer, and all values were within ±0.4% of the calculated values. High-resolution mass spectra (HRMS) were recorded on a JEOL JMS-SX102A mass spectrometer. GC-MS analyses were performed on a Shimadzu GCMS-QP2010. All reagents and solvents were purchased from Aldrich, Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan), Wako Pure Chemical Industries, Ltd. (Osaka, Japan), or Kanto Chemical Co., Inc. (Tokyo, Japan), and used without purification. Flash column chromatography was performed using silica gel 60 (particle size 0.046–0.063 mm) supplied by Merck & Co., Inc. (NJ, U.S.A.).

1-Cyclohexyl-3-methyl-1*H*-pyrazol-5(4*H*)-one (1).



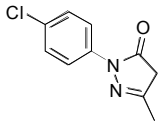
To a suspension of ethyl acetoacetate (**24a,** 1.30 g, 10.0 mmol) and cyclohexylhydrazine hydrochloride (1.14 g, 10.0 mmol) in EtOH (10 mL) was added Et_3N (1.60 mL, 11.5 mmol). The mixture was stirred at reflux temperature for 19 h. The solvent was removed by evaporation, and the residue was extracted with AcOEt. The organic layer was washed with saturated aqueous $NaHCO_3$, water, and brine, and dried over Na_2SO_4. Filtration and concentration *in vacuo*, and purification by silica gel flash chromatography (AcOEt only) gave 745 g (42%) of **1** as a white solid. The crude solid was recrystallized from EtOH and collected by filtration to give white crystals: mp 152–153 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz, δ; ppm) 3.99 (1H, m), 3.20 (2H, s), 2.10 (3H, s), 1.50 (10H, m); ^{13}C-NMR ($CDCl_3$, 500 MHz, δ; ppm) 171.5, 155.0, 52.4, 42.2, 31.0, 25.5, 25.2, 17.0; MS (EI) m/z: 180 (M^+); Anal. Calcd for $C_{10}H_{16}N_2O$: C, 66.63; H, 8.95; N, 15.54. Found: C, 66.56; H, 8.81; N, 15.58.

1-(4-Methoxyphenyl)-3-methyl-1*H*-pyrazol-5(4*H*)-one (2).



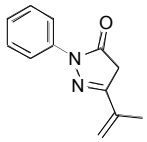
Compound **2** was prepared from ethyl acetoacetate (**24a**) and 4-methoxyphenylhydrazine hydrochloride in a 36% yield using the procedure described for **1**. In this case, AcOH was used instead of EtOH. The crude solid was recrystallized from THF and *n*-hexane, and collected by filtration to give yellow crystals: mp 127–128 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.72 (2H, d, J = 8.3 Hz), 6.92 (2H, d, J = 8.5 Hz), 3.81 (3H, s), 3.41 (2H, s), 2.19 (3H, s); MS (EI) m/z: 204 (M^+); Anal. Calcd for $C_{11}H_{12}N_2O_2$: C, 64.69; H, 5.92; N, 13.72. Found: C, 64.73; H, 6.00; N, 13.67.

1-(4-Chlorophenyl)-3-methyl-1*H*-pyrazol-5(4*H*)-one (3).



Compound **3** was prepared from ethyl acetoacetate (**24a**) and 4-chlorophenylhydrazine hydrochloride in a 60% yield using the procedure described for **1**. The crude solid was recrystallized from EtOH and collected by filtration to give white crystals: mp 173–174 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.85 (2H, d, J = 8.8 Hz), 7.35 (2H, d, J = 8.8 Hz), 3.44 (2H, s), 2.20 (3H, s); MS (EI) m/z: 208 (M^+); Anal. Calcd for $C_{10}H_9ClN_2O$: C, 57.57; H, 4.35; N, 13.43. Found: C, 57.52; H, 4.40; N, 13.58.

1-Phenyl-3-(prop-1-en-2-yl)-1*H*-pyrazol-5(4*H*)-one (4).

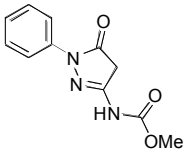


Step 1: Preparation of ethyl 4-methyl-3-oxopent-4-enoate (24b). To a suspension of malonic acid monoethyl ester potassium salt (9.80 g, 57.6 mmol), $MgCl_2$ (6.00 g, 63.0 mmol) and Et_3N (17.5 mL, 126 mmol) in MeCN (100 mL) was added methacryloyl chloride (**20a**, 3.00 g, 28.7 mmol) dropwise with cooling in an ice-water bath. After 30 min stirring at 0 °C, 2 M aqueous HCl was added to the mixture and stirred at 0 °C for 30 min. The mixture was poured into ice-water and extracted with AcOEt. The organic layer was separated, washed with water, saturated aqueous $NaHCO_3$, and brine, and dried over Na_2SO_4. Filtration and concentration *in vacuo* and purification by silica gel flash chromatography (*n*-hexane/AcOEt = 5/1) gave 2.52 g (43%) of **24b** as a colorless oil; ^{1}H-NMR ($CDCl_3$, 400 MHz, δ; ppm) 5.96 (1H, s), 5.93 (1H, s), 5.90 (1H, s), 5.89 (1H, s), 5.35 (0.3H, s), 4.23 (2H, q, J = 7.1 Hz), 4.21 (2H, q, J = 7.1 Hz), 3.74 (1.4H, s), 1.90 (3H, s), 1.31 (3H, t, J = 7.3 Hz), 1.27 (3H, t, J = 7.3 Hz).

Step 2: Preparation of 1-phenyl-3-(prop-1-en-2-yl)-1*H*-pyrazol-5(4*H*)-one (4). Compound **4** was prepared from **24b** obtained above and phenylhydrazine in an 11% yield using the procedure described for **1**. In this case, AcOH was used instead of EtOH and Et_3N was not used. The crude solid was recrystallized from AcOEt and *n*-hexane, and collected by filtration to give yellow crystals: mp 56–57 °C; IR (KBr, cm^{-1}) 3435, 1718, 1597, 4499, 1377, 758, 692;

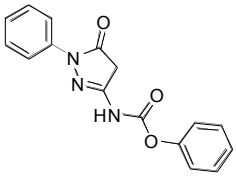
^{1}H-NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.90 (2H, d, *J* = 7.8 Hz), 7.41 (2H, t, *J* = 7.6 Hz), 7.20 (1H, t, *J* = 7.3 Hz), 5.43 (1H, s), 5.34 (1H, s), 3.64 (2H, s), 2.11 (3H, s); HRMS Calcd for $C_{12}H_{12}N_2O$: 200.095, Found: 200.094; Anal. Calcd for $C_{12}H_{12}N_2O \cdot 0.2H_2O$: C, 70.71; H, 6.13; N, 13.74. Found: C, 70.97; H, 5.99; N, 13.62.

Methyl 5-oxo-1-phenyl-4,5-dihydro-1*H*-pyrazol-3-ylcarbamate (5).



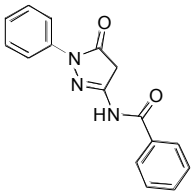
To a suspension of 3-amino-1-phenyl-5-pyrazolone (**25**, 1.75 g, 10.0 mmol) in pyridine (15 mL) was added dropwise chloroformic acid methyl ester (0.95 g, 10 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h and stirred at 50 °C for 2 h. The reaction mixture was poured into water, and the precipitated solid was collected by filtration and washed with water and EtOH. The crude solid was recrystallized from MeOH and $CHCl_3$ and collected by filtration to give 545 mg (23%) of **5** as white crystals: mp 231–232 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.82 (2H, d, *J* = 7.6 Hz), 7.38 (2H, t, *J* = 8.0 Hz), 7.20 (2H, t, *J* = 7.3 Hz), 4.00 (2H, s), 3.82 (3H, s); ^{1}H-NMR (DMSO-d_6, 500 MHz, δ; ppm) 11.8 (1H, s), 11.0 (0.3H, s), 9.99 (1H, br), 7.79 (0.6H, d, *J* = 8.5 Hz), 7.68 (2H, d, *J* = 7.9 Hz), 7.43–7.39 (2.6H, m), 7.20 (1H, t, *J* = 7.3 Hz), 7.15 (0.3H, t, *J* = 7.6 Hz), 5.77 (1H, s), 4.03 (0.7H, s), 3.68 (0.9H, s), 3.63 (3H, s); ^{13}C-NMR (DMSO-d_6, 500 MHz, δ; ppm) 168.1, 153.9, 153.4, 152.2, 151.9, 147.1, 138.7, 138.1, 128.7, 124.8, 124.0, 120.3, 117.7, 79.6, 52.3, 51.6; MS (EI) m/z: 233 (M^+); Anal. Calcd for $C_{11}H_{11}N_3O_3$: C, 56.65; H, 4.75; N, 18.02. Found: C, 56.84; H, 4.85; N, 17.89.

Phenyl 5-oxo-1-phenyl-4,5-dihydro-1*H*-pyrazol-3-ylcarbamate (6).



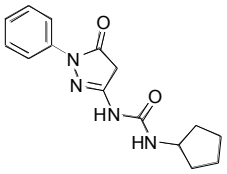
Compound **6** was prepared from 3-amino-1-phenyl-5-pyrazolone (**25**) and chloroformic acid phenyl ester in a 21% yield using the procedure described for **5**. The crude solid was recrystallized from AcOEt and collected by filtration to give white crystals: mp 225–226 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.84 (2H, d, J = 8.5 Hz), 7.69 (1H, s), 7.42 (2H, t, *J* = 8.5 Hz), 7.40 (2H, t, *J* = 7.5 Hz), 7.29 (1H, t, *J* = 7.3 Hz), 7.19 (3H, t, *J* = 8.3 Hz); MS (EI) m/z: 295 (M^+); Anal. Calcd for $C_{16}H_{13}N_3O_3$: C, 65.08; H, 4.44; N, 14.23. Found: C, 65.17; H, 4.71; N, 14.40.

***N*-(5-oxo-1-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl) benzamide (7).**



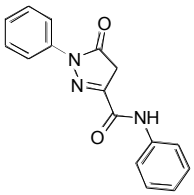
To a suspension of 3-amino-1-phenyl-2-pyrazolin-5-one (25, 351 mg, 2.00 mmol) in dioxane (5.5 mL) was added benzoyl chloride (0.25 mL, 2.2 mmol) and the mixture was stirred at room temperature for 3.5 h. The reaction mixture was poured into water and extracted with AcOEt. The organic layer was separated, washed with brine, and dried over Na_2SO_4. Filtration and concentration *in vacuo* and purification by silica gel flash chromatography (*n*-hexane/AcOEt = 3/1) gave 83 mg (15%) of **7** as a light brown solid. The crude solid was recrystallized from AcOEt and collected by filtration to give light brown crystals: mp 218–219 °C; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ; ppm) 8.58 (1H, s), 7.89 (2H, d, J = 7.3 Hz), 7.85 (2H, d, J = 7.5 Hz), 7.64 (1H, t, J = 7.5 Hz), 7.54 (2H, t, J = 7.7 Hz), 7.41 (2H, t, J = 8.1 Hz), 7.19 (1H, t, J = 7.5 Hz), 4.23 (2H, s); ^{13}C-NMR (DMSO-d_6, 600 MHz, δ; ppm) 164.6, 152.1, 147.1, 138.7, 134.0, 131.5, 128.8, 128.2, 127.7, 125.0, 120.6, 81.6; MS (EI) m/z: 279 (M^+); Anal. Calcd for $C_{16}H_{13}N_3O_2$: C, 68.81; H, 4.69; N, 15.05. Found: C, 68.93; H, 4.89; N, 14.87.

1-Cyclopentyl-3-(5-oxo-1-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl)urea (8).



To a suspension of compound **6** (295 mg, 1.00 mmol) in xylene was added cyclopentylamine (85 mg, 1.0 mmol) and stirred at reflux temperature for 3 h. The reaction mixture was poured into EtOH and the precipitated solid was collected by filtration, and washed with water and EtOH to give 119 mg (42%) of **8** as a white solid. The crude solid was recrystallized from THF and *n*-hexane and collected by filtration to give white crystals: mp 184–185 °C; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ; ppm) 8.75 (1H, s), 7.76 (2H, d, J = 8.5 Hz), 7.41 (2H, t, J = 8.1 Hz), 7.19 (1H, t, J = 7.5Hz), 4.19 (1H, sext, J = 6.6 Hz), 3.65 (2H, s), 2.05–2.00 (2H, m), 1.78–1.67 (4H, m), 1.58–1.52 (2H, m); ^{13}C-NMR (DMSO-d_6, 500 MHz, δ; ppm) 167.8, 152.7, 152.5, 138.2, 128.8, 123.9, 117.5, 78.6, 51.1, 32.7, 23.0; MS (EI) m/z: 286 (M^+); Anal. Calcd for $C_{15}H_{18}N_4O_2$: C, 62.92; H, 6.34; N, 19.57. Found: C, 62.58; H, 6.36; N, 19.60.

5-Oxo-*N*,1-diphenyl-4,5-dihydro-1*H*-pyrazole-3-carboxamide (9).

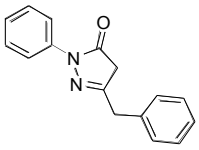


Step 1: Preparation of 5-oxo-1-phenyl-4,5-dihydro-1*H*-pyrazole-3-carbonyl chloride (27). To a suspension of 5-oxo-1-phenyl-2-pyrazolin-3-carboxylic acid (**26**, 818 mg, 4.01 mmol) in CH_2Cl_2 (15 mL) was added oxalyl chloride

(0.70 mL, 8.2 mmol) dropwise and cat. DMF. The mixture was stirred at room temperature for 35 min. The reaction mixture was concentrated *in vacuo* and washed with CH_2Cl_2 three times to give a brown solid.

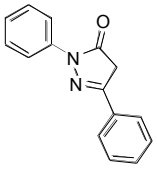
Step 2: Preparation of 5-oxo-*N*,1-diphenyl-4,5-dihydro-1*H*-pyrazole-3-carboxamide (9). To a suspension of **27** obtained above in CH_2Cl_2 (16 mL) was added aniline (2.0 mL, 22 mmol) and the mixture was stirred at room temperature for 19 h. The reaction mixture was poured into 2 M aqueous HCl with cooling in an ice-water bath and extracted with AcOEt. The organic layer was separated, washed with brine, and dried over Na_2SO_4. Filtration and concentration *in vacuo* and purification by silica gel flash chromatography (*n*-hexane/AcOEt = 4/1 → 1/1) gave 825 mg (74%, two steps) of **9** as a light brown solid. The crude solid was recrystallized from AcOEt and *n*-hexane and collected by filtration to give light yellow crystals: mp 214–215 °C; ^{1}H-NMR (DMSO-d_6, 500 MHz, δ; ppm) 12.1 (1H, br), 9.93 (1H, br), 7.85 (2H, d, J = 8.5 Hz), 7.80 (2H, d, J = 7.6 Hz), 7.53 (2H, t, J = 8.1 Hz), 7.37 (1H, t, J = 7.5 Hz), 7.34 (2H, t, J = 7.9 Hz), 7.08 (1H, t, J = 7.5 Hz), 6.02 (1H, s); ^{13}C-NMR (DMSO-d_6, 600 MHz, δ; ppm) 160.1, 153.7, 145.7, 138.6, 138.1, 128.9, 128.5, 126.7, 123.4, 122.1, 120.1, 87.6; MS (EI) m/z: 279 (M^+); Anal. Calcd for $C_{16}H_{13}N_3O_2$: C, 68.81; H, 4.69; N, 15.05. Found: C, 69.04; H, 4.75; N, 15.01.

3-Benzyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one (10).



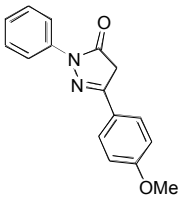
Compound **10** was prepared from phenylacetyl chloride (**20b**) and phenylhydrazine in a 23% yield using the procedure described for **4**. The crude solid was recrystallized from EtOH and collected by filtration to give white crystals: mp 215–216 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.88 (2H, dd, J = 8.8, 1.2 Hz), 7.40 (2H, t, J = 7.6 Hz), 7.36 (2H, t, J = 7.6 Hz), 7.29 (1H, t, J = 7.3 Hz), 7.27 (2H, dd, J = 8.0, 1.5 Hz), 7.19 (1H, t, J = 7.6 Hz), 3.83 (2H, s), 3.33 (2H, s); MS (EI) m/z: 250 (M^+); Anal. Calcd for $C_{16}H_{14}N_2O$: C, 76.78; H, 5.64; N, 11.19. Found: C, 76.74; H, 5.7; N, 10.99.

1,3-Diphenyl-1*H*-pyrazol-5(4*H*)-one (11).



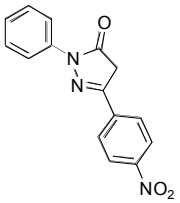
Compound **11** was prepared from ethyl acetoacetate (**24a**) and phenylhydrazine in a 66% yield using the procedure described for **1**. In this case, Et_3N was not used. The crude solid was recrystallized from EtOH and collected by filtration to give white crystals: mp 136–138 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz, δ; ppm) 8.18 (2H, d, J = 6.8 Hz), 7.78–7.74 (2H, m), 7.48–7.41 (5H, m), 7.22 (1H, t, J = 6.8 Hz), 3.84 (2H, s); MS (EI) m/z: 236 (M^+); Anal. Calcd for $C_{15}H_{12}N_2O$: C, 76.25; H, 5.12; N, 11.86. Found: C, 76.13; H, 5.17; N, 11.99.

3-(4-Methoxyohenyl)-1-phenyl-1*H*-pyrazol-5(4*H*)-one (12).



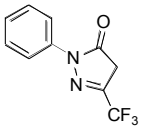
To a solution of 4-methoxybenzoyl acetic acid ethyl ester (**24e**, 2.22 g, 10.0 mmol) in EtOH (10 mL) was added phenylhidrazine (1.08 g, 10.0 mmol). The mixture was stirred at reflux temperature for 15 h. After cooling, the precipitated solid was collected by filtration to give 1.32 g (85%) of **12** as a yellow solid. The crude solid was recrystallized from EtOH and THF and *n*-hexane and collected by filtration to give yellow crystals: mp 141–142 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.99 (2H, d, J = 7.5 Hz), 7.73 (2H, d, J = 8.8 Hz), 7.44 (2H, t, J = 8.5 Hz), 7.23 (1H, t, J = 7.3 Hz), 6.98 (2H, d, J = 8.8 Hz), 3.87 (3H, s), 3.84 (2H, s); MS (EI) m/z: 266 (M^+); Anal. Calcd for $C_{16}H_{14}N_2O_2$: C, 72.16; H, 5.30; N, 10.52. Found: C, 71.88; H, 5.30; N, 10.64.

3-(4-Nitrophenyl)-1-phenyl-1*H*-pyrazol-5(4*H*)-one (13).



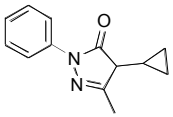
Compound **13** was prepared from 4-nitrobenzoyl acetic acid ethyl ester (**24f**) and phenylhydrazine in an 85% yield using the procedure described for **12**. The crude solid was recrystallized from EtOH and THF and collected by filtration to give yellow crystals: mp 190–191 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz, δ; ppm) 8.32 (1H, d, J = 9.1 Hz), 7.95 (2H, d, J = 8.7Hz), 7.46 (2H, d, J = 8.7 Hz), 7.46 (2H, t, J = 7.6 Hz), 3.91 (2H, s); ^{13}C-NMR (DMSO-d_6, 500 MHz, δ; ppm) 154.2, 147.5, 146.6, 139.8, 138.5, 129.0, 126.3, 125.8, 124.0, 121.5, 86.1; MS (EI) m/z: 291 (M^+); Anal. Calcd for $C_{15}H_{11}N_3O_3$: C, 64.05; H, 3.94; N, 14.94. Found: C, 63.77; H, 4.02; N, 15.12.

1-Phenyl-3-trifluoromethyl-1*H*-pyrazol-5(4*H*)-one (14).



Compound **14** was prepared from trifluoroacetoacetic acid ethyl ester (**24g**) and phenylhydrazine in a 75% yield using the procedure described for **12**. In this case, AcOH was used instead of EtOH. The crude solid was recrystallized from AcOEt and *n*-hexane, and collected by filtration to give white crystals: mp 195–196 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.80 (2H, d, J = 7.6 Hz), 7.66 (2H, d, J = 7.8 Hz), 6.61 (1H, s), 5.91 (1H, s), 3.71 (2H, s); ^{13}C-NMR (DMSO-d_6, 500 MHz, δ; ppm) 153.7, 140.4 (q, $^3J_{C-F}$ = 37.6 Hz), 137.7, 129.1, 127.2, 122.3, 85.6; MS (EI) m/z: 228 (M^+); Anal. Calcd for $C_{10}H_7F_3N_2O$: C, 52.64; H, 3.09; N, 12.28. Found: C, 52.76; H, 3.17; N, 12.65.

4-Cyclopropyl-3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one (15).

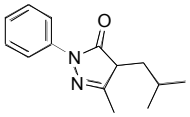


Step 1: Preparation of 2-cyclopropyl-3-oxobutyronitrile (22). To lithium *N*,*N*-diisoprorylamide (1.5 M solution in cyclohexane, 9.0 mL, 14 mmol) was added a solution of cyclopropylacetonitrile (**21**, 1.00 g, 12.3 mmol) in THF (10 mL) dropwise with cooling in a dry ice-acetone bath. The mixture was stirred for 10 min and a solution of acetic anhydride (700 mg, 6.86 mmol) in THF (10 mL) was added dropwise at –78 °C. The mixture was stirred at room temperature for 10 min, poured into 2 M aqueous HCl, and extracted with AcOEt. The organic layer was separated, washed with brine, and dried over Na_2SO_4. Filtration and concentration *in vacuo* and purification by silica gel flash chromatography (*n*-hexane/AcOEt = 5/2) gave 593 mg (78%) of **22** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 400 MHz, δ, ppm) 3.17 (1H, d, *J* = 7.6 Hz), 2.41 (3H, s), 1.30–1.22 (1H, m), 0.83–0.75 (2H, m), 0.58–0.52 (2H, m).

Step 2: Preparation of ethyl 2-cyclopropyl-3-oxobutanoate (24h). To a solution of **22** (593 mg, 3.48 mmol) obtained above in EtOH (4.80 mL, 82.3 mmol) was added acetyl chloride (2.97 mL, 41.8 mmol) dropwise with cooling in an ice-water bath. The mixture was stirred overnight at room temperature. The mixture was concentrated, and concentrated HCl (0.3 mL) was added to a solution of the residue in EtOH (5.5 mL). The mixture was heated at 40 °C for 2 h, poured into 2 M aqueous HCl and extracted with AcOEt. The organic layer was separated, washed with water and brine, and dried over Na_2SO_4. Filtration and concentration *in vacuo* and purification by silica gel flash chromatography (*n*-hexane/AcOEt = 4/1) gave 527 mg (89%) of **24h** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 400 MHz, δ, ppm) 4.21 (2H, q, *J* = 7.3 Hz), 2.61 (1H, d, *J* = 10.2 Hz), 2.29 (3H, s), 1.35–1.25 (1H, m), 1.28 (3H, t, *J* = 7.3 Hz), 0.70–0.67 (2H, m), 0.31–0.27 (2H, m).

Step 3: 4-cyclopropyl-3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one (15). Compound **15** was prepared from **24h** obtained above and phenylhydrazine in an 81% yield using the procedure described for **12**. In this case, AcOH was used instead of EtOH. The crude solid was recrystallized from AcOEt and *n*-hexane, and collected by filtration to give white crystals: mp 150–152 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz, δ, ppm) 7.89 and 7.67 (2H, each d, J = 7.8 Hz), 7.39 and 7.37 (2H, each t, *J* = 7.6 Hz), 7.17 (1H, t, *J* = 7.3 Hz), 6.75 (0.4H, br), 2.63 (0.6H, d, *J* = 9.0 Hz), 2.24 (3H, s), 1.40–1.30 (0.4H, m), 1.10–0.55 (4.6H, m); MS (EI) m/z: 214 (M^+); Anal. Calcd for $C_{13}H_{14}N_2O$: C, 72.87; H, 6.59; N, 13.07. Found: C, 72.71; H, 6.58; N, 13.04.

4-Isobutyl-3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one (16).

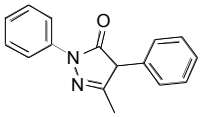


Step 1: Preparation of ethyl 2-acetyl-4-methylpentanoate (24i). To NaOEt in EtOH (20%, 8.70 g, 25.6 mmol) was added ethyl acetoacetate (**24a** 3.00 g, 23.1 mmol) all at once and the mixture was warmed to 80 °C. A solution of isobutyl iodide (5.10 g 27.7 mmol) in THF (5.0 mL) was added dropwise over a 2 h period, and the reaction mixture was kept at 80 °C for an additional 2 h. After cooling, the reaction mixture was poured into 2 M aqueous HCl-ice and extracted with AcOEt. The organic layer was separated, washed with water and brine, and dried over Na_2SO_4. Filtration and concentration *in vacuo* and purification by silica gel flash chromatography (*n*-hexane/AcOEt = 20/1)

gave 1.83 g (43%) of **24i** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 400 MHz, δ; ppm) 12.91 (0.2H, s), 4.20 and 4.19 (2H, each q, J = 7.1 Hz), 3.50 (0.8H, dd, J = 8.3, 6.6 Hz), 2.23 and 2.00 (3H, each s), 2.05 (0.4H, d, J = 7.1 Hz), 1.80–1.76 (0.8H, m), 1.72–1.66 (0.8H, m), 1.56–1.51 (1H, m), 1.31 and 1.27 (3H, each t, J = 7.1 Hz), 0.92, 0.91 and 0.87 (6H, each d, J = 6.7 Hz).

Step 2: 4-isobutyl-3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one (16). Compound **16** was prepared from **24i** obtained above and phenylhydrazine in a 70% yield using the procedure described in **12**. In this case, AcOH was used instead of EtOH. The crude solid was recrystallized from AcOEt and *n*-hexane, and collected by filtration to give white crystals: mp 117–119 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.89 and 7.72 (2H, each d, J = 7.8 Hz), 7.39 (2H, t, J = 7.6 Hz), 7.17 and 7.15 (1H, each t, J = 7.3 Hz), 6.80 (0.3H, br), 3.26 (0.7H, t, J = 6.3 Hz), 2.18–2.15 (3.6H, m), 2.01–1.94 (1H, m), 1.78 (1.4H, t, J = 7.3 Hz), 0.962, 0.958, and 0.920 (6H, each d, J = 6.6 Hz); MS (EI) m/z: 230 (M^+); Anal. Calcd for $C_{14}H_{18}N_2O \cdot 0.2H_2O$: C, 73.01; H, 7.88; N, 12.16. Found: C, 73.26; H, 7.93; N, 12.38.

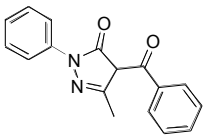
3-Methyl-1,4-diphenyl-1*H*-pyrazol-5(4*H*)-one (17).



Step 1: Preparation of ethyl 3-oxo-2-phenylbutanoate (24j). To a suspension of sodium hydride (60%, 1.50 g, 37.5 mmol) in THF (30 mL) was added a solution of phenylacetic acid ethyl ester (**23a**, 5.00 g, 30.5 mmol) dropwise with cooling in an ice-water bath. The solution was stirred at 60 °C for 30 min, followed by the addition of acetic anhydride (3.20 mL, 33.9 mmol) in THF (10 mL) at 0 °C. The solution was stirred at room temperature for 1 h. The reaction mixture was poured into ice-water and extracted with AcOEt. The organic layer was separated, washed with water, saturated aqueous $NaHCO_3$, and brine, and dried over Na_2SO_4. Filtration and concentration *in vacuo* and purification by silica gel flash chromatography (*n*-hexane/AcOEt = 10/1) gave 3.23 g (51%) of **24j** as a pale yellow oil: ^{1}H-NMR ($CDCl_3$, 400 MHz, δ, ppm) 7.52–7.10 (5H, m), 4.15 (2H, q, J = 7.1 Hz), 3.57 (0.5H, s), 2.24 and 1.90 (3H, each s), 1.22 and 1.20 (3H, each t, J = 7.1 Hz).

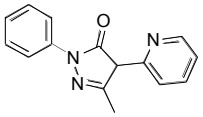
Step 2: 3-methyl-1,4-diphenyl-1*H*-pyrazol-5(4*H*)-one (17). Compound **17** was prepared from **24j** obtained above and phenylhydrazine in 13% yield using the procedure described for **12**. The crude solid was recrystallized from EtOH and collected by filtration to give white crystals: mp 203–205 °C; ^{1}H-NMR (DMSO-d_6, 400 MHz, δ, ppm) 11.4 (1H, br), 7.75 (2H, d, J = 8.0 Hz), 7.65–7.50 (2H, m), 7.48 (2H, t, J = 7.7 Hz), 7.39 (2H, t, J = 7.6 Hz), 7.25–7.20 (2H, m), 2.30 (3H, s); ^{13}C-NMR (DMSO-d_6, 500 MHz, δ; ppm) 146.5, 132.4, 128.9, 128.2, 127.6, 127.7, 125.6, 124.9, 124.8, 119.0, 12.6; MS (EI) m/z: 250 (M^+); Anal. Calcd for $C_{13}H_{14}N_2O$: C, 76.78; H, 5.64; N, 11.19. Found: C, 76.56; H, 5.73; N, 11.18.

4-Benzoyl-3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one (18).



To a suspension of 3-methyl-1-phenyl-2-pyrazolin-5-one (**28**, 349 mg, 2.00 mmol) in dioxane (2.5 mL) was added $Ca(OH)_2$ (221 mg, 2.98 mmol) and benzoyl chloride (0.25 mL, 2.2 mmol), and the mixture was stirred at 100 °C for 1 h. The reaction mixture was poured into 2 M aqueous HCl and extracted with AcOEt. The organic layer was separated, washed with brine, and dried over Na_2SO_4. Filtration and concentration i*n vacuo* and purification by silica gel flash chromatography (*n*-hexane/AcOEt = 9/1) gave 440 mg (79%) of **18** as a yellow solid. The crude solid was recrystallized from AcOEt and *n*-hexane, and collected by filtration to give light yellow crystals: mp 90–91 °C; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ; ppm) 7.88 (2H, d, *J* = 8.1 Hz), 7.65 (2H, d, *J* = 7.6 Hz), 7.58 (1H, t, *J* = 7.5 Hz), 7.52 (2H, t, *J* = 7.5 Hz), 7.47 (2H, t, *J* = 8.1 Hz), 7.31 (1H, t, *J* = 7.3 Hz), 2.10 (3H, s); ^{13}C-NMR ($CDCl_3$, 600 MHz, δ; ppm) 191.9, 161.7, 148.0, 137.8, 137.5, 131.8, 129.1, 128.5, 127.9, 126.7, 120.9, 103.7, 15.8; MS (EI) m/z: 278 (M^+); Anal. Calcd for $C_{17}H_{14}N_2O_2$: C, 73.37; H, 5.07; N, 10.07. Found: C, 73.39; H, 4.84; N, 10.02.

3-Methyl-1-phenyl-4-(pyridine-2-yl)-1*H*-pyrazol-5(4*H*)-one (19).



Step 1: Preparation of ethyl 3-oxo-2-(pyridine-2-yl)butanoate (24k). To a suspension of sodium hydride (60%, 600 mg, 15.0 mmol) in THF (8.0 mL) was added 2-pyridylacetic acid ethyl ester (**23b**, 0.45 mL, 3.0 mmol) dropwise with cooling in an ice-water bath. The mixture was stirred at 60 °C for 35 min, followed by the addition of acetic anhydride (1.25 mL, 13.2 mmol) in THF (3.0 mL) at 0 °C. The mixture was stirred at room temperature for 16 h. The reaction mixture was poured into ice-water, neutralized with 2 M aqueous HCl, and extracted with AcOEt. The organic layer was separated, washed with brine, and dried over Na_2SO_4. Filtration and concentration *in vacuo* and purification by silica gel flash chromatography (*n*-hexane/AcOEt = 5/1) gave 639 mg of **24k** as a yellow oil.

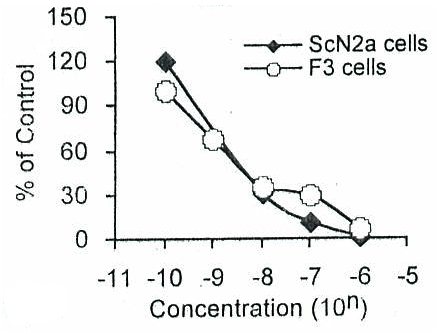
Step 2: Preparation of 3-methyl-1-phenyl-4(pyridine-2-yl)-1*H*-pyrazol-5(4*H*)-one (19). Compound **19** was prepared from **24k** and phenylhydrazine in an 8% yield (two steps) using the procedure described for **12**. The crude solid was recrystallized from AcOEt and collected by filtration to give yellow crystals: mp 184–185 °C; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ; ppm) 8.04 (2H, d, *J* = 7.6 Hz), 7.91 (1H, d, *J* = 5.8 Hz), 7.77 (1H, t, *J* = 7.9 Hz), 7.47 (1H, d, *J* = 8.8 Hz), 7.41 (2H, t, *J* = 7.9 Hz), 7.16 (1H, t, *J* = 7.5 Hz), 6.90 (1H, t, *J* = 6.7 Hz), 2.50 (3H, s); ^{13}C-NMR ($CDCl_3$, 600 MHz, δ; ppm) 151.6, 145.6, 140.1, 139.5, 137.2, 128.7, 124.4, 119.7, 118.9, 115.0, 93.3, 16.6; MS (EI) m/z: 251 (M^+); Anal. Calcd for $C_{15}H1_3N_3O$: C, 71.70; H, 5.21; N, 16.72. Found: C, 71.93; H, 5.29; N, 16.53.

Biological assays:

Anti-prion activity.

Anti-prion activities of each compound were assayed by measuring the 50%-inhibitory concentration (IC_{50}) for PrP-res formation in ScN2a and F3 cells by Western blot analysis.[4–6] Test compounds were dissolved in 100% DMSO or 95% ethanol just before use and added to the medium. The final concentration of either DMSO or ethanol in the medium was less than 0.2%.

Figure S1. Densitometric quantitation of proteinase-resistant prion protein derived from cells treated with compound **13**. Non-treated ScN2a and F3 cells were used as controls.



Cytotoxicity assay.

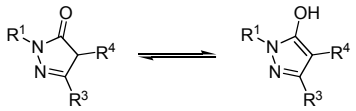
A human neuroblastoma (NB69) cell line was employed to evaluate the cytotoxic effects of the synthesized compounds. NB69 cells were grown in 10 cm dish, and were seeded (2×10^4 cells/well) in 96-well plate when cells achieved 80% confluency. After 24 h, the cells were incubated with various concentrations of compounds (10^{-10} M to 10^{-6} M) for additional 24 h. Cytotoxicity was measured by using MTT assay. MTT (2.5 mg/mL) in PBS was added to the culture media (10 μL/well). After incubation for 4 h, the medium were removed, and cell lysis solution (20% SDS:DMF = 1:1) was added (100 μL/well). The lysate was stand at room temperature overnight, and absorbance at 570 nm was measured by a photometer. Relative survival in comparison to the control treated with vehicle (DMSO) was then determined.

SOD-like activity assay.

SOD-like activity of pyrazolone derivatives was quantified by using SOD-like assay kit-WST (Dojindo Laboratories, Kumamoto, Japan).

To prepare concentrated compound solutions, synthesized or purchased pyrazolone derivatives were dissolved using the same method as described below (see "Electrochemical measurement"). In this case, 0.9% NaCl solution was used instead of 50 mM NaCl solution to dilute the concentrated solution.

Table S1. Cytotoxicity of pyrazolone derivatives.



Compound	R^1	R^3	R^4	Cytotoxicity	
				LD_{50} (M)[a]	MC (M)[c]
Edaravone	Ph	CH_3	H	—	—
1	Cyclohexyl	CH_3	H	$>10^{-6}$	$>10^{-6}$
2	4-CH_3OPh-	CH_3	H	$>10^{-6}$	$>10^{-7}$
3	4-ClPh-	CH_3	H	$>10^{-6}$	$>10^{-7}$
4	Ph	Isopropenyl	H	$>10^{-6}$	$>10^{-6}$
5	Ph	CH_3OCONH-	H	$>10^{-6}$	$>10^{-6}$
6	Ph	PhOCONH-	H	n.d.[b]	$>10^{-6}$
7	Ph	PhCONH-	H	$>10^{-6}$	$>10^{-6}$
8	Ph	CyclopentylNHCONH-	H	$>10^{-6}$	$>10^{-6}$
9	Ph	PhNHCO-	H	$>10^{-6}$	$>10^{-6}$
10	Ph	$PhCH_2$-	H	3×10^{-7}	$>10^{-6}$
11	Ph	Ph	H	$>10^{-6}$	$>10^{-7}$
12	Ph	4-CH_3OPh-	H	$>10^{-6}$	$>10^{-7}$
13	Ph	4-NO_2Ph-	H	$>10^{-6}$	$>10^{-6}$
14	Ph	CF_3	H	$>10^{-6}$	$>10^{-6}$
15	Ph	CH_3	Cyclopropyl	$>10^{-6}$	$>10^{-6}$
16	Ph	CH_3	Isobutyl	$>10^{-6}$	$>10^{-6}$
17	Ph	CH_3	Ph	$>10^{-6}$	$>10^{-7}$
18	Ph	CH_3	PhCO-	$>10^{-6}$	$>10^{-6}$
19	Ph	CH_3	2-Pyridyl	$>10^{-6}$	$>10^{-6}$

[a] LD_{50}, approximate concentration of compound required to reduce relative survival to 50% of controlled NB69 cells. [b] n.d., not determined. [c] MC, maximal concentration that does not obviously affect the rate of ScN2a and F3 cells' growth to confluence.

Physicochemical analysis:

Chemicals. Hydrogen peroxide (H_2O_2) and 2-amino-2-hydroxymethyl-1,3-propanediol (Tris) were purchased from Wako Pure Chemical Industries, and 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) was from Dojindo Laboratories (Kumamoto, Japan). All reagents used for the measurement of radical scavenging ability and electrochemical properties were of analytical or spectrometry grade. All other reagents and solvents were purchased from Tokyo Chemical Industry Co., Ltd., Wako Pure Chemical Industries, Ltd., or Kanto Chemical Co.

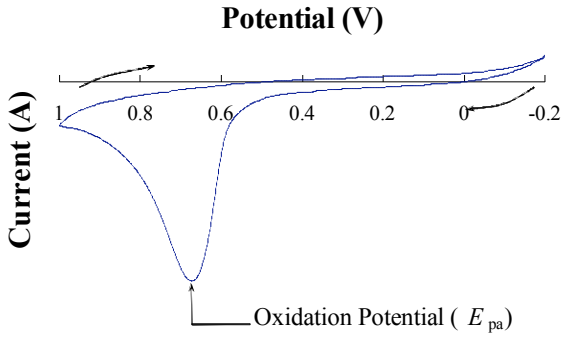
Electrochemical measurement.

Electrochemical experiments were carried out in deoxygenated salt solution (50 mM NaCl at a pH range of 7 to 8). The electrochemical instrumentation used for the measurements included an ALS/CH Instruments Electrochemical Analyzer model 612A connected to a PC. Platinum electrodes (ALS/CH Instruments) were used as working and counter electrodes. Potentials were measured relative to the Ag/AgCl/NaCl reference electrode (ALS/CH Instruments).

The electrochemical technique used was cyclic voltammetry employing a scan rate of 50 mV/s. Anodic peak potentials and currents were recorded as the relative oxidizing potentials and amounts of oxidizing species.

To prepare concentrated solutions, synthesized and purchased pyrazolone derivatives were dissolved in a small amount of 1 M NaOH, and the alkaline solutions were diluted with MilliQ water and neutralized with 1 M HCl. For electrochemical measurements, the concentrated solutions were diluted to the desired concentrations with 50 mM NaCl solution.

Figure S2. Cyclic voltammogram of compound **14** as a representative voltammogram of synthesized edaravone derivatives. The oxidation current was observed, but the voltammogram was irreversible.



ESR spin trapping experiments.

Hydrogen peroxide (25 mM) in Tris-HCl buffer (pH7.4) containing 12.5 mM NaCl was irradiated with 200 mJ/cm^2 of ultraviolet light in the presence of DMPO (25 mM) and various concentrations of a test compound, using a UV-crosslinker (American Life Science). The resulting solutions were subjected to ESR measurements. ESR measurements, which were carried out on a JES-RE2X ESR spectrometer (JEOL, Co., Ltd., Tokyo, Japan), with a LABOTEC LLC-04B as a flat cell. The instrument was operated at 9.4 GHz and 337 mT field modulation, and was equipped with a TE102 cavity. Typical spectrometer parameters were: microwave power, 10 mW; modulation width, 0.063 mT; time constant, 0.03 s; sweep width, 7.5 mT; sweep time, 1 min; gain, 320. Data were collected using WinRad software (Radical Research Inc., Tokyo, Japan).

The concentration of 50% inhibition of the DMPO-OH adduct formation (IC_{50}) was calculated from the signal intensities of ESR spectra of the DMPO-OH adduct.

Figure S3. ESR spectra for hydroxyl radical adducts of DMPO in the absence of antioxidant (A), in the presence of 0.25 mM of edaravone (B), and in the presence of 0.25 mM of **13** (C).

Copper-complexing study.

UV-Visible absorption spectra of derivatives with or without $Cu(ClO_4)_2$ were recorded on an Agilent 8453 spectrophotometer (Agilent Technologies, CA, U.S.A.).

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