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Endoplasmic reticulum (ER) stress protecting compounds from the mushroom *Mycoleptodonoides aitchisonii*

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ABSTRACT

Two novel compounds, 3-(hydroxymethyl)-4-methylfuran-2(5*H*)-one (**1**) and (3*R*,4*S*,1′*R*)-3-(1′-hydroxyethyl)-4-methyldihydrofuran-2(3*H*)-one (**2**), were isolated along with two known ones (**3** and **4**) from an edible mushroom *Mycoleptodonoides aitchisonii*. The structures of **1**–**4** were determined by the interpretation of spectroscopic data. Compounds **1**–**4** showed protective activity against endoplasmic reticulum (ER) stress-dependent cell death.

1. Introduction

Neuronal cell death is an essential feature of neurodegenerative diseases. Many types of neuronal cell death are induced by endoplasmic reticulum (ER) stress. ER is an extensive membranous network that provides a unique environment for the synthesis, folding, and modification of secretory and cell surface proteins. Certain pathological stress conditions can disrupt homeostasis in the ER, causing loss of the ER intraluminal oxidative environment and depletion of intracellular calcium stores, and lead to accumulation of misfolded proteins in the ER.[1] The condition is referred to as ER stress, which results in apoptosis of neural cells in the brain. It is shown as a major cause for a wide variety of neurological disorders such as Alzheimer's disease, Parkinson's disease, and polyglutamine disease.[2–4] ER stress has been reported to cause not only neurodegenerative diseases but also some other diseases, such as diabetes, atherosclerosis, or heart and liver disease.[5] Therefore, the protective activity against ER stress is an important target for the cure or prevention of these diseases. ER stress responses can be mainly divided into two phases, cell survival signals and cell death signals. The former survival signals include up-regulation of ER chaperones, translational repression, and stimulation of ER-associated degradation (ERAD). When the functions of ER are severely impaired, the latter cell death signals, such as up-regulation of Chop [C/EBP (CCAAT/enhancer-binding protein) homologous protein] and activation of caspase-12, are induced.[6] Thus, two protective mechanisms can be considered, namely stimulation of protecting signals and inhibition of cell death signals. Regarding the former mechanism, some chemical and pharmacological chaperones, which are small molecules functioning like proteinous molecular chaperones, have been developped.[7] Concerning the latter mechanism, we previously reported the protective function of dilinoleoylphosphatidylethanolamine (DLPE) that was isolated from the mushroom *Hericium erinaceum*.[8] DLPE inhibited one of the cell death signals without affecting protective signals (unpublished data). However, the activity of the compound was very weak.

The demand for new protective substances prompted us to screen the protective activity of the mushroom extracts, and we found the protective activity in the extract of the mushroom *Mycoleptodonoides aitchisonii*, whose enhancing effect on the synthesis of NGF and catecholamine metabolites in the rat brain had been reported.[9,10] We tried to isolate the active principles and succeed in isolation of two new compounds and two known ones.

2. Results and discussion

The fresh fruiting bodies of *M. aitchisonii* were extracted with EtOH and acetone, successively. The extract of the mushroom was divided into a CH_2Cl_2 soluble fraction, an EtOAc soluble fraction, and a water soluble fraction. Since only the CH_2Cl_2 soluble fraction showed the protective activity against ER stress-dependent cell death, this fraction was repeatedly subjected to column

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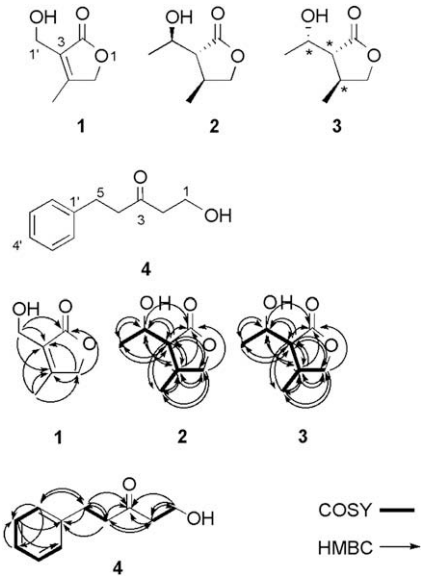

Figure 1. COSY and HMBC correlations of **1**–**4**.

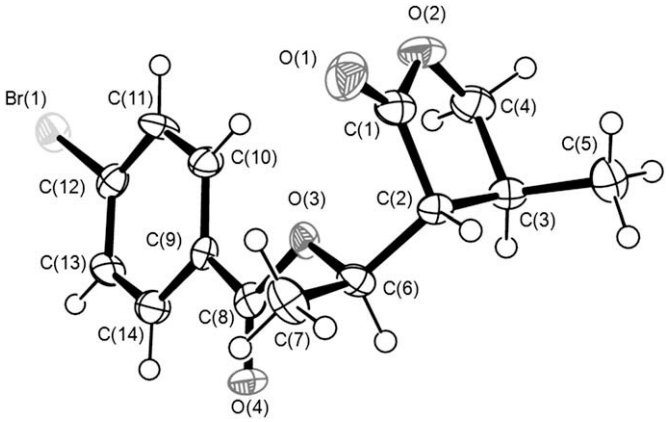

Figure 2. A stereo ORTEP of *p*-bromobenzoate of **2**.

chromatography, being guided by the result of the bioassay. As a consequence, two new compounds (**1** and **2**) and two known ones (**3** and **4**) were purified.

Compound **1** was purified as colorless oil. Its molecular formula was determined as $C_6H_8O_3$ by HRESIMS. The structure of **1** was elucidated by interpretation of 2D NMR spectra including HMBC and HMQC (Fig. 1). The complete assignment of all the protons and carbons was accomplished as shown in Table 1. The furanone ring was constructed by the HMBC correlations (H5/C2, H5/C3, H5/C4). The connection between the hydroxymethyl group and furanone ring was determined by the HMBC correlation (H1′/C2, H1′/C3, H1′/C4). The methyl was determined to be connected to C4 by the HMBC (C4-*Me*/C3, C4-*Me*/C4, C4-*Me*/C5). All the data allowed us to conclude that **1** was 3-hydroxymethyl-4-methylfuran-2(5*H*)-one.

Compound **2** was isolated as colorless oil. Its molecular formula was determined as $C_7H_{12}O_3$ by HRESIMS. The structure elucidation using NMR was accomplished in the same manner as **1** (Fig. 1 and Table 1). The γ-lactone moiety was elucidated by the HMBC correlations (H3/C2, H3/C4, H3/C5, H4/C2, H4/C3, H4/C5, H5/C2, H5/C3, H5/C4). The presence of the hydroxyethyl group was elucidated by downfield-shifted chemical shift of position 1′ (δ_H 3.93, δ_C 67.5) and the COSY correlation (H1′/H2′). The connection between the hydroxyethyl and the γ-lactone moieties was determined by the COSY correlation (H1′/H2′) and the HMBC correlations (H1′/C2, H1′/C3, H1′/C4, H2′/C3). The position of C4-Me was determined by the COSY (H4/C4-*Me*) and the HMBC (C4-*Me*/C3, C4-*Me*/C4, C4-*Me*/C5) correlations. As a result, the plane structure of **2** was determined to be 3-(1′-hydroxyethyl)-4-methyldihydrofuran-2(3*H*)-one. The absolute configuration of **2** was determined as 3*R*,4*S*,1′*R* by X-ray crystallography analysis on its *p*-bromobenzoate (Fig. 2).

Compound **3** was purified as colorless oil. This compound, which was an diastereomer of **2**, has been reported and the structure including relative stereochemistry was determined as (3*R**,4*S**,1′*S**)-3-hydroxyethyl-4-methyldihydrofuran-2(3*H*)-one by X-ray crystallography analysis.[11] In this study, the plane structure was confirmed using NMR and HRESIMS (Fig. 1 and Table 1).

Compound **4** was isolated as colorless oil. Its molecular formula was determined as $C_{11}H_{14}O_2$ by HRESIMS. The structure of **4** was elucidated by interpretation of 2D NMR spectra including COSY, HMBC, and HMQC (Fig. 1). The complete assignment of all the protons and carbons was accomplished as shown in Table 1. The presence of the benzene ring was suggested by the COSY correlations (bold line in Fig. 1) and the HMBC correlations (H2′/C3′, H2′/C4′, H3′/C1′, H3′/C6′, H4′/C3′). The 1-hydroxy-3-pentanone moiety was elucidated by the COSY correlations (H1/H2, H4/H5) and the HMBC correlation (H1/C2, H1/C3, H2/C1, H2/C3, H2/C4, H4/C2, H4/C3, H4/C5, H5/C3, H5/C4). The connection between the benzene and pentanone moieties was determined by the HMBC correlations (H4/C1′, H5/C1′, H5/C2′, H2′/C5). Compound **4** has been previously reported as an intermediate of organic synthesis but its spectroscopic data have not been given in the report.[12] This is the first report of isolation of **4** from nature.

Compounds **1**–**4** were subjected to the protective activity assay against ER stress-dependent cell death caused by tunicamycin or thapsigargin (Fig. 3). ER stress was induced by addition of tunicamycin or thapsigargin into the culture medium of Neuro2a cells in the presence or absence of these compounds. Tunicamycin is an inhibitor of N-glycosylation to glycoproteins in the ER and causes

Table 1
^{1}H and ^{13}C NMR data for **1** to **4** (in $CDCl_3$)

Position	**1**		**2**		**3**		Position	**4**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C		δ_H	δ_C
1	—	—	—	—	—		1	3.82 (2H, t, 5.3)	57.8
2	—	174.6	—	179.1	—	178.5	2	2.63 (2H, t, 5.3)	44.6
3	—	125.9	2.11 (1H, dd, 6.9, 9.9)	53.0	2.28 (1H, dd, 3.8, 9.3)	53.7	3	—	210.6
4	—	159.5	2.41 (1H, m)	33.1	2.65 (1H, m)	31.0	4	2.76 (2H, t, 7.5)	44.8
5	4.66 (2H, br s)	72.9	4.37 (1H, dd, 8.4, 9.1)	72.9	4.39 (1H, dd, 8.3, 8.9)	73.2	5	2.90 (2H, t, 7.5)	29.5
	—		3.71 (1H, dd, 9.1, 9.1)		3.73 (1H, dd, 8.9, 8.9)				
1′	4.36 (2H, d, 5.5)	54.6	3.93 (1H, m)	67.5	4.28 (1H, m)	66.0	1′	—	140.7
2′	—	—	1.29 (3H, d, 6.4)	20.8	1.29 (3H, d, 6.9)	20.4	2′6′	7.16 (2H, d, 7.6)	128.3
4-CH_3	2.09 (3H, s)	12.2	1.15 (3H, d, 6.5)	17.2	1.18 (3H, d, 6.9)	18.0	3′5′	7.26 (2H, dd, 7.6, 7.9)	128.5
							4′	7.18 (1H, t, 7.9)	126.2

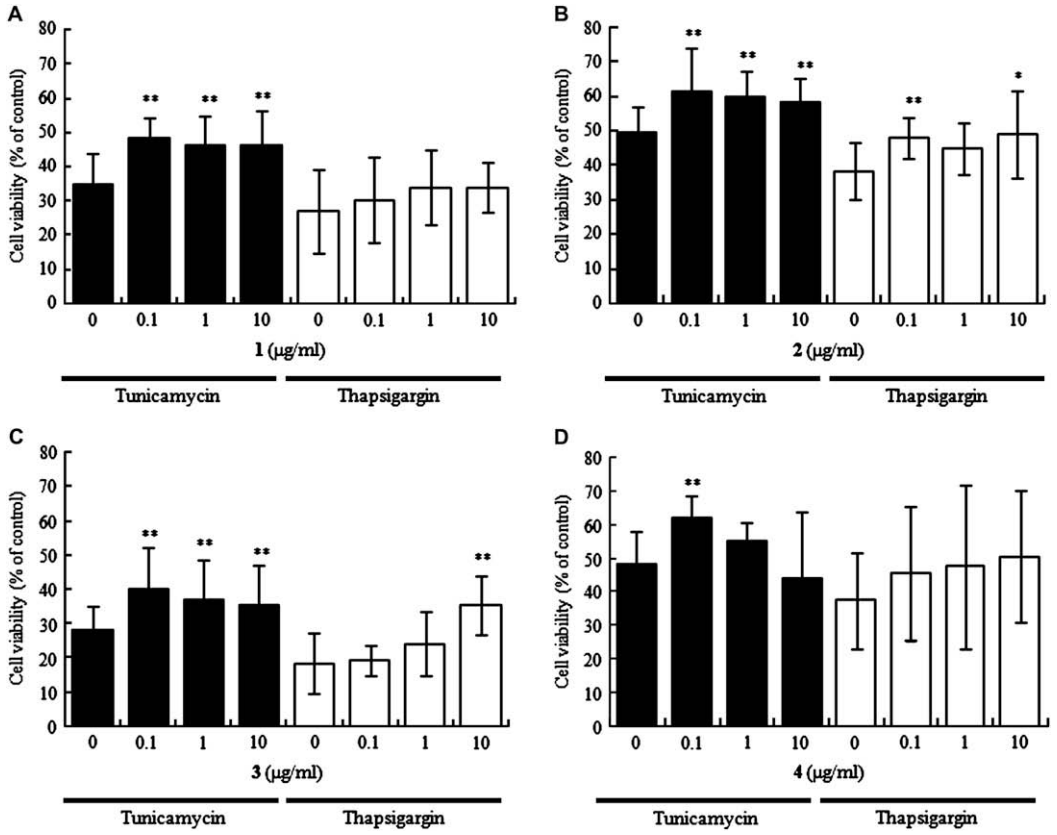

Figure 3. Protective effects of **1**–**4** on ER stress-induced cell death. Neuro2a cells were incubated with various concentrations of compounds **1** (A), **2** (B), **3** (C), and **4** (D) in the presence of 0.5 mg/ml of tunicamycin (closed column) or 20 nM thapsigargin (open column) for 24 h. After treatment, cell viabilities were analyzed by MTT assay. Data are the mean ± SE of two cultures (*$p<0.05$, **$p<0.01$ vs control using Student's *t*-test).

protein misfolding in the ER. Thapsigargin is an ER Ca^{2+}-ATPase inhibitor that causes Ca^{2+} depletion in the ER. Compounds **1**–**4** showed the protective activity against tunicamycin toxicity at 0.1 μg/mL. In the case of the assay using thapsigargin, **2** and **3** showed the activity at 0.1 μg/mL and 10 μg/mL, respectively. However, **1** and **4** did not show any activity even at the concentrations up to 10 μg/mL. Although it has been known that tunicamycin and thapsigargin share common ER stress associated signals,[5] a few paper reported that tunicamycin and thapsigargin induced distinct signaling.[13,14] Our data suggested that **2** and **3** protected the cells by affecting the common ER stress signaling pathway, and **1** and **4** inhibited the tunicamycin specific stress signaling.

3. Experimental

3.1. General

^{1}H NMR spectra (one- and two-dimensional) were recorded on a JEOL lambda-500 spectrometer at 500 MHz, while ^{13}C NMR spectra were recorded on the same instrument at 125 MHz. The HRESIMS spectra were measured on a JMS-T100LC mass spectrometer. A JASCO grating infrared spectrophotometer was used to record the IR spectra. The $[\alpha]_D$ spectra were measured by using a JASCO DIP-1000 spectropolarimeter. HPLC separations were performed with a JASCO Gulliver system using normal-phase HPLC columns (Senshu PAK AQ, Senshu scientific Co., Ltd, Japan, Develosil silica-hilic, Nomura chemical Co., Ltd, Japan). Silica gel plate (Merck F_{254}) and silica gel 60 N (Merck 100–200 mesh) were used for analytical TLC and for flash column chromatography, respectively.

3.2. Fungus materials

Mature fruiting bodies of *M. aitchisonii* were collected at Narusawa village, Yamanashi Prefecture in Japan, in August 2006.

3.3. Extraction and isolation

The fresh fruiting bodies of *M. aitchisonii* (13.0 kg) were extracted with EtOH (21 L, three times) and then acetone (10 L). The combined solution was under reduced pressure and partitioned between CH_2Cl_2 and H_2O and then between EtOAc and H_2O. The CH_2Cl_2 soluble part (73.6 g) was fractionated by silica gel flash column chromatography (hexane/CH_2Cl_2 7:3; hexane/EtOAc 9:1, 5:5; CH_2Cl_2/MeOH 8:2, 5:5, 0:10, 2 L each) to obtain 26 fractions, and fraction 19 (4.9 g) was further separated by silica gel flash column chromatography (CH_2Cl_2/acetone 9:1, 7:3, 3:7; acetone/MeOH 5:5; and MeOH, 1 L each), and then fraction 19-8 (1.0 g) was further separated by silica gel flash column chromatography (CH_2Cl_2/acetone 9:1, 8:2; CH_2Cl_2/MeOH 9:1, 8:2; and EtOH, 1 L each). Compound **1** (5.7 mg) was purified from fraction 19-8-6 (613.2 mg) by normal-phase HPLC (Develosil silica-hilic, $CHCl_3$/MeOH 98:2). Similarly, fraction 19-7 (823.0 mg) was further separated by silica gel flash column chromatography (CH_2Cl_2/acetone 9:1, 7:3, 3:7; CH_2Cl_2/MeOH 9:1, 8:2; and EtOH, 1 L each), and 11 fractions were obtained. Fraction 19-7-6 (140.7 mg) was further separated by normal-phase HPLC (Senshu PAK AQ, hexane/$CHCl_3$ 9:1) to afford compounds **2** (31.8 mg) and **4** (1.9 mg). Compound **3** (7.6 mg) was obtained from fraction 19-7-7 (131.5 mg) by normal-phase HPLC (Senshu PAK AQ, hexane/$CHCl_3$ 9:1).

3.3.1. Compound ***1***

Colorless oil; IR (neat): 3397, 1734 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; ESIMS *m*/*z* 151 $[M+Na]^+$; HRESIMS *m*/*z* 151.0348 $[M+Na]^+$ (calcd for $C_6H_8NaO_3$, 151.0371).

3.3.2. Compound ***2***

Colorless oil; $[\alpha]_D^{27}$ −32.2 (*c* 1.00, $CHCl_3$); IR (neat): 3463, 1759 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; ESIMS *m*/*z* 167 $[M+Na]^+$; HRESIMS *m*/*z* 167.0639 $[M+Na]^+$ (calcd for $C_7H_{12}NaO_3$, 167.0684).

3.3.3. Compound ***3***

Colorless oil; $[\alpha]_D^{27}$ +33.0 (*c* 0.25, $CHCl_3$); IR (neat): 3461, 1756 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; ESIMS *m*/*z* 167 $[M+Na]^+$; HRESIMS *m*/*z* 167.0639 $[M+Na]^+$ (calcd for $C_7H_{12}NaO_3$, 167.0684).

3.3.4. Compound ***4***

Colorless oil; IR (neat): 3412, 1709 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; ESIMS *m*/*z* 201 $[M+Na]^+$; HRESIMS *m*/*z* 201.0892 $[M+Na]^+$ (calcd for $C_{11}H_{14}NaO_2$, 201.0878).

3.4. Preparation of *p*-bromobenzoate of 2

Compound **2** (2.0 mg) was dissolved in 0.25 mL anhydrous pyridine in a 4 mL vial and *p*-bromobenzoyl chloride (15.1 mg) was added to the solution. After shaking at 50 °C for 2 weeks, the reaction mixture was evaporated to dryness under reduced pressure. The products were then purified by normal-phase HPLC (Senshu PAK AQ, hexane/$CHCl_3$ 1:1) to give the *p*-bromobenzoate (3.5 mg) of **2**. *p*-Bromobenzoate of **2**: ^1H NMR (500 MHz, in $CDCl_3$) δ 7.85 (2H, d, *J*=8.5 Hz, COC_6H_4-*p*-Br), 7.56 (2H, d, *J*=8.5 Hz, COC_6H_4-*p*-Br), 5.51 (1H, m, H1′), 4.35 (1H, dd, *J*=8.1, 8.5 Hz, H5), 3.74 (1H, dd, *J*=8.5, 8.5 Hz, H5), 2.54 (2H, m, H3 and H4), 1.54 (3H, d, *J*=6.4 Hz, H2′), 1.19 (3H, d, *J*=6.4 Hz, 4-CH_3); ESIMS *m*/*z* 349 $[M+Na]^+$; HRESIMS *m*/*z* 349.0019 $[M+Na]^+$ (calcd for $C_{14}H_{15}BrNaO_4$, 349.0051).

3.5. X-ray crystallography analysis

Crystal data for **2**: $C_{14}H_{15}BrO_4$, *M*=327.17, orthorhombic, *a*=7.3821(10) Å, *b*=10.0598(13) Å, *c*=18.504(3) Å, *V*=1374.1(3) $Å^3$, *T*=150 K, space group $P2_12_12_1$, *Z*=4, λ=0.71073 Å, μ(Mo Kα)=2.998 mm^{-1}, *F*(000)=664. The size of the crystal used for measurements was 0.25×0.09×0.05 mm. Diffraction data were collected on a Rigaku AFC8 diffractometer with Satern70 CCD detector. 5938 reflections were collected in the range $2.30<\theta<27.48$, of which 3066 unique (R_{int}=0.0619) reflections. The structure was refined by full-matrix least-squares procedure on F^2 values using all unique reflections. The final *R* indices were $R(F)$=0.0583, $wR(F^2)$=0.0769 (1865 reflections with $I>2\sigma(I)$) with goodness-of-fit=0.971. Crystallographic data for **2** has been deposited at The Cambridge Crystallographic Data Centre and allocated the deposition number, CCDC 701134. The data can be obtained free of charge via www.ccdc.cam.ac.uk/products/csd/request.

3.6. Cell culture

Neuro2a cells were obtained from the Health Science Research Resources Bank, Japan and maintained in the Dulbecco's modified Eagles medium (D-MEM) (SIGMA, USA) supplemented with 10% fetal bovine serum (FBS), unless particularly noted.

3.7. Cell viability

Cell viability analysis was performed by 3-(4,5-dimethyl-2-thiazolyl) 2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay.

MTT assay was performed as follows. Neuro2a cells were cultured in 96-well plates at cell density 5000 cells/well. After one-day cultivation, the cells were cultured in D-MEM without FBS, and 0.5 μg/ml of tunicamycin (or 20 nM thapsigargin) and varying concentrations of compounds **1**–**4** were applied to the medium. The cells were incubated for 24 h, and then the viability was measured by MTT assay, as described previously.[15] Briefly, 0.25 mg/ml of MTT in D-MEM without FBS were added onto the cells and incubated for 2 h. The incubation was terminated by addition of 20% SDS (v/w) and 50% dimethylformamide (v/v) in water. The absorbance at 570 nm of the reaction mixture was measured by a microplate reader (Molecular Devices, USA).

3.8. Statistical analysis

Data were statistically analyzed using Student's *t*-test to determine significant difference in the data among the groups. *P* values less than 0.05 were considered significant. The values are expressed as mean±SE.

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References and notes

1. Kaufman, R. J. *Genes Dev.* **1999**, *13*, 1211–1233.
2. Katayama, T.; Imaizumi, K.; Sato, N.; Miyoshi, K.; Kudo, T.; Hitomi, J.; Morihara, T.; Yoneda, T.; Gomi, F.; Mori, Y.; Nakano, Y.; Takeda, J.; Tsuda, T.; Itoyama, Y.; Murayama, O.; Takashima, A.; St George-Hyslop, P.; Takeda, M.; Tohyama, M. *Nat. Cell Biol.* **1999**, *1*, 479–485.
3. Tamatani, M.; Matsuyama, T.; Yamaguchi, A.; Mitsuda, N.; Tsukamoto, Y.; Taniguchi, M.; Che, Y. H.; Ozawa, K.; Hori, O.; Nishimura, H.; Yamashita, A.; Okabe, M.; Yanagi, H.; Stern, D. M.; Ogawa, S.; Tohyama, M. *Nat. Med.* **2001**, *7*, 317–323.
4. Kouroku, Y.; Fujita, E.; Jimbo, A.; Kikuchi, T.; Yamagata, T.; Momoi, M. Y.; Kominami, E.; Kuida, K.; Sakamaki, K.; Yonehara, S.; Momoi, T. *Hum. Mol. Genet.* **2002**, *11*, 1505–1515.
5. Yoshida, H. *FEBS J.* **2007**, *274*, 630–658.
6. Oyadomari, S.; Mori, M. *Cell Death Differ.* **2004**, *11*, 381–389.
7. Loo, T. W.; Clarke, D. M. *Expert Rev. Mol. Med.* **2007**, *9*, 1–18.
8. Nagai, K.; Chiba, A.; Nishino, T.; Kubota, T.; Kawagishi, H. *J. Nutr. Biochem.* **2006**, *17*, 525–530.
9. Okuyama, S.; Lam, N. V.; Hatakeyama, T.; Terashima, T.; Yamagata, K.; Yokogoshi, H. *Nutr. Neurosci.* **2004**, *7*, 341–349.
10. Okuyama, S.; Sawasaki, E.; Yokogoshi, H. *Nutr. Neurosci.* **2004**, *7*, 107–111.
11. Fujii, I.; Kawagishi, H.; Hirayama, N. *Anal. Sci.* **1998**, *14*, 867–868.
12. Marcantoni, E.; Nobili, F.; Bartoli, G.; Bosco, M.; Sambri, L. *J. Org. Chem.* **1997**, *62*, 4183–4184.
13. Hsieh, Y. H.; Su, I. J.; Lei, H. Y.; Lai, M. D.; Chang, W. W.; Huang, W. *Biochem. Biophys. Res. Commun.* **2007**, *359*, 643–648.
14. Sasaya, H.; Utsumi, T.; Shimoke, K.; Nakayama, H.; Matsumura, Y.; Fukunaga, K.; Ikeuchi, T. *J. Biochem.* **2008**, *144*, 251–257.
15. Liu, Y.; Peterson, D. A.; Kimura, H.; Schubert, D. *J. Neurochem.* **1997**, *69*, 581–593.