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Neuroprotective constituent from the seeds of *Alpinia katsumadai* Hayata

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ABSTRACT

Two new compounds, named as rhamnocitrin-3-*O*-β-D-glucopyranosyl-4′-*O*-β-D-galactosyl-(1→3)-*O*-*β*-D-glucopyranoside (**1**), and (3*R*)-5,6,7-trihydroxy-3-isopropyl-3-methylisochroman-1-one (**2**), were isolated from the seeds of *Alpinia katsumadai* Hayata. Their structures were elucidated on the basis of spectroscopic analysis. Additionally, compounds **2** exhibited potent neuroprotective activity on 1-methyl-4-phenylpyridinium-induced oxidative damage in PC12 cells.

1. Introduction

Alpinia katsumadai Hayata is mainly distributed in the Southern and Southeast Asia (Li et al., 2010; Ngo and Brown, 1998). Previous chemical investigations on this plant have resulted in the isolation of various constituents such as flavonoids, kavalactones, diarylheptanoids, stilbenes, monoterpenes and sesquiterpenes (Nam and Seo, 2012). Some of these compounds have anti-emetic, antiviral, anti-oxidant, anti-tumor and cytoprotective activities (Grienke et al., 2010; Jeong et al., 2007; Li et al., 2011; Xu et al., 2013; Yang et al., 1999). This paper described the isolation and characterization of two new compounds, rhamnocitrin-3-*O*-β-D-glucopyranosyl-4′-*O*-β-D-galactosyl-(1→3)-*O*-*β*-D-glucopyranoside (**1**), and (3*R*)-5,6,7-trihydroxy-3-isopropyl-3-methylisochroman-1-one (**2**) from the seeds of *Alpinia katsumadai* Hayata (Fig. 1). Oxidative stress has been considered as a main factor in the pathogenesis of neural diseases, which can be caused by cytotoxic agents (Lee et al., 2005; Luo et al., 2012). Exposure of neurons to such cytotoxic agents will result in the activation of intracellular toxic events to increase the mitochondrial membrane permeability, release cytochrome-c from the mitochondrial to nucleus, activate caspase-related apoptotic proteins and facilitate the formation of apoptosome complex, finally resulting in DNA damage and neuronal cell death (Leuner et al., 2007). Supplementation of exogenous antioxidants has their effects in curtailing the oxidative damage to cellular macromolecules (Wu et al., 2015). This study was conducted to find new neuroprotective agents against oxidative stress-induced neurodegeneration.

2. Results and discussion

Compound **1** was obtained as a yellow amorphous powder. Its molecular formula $C_{34}H_{42}O_{21}$ was deduced from HR-ESI–MS. The IR spectrum showed the presence of the carbonyl group(s) (1675 cm^{-1}), hydroxyl group(s) (3365 cm^{-1}), and aromatic ring (s) (1553, 1462 cm^{-1}). The UV spectrum at 262 and 338 nm showed the characteristic signals of a flavonoid. The ^{1}H NMR data of **1** (Table 1) showed the signal of hydroxyl group at δ 12.59 (1H, s, H-5). The spectrum showed four aromatic protons at δ 8.17 (2H, d, J = 9.0 Hz, H-2′, H-6′) and 7.11 (2H, d, J = 9.0 Hz, H-3′, H-5′), suggesting an AA′BB′ system. Additionally, the ^{1}H NMR spectrum also displayed two *meta*-coupled alkene protons at δ 6.71 (1H, d, J = 2.0 Hz, H-8) and 6.42 (1H, d, J = 2.0 Hz, H-6). The acid hydrolysis with HPLC analysis confirmed the presence of D-galactose and D-glucose in the ratio of 1:2 (Tanaka et al., 2007). The proton signals at δ 5.51 (1H, d, J = 7.5 Hz, H-1″), 5.07 (1H, d, J = 7.5 Hz, H-1‴), and 4.25 (1H, d, J = 8.0 Hz, H-1⁗) were attributed to three sugar moieties in the ^{1}H NMR spectrum, which further confirmed that these three sugar residues were β-pyranosyl configurations. One β-D-glucosyl moiety was linked to the aglycone at C-3, indicated by

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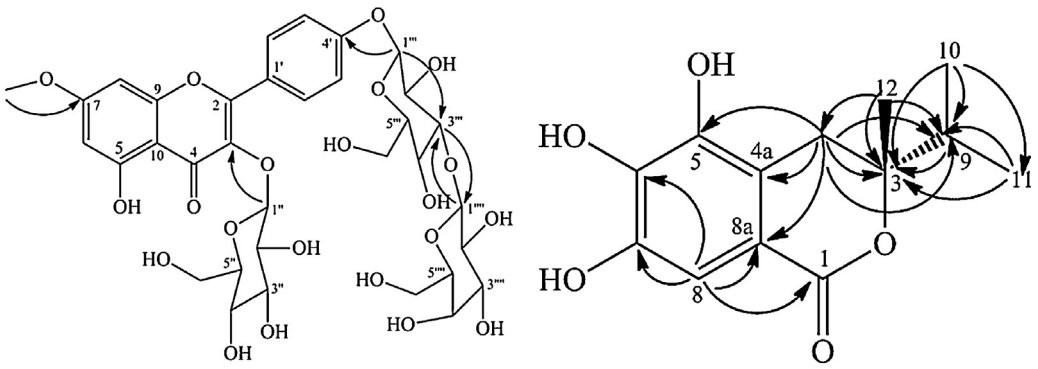

1 **2**

Fig. 1. Structure and key HMBC correlations of compounds **1** and **2**.

Table 1
^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectral data of compounds **1** and **2** in DMSO-d_6 (δ in ppm, J in Hz).

Position	**1**		Position	**2**
	δ_H δ_C			δ_H δ_C
2	–	157.6	1	−164.3
3	–	134.1	3	−84.1
4	–	178.9	4	2.71 (1H, d, J = 13.5) 28.6
5-OH	12.59 (1H, s)	159.5		2.76 (1H, d, J = 13.5)
6	6.42 (1H, d, J = 2.0)	98.9	4a	−113.6
7	–	165.4	5	−143.1
8	6.71 (1H, d, J = 2.0)	93.8	6	−146.3
9	–	156.3	7	−138.8
10	–	105.2	8	6.96 (1H, s) 107.3
1′	–	124.8	8a	−115.8
2′	8.17 (1H, d, J = 9.0)	131.3	9	1.92 (1H, s) 35.8
3′	7.11 (1H, d, J = 9.0)	116.8	10	0.93 (3H, d, J = 6.5) 15.6
4′	–	161.1	11	0.82 (3H, d, J = 6.5) 17.1
5′	7.11 (1H, d, J = 9.0)	116.8	12	1.18 (3H, s) 21.8
6′	8.17 (1H, d, J = 9.0)	131.3	5,6,7-OH	9.52, 9.37, 8.81 (*brs*)
7-OMe	3.89 (3H, s)	56.0		
3-Glc				
1″	5.51 (1H, d, J = 7.5)	101.7		
2″	3.35 (1H, m)	74.9		
3″	3.24 (1H, m)	76.6		
4″	3.19 (1H, m)	70.9		
5″	3.49 (1Hm)	77.2		
6″	3.68 (1H, br d, J = 11.0)	60.3		
4′-Glc	3.52 (1H, m)			
1‴	5.07 (1H, d, J = 7.5)	100.8		
2‴	2.99 (1H, m)	73.3		
3‴	3.31 (1H, m)	80.0		
4‴	3.02 (1H, m)	70.1		
5‴	3.34 (1H, m)	77.8		
6‴	3.61 (1H, m)	61.9		
	3.46 (1H, m)			
3‴-Gal				
1⁗	4.25 (1H, d, J = 8.0)	103.2		
2⁗	3.36 (1H, m)	75.8		
3⁗	3.23 (1H, m)	73.3		
4⁗	3.15 (1H, m)	77.6		
5⁗	3.45 (1H, m)	77.1		
6⁗	3.75 (1H, m)	61.9		
	3.39 (1H, m)			

the HMBC correlation from δ 5.51 (H-1″) to δ 134.1 (C-3). The HMBC correlations from δ 5.07 (H-1‴) to δ 161.1 (C-4′), from δ 4.25 (H-1⁗) to δ 80.0 (C-3‴), and from δ 3.31 (H-3‴) to δ 103.2 (C-1⁗) exhibited that the remaining β-D-glucosyl unit was attached to C-4′, and the β-D-galactosyl moiety was linked to C-3‴. The HMBC correlation from a methoxyl proton signal δ 3.89 (3H, s) to δ 165.4 (C-7) revealed that a methoxyl unit was linked to aglycone at C-7. Therefore, the structure of compound **1** was elucidated as rhamnocitrin-3-*O*-β-D-glucopyranosyl-4′-*O*-β-D-galactosyl-(1 → 3)-*O*-*β*-D-glucopyranoside.

Compound **2** was obtained as a white amorphous powder. Its HR-ESI–MS and NMR data gave the molecular formula $C_{13}H_{16}O_5$, which indicated 6 ° of unsaturation. Compound **2** appearing purple in the 10% ethanol sulfate solution and showing positive reaction with $FeCl_3$-$K_3[Fe(CN)_6]$, together with the IR spectrum at 3361, 3283 cm^{-1}, indicated the presence of phenolic hydroxyl group. The UV spectrum at 233 and 281 nm and the IR spectrum (1711, 1633, 1451, 1342, 859 cm^{-1}) showed the presence of the aromatic ring and conjugated ketone. The ^{1}H NMR spectrum (Table 1) of **2** exhibited one aryl proton signal at δ 6.96 (1H, s, H-8), two methylene signals at δ 2.71 (1H, d, J = 13.5 Hz, H-4α) and δ 2.76 (1H, d, J = 13.5 Hz, H-4β), one methine signal at δ 1.92 (1H, d, H-9), three methyls at δ 0.93 (3H, d, J = 6.5 Hz, H-10), δ 0.82 (3H, d, J = 6.5 Hz, H-11), δ 1.18 (3H, s, H-12), and three hydroxyl groups at δ 9.52 (1H, brs, H-5), δ 9.37 (1H, brs, H-6), δ 8.81 (1H, brs, H-7). ^{13}C NMR spectrum (Table 1) showed 13 signals, associated with one carbonyl (δ 164.3, C-1); six olefinic carbons including one methine (δ 107.3, C-8), two quaternary carbons (δ 113.6, C-4a; 115.8, C-8a) and three oxygenated quaternary olefinic carbons (δ 143.1, C-5; 146.3, C-6; 138.8, C-7); one quaternary carbon connecting to oxygen (δ 84.1, C-3); one methine (δ 35.8, C-9); one methylene (δ 28.6, C-4) and three methyls (δ 15.6, C-10; 17.1, C-11; 21.8, C-12). The correlation between H-9 and H-10/H-11 in ^{1}H-^{1}H COSY spectrum, and the correlation between H-10, H-11 and C-9, H-10 and C-11 in HMBC spectrum suggested the presence of an isopropyl group. In the HMBC spectrum (Fig. 1), the correlation between H-8 and C-8a/C-7/C-1, indicated the benzene ring connecting an ester. The correlation between H-4 and C-4a/C-8a/C-5, indicated the methylene at C-4a. The correlation between H-12 and C-3, H-9, H-10, H-11 and C-3, indicated both the methyl and isopropyl group at C-3. Based on the ^{13}C NMR and HMBC, C-3 was confirmed as quaternary carbon connecting to oxygen. Combining the unsaturation degree, the lactone ring was deduced in compound **2**. The correlation between H-12 and C4/C-9, H-4 and C-1/C-9 further established the structure of compound **2**. The absolute configuration at C-3 was assigned by comparing the optical rotation of compound **2** ([α]20 D = −55.7) and (3*R*)-3,4-dihydro-3-methyl-5,6,8-trihydroxy-1H-2-benzopyran-1-one ([α]20 D = −61.7) (Krohn et al., 1997). Based on these data, compound **2** was elucidated as (3*R*)-5,6,7-trihydroxy-3-isopropyl-3-methylisochroman-1-one.

Overproduction of ROS can cause lipid peroxidation, protein denaturation, nucleic acid damage, and mitochondrial dysfunction,

thereby impairing cellular function and integrity (Adibhatla and Hatcher, 2010; Wang and Michaelis, 2010). This type of damage has been considered as a major cause of neuronal damages in some neurodegenerative disorders (Birben et al., 2012; Surendran and Rajasankar, 2010). Therefore, the imbalance between the intracellular oxidative and anti-oxidative defense systems, requires the supplement of external antioxidants to eliminate ROS as the potential neuroprotective therapeutics (Xin et al., 2014).

In this study, we demonstrated that compound **2** increased the viability of PC12 cells, and inhibited cellular apoptosis in MPP^+-induced oxidative stress model (Figs. 2–5). Compound **2** effectively protected PC12 cells against MPP^+-induced injury, most possibly through mitochondria pathway, closely related to the presence of hydroxyl groups with powerful antioxidant action and effective free radical-scavenging ability. These results warrant further study as the potential agent for the treatment of neurodegenerative disease.

3. Conclusions

We isolated and identified two new compounds, rhamnocitrin-3-*O*-β-D-glucopyranosyl-4′-*O*-β-D-galactosyl-(1 → 3)-*O*-*β*-D-glucopyranoside (**1**), and (3*R*)-5,6,7-trihydroxy-3-isopropyl-3-methylisochroman-1-one (**2**), from the seeds of *Alpinia katsumadai* Hayata for the first time. Compound **2** exhibited potent neuroprotective activity against MPP^+-induced damage in PC12 cells through preventing apoptosis via mitochondria pathway.

4. Experimental

4.1. General experimental procedure

Optical rotations were measured on P-2000 polarimeter (JASCO). IR spectra were recorded on a PerkinElmer Spectrum One FT-IR spectrometer with KBr pellets. The UV spectra were recorded using a Shimadzu UVmini-1240 spectrophotometer with PhotoMultiplier Tube. The NMR spectra were performed on a Bruker AV-500 spectrometer, using TMS as an internal standard. HR-ESI–MS spectra were measured on a Waters API QSTAR Pular-1 mass spectrometer. HPLC was performed by Shimadzu apparatus (LC-6AD pump, SPD-20A UV detector, YMC ODS-A (20 mm × 250 mm, 10 μm), detection at UV 265 nm). Column chromatography was performed with D101 macroporous resin and reversed-phase C_{18} silica gel (Merck, Germany), and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden).

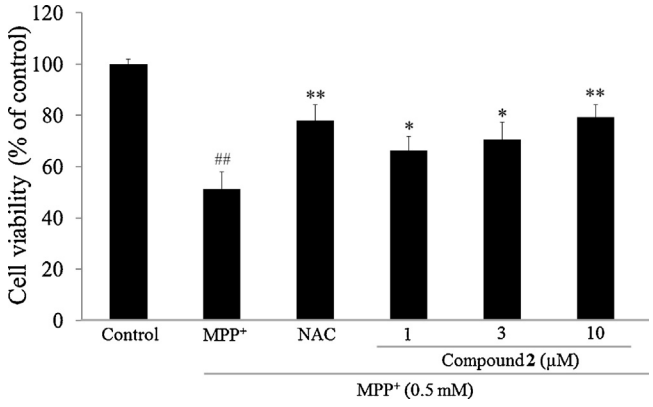

Fig. 2. Effect of compound **2** on the cell viability of MPP^+-damaged PC12 cells by MTT assay. Pre-incubation with compound **2** or NAC (positive control) for 24 h protected PC12 cells from MPP^+ toxicity (0.5 mM, 24 h). NAC: N-acetyl-L-cysteine. Values were expressed as mean ± SD ($n = 5$). $^{\#\#}P < 0.01$ *vs* control group; $^{*}P < 0.05$, $^{**}P < 0.01$ *vs* MPP^+ group.

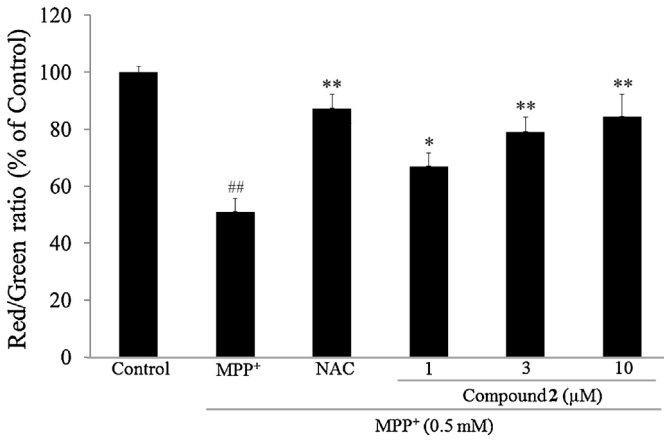

Fig. 3. Effect of compound **2** on mitochondrial membrane potential in MPP^+-damaged PC12 cells assayed by JC-1. Values were expressed as mean ± SD ($n = 5$). $^{\#\#}P < 0.01$ *vs* control group; $^{*}P < 0.05$, $^{**}P < 0.01$ *vs* MPP^+ group.

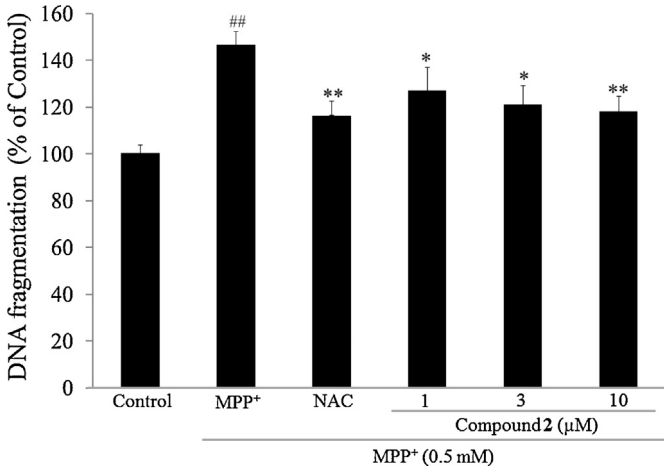

Fig. 4. Effect of compound **2** on DNA fragmentation of MPP^+-damaged PC12 cells. Values were expressed as mean ± SD ($n = 5$). $^{\#\#}P < 0.01$ *vs* control group; $^{*}P < 0.05$, $^{**}P < 0.01$ *vs* MPP^+ group.

4.2. Plant materials

Alpinia katsumadai Hayata was purchased from Anguo City, Hebei province, China, in June 22nd 2015, and authenticated by Prof. Jinkai Liu, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. 20150622) was deposited at The First Affiliated Hospital of Shantou University Medical College.

4.3. Extraction and isolation

Air-dried seeds of *Alpinia katsumadai* Hayata (20.0 kg) were pulverized, and then extracted with 90% ethanol (50 L × 3 × 4 h) under reflux. The plant residue was extracted with distilled water and the solvent was evaporated to afford a residue (A, 1066 g). The 90% ethanol collected was evaporated to yield a crude extract (B, 770 g). The residue A was chromatographed on a D101 macroporous resin (HPD-110, 20 L) column (20 cm × 200 cm) with gradient elution of H_2O/ethanol (100:0–0:100, 50 L) to yield 6 fractions (Fr. 1–6). Fr. 3 (353 g) was chromatographed on a RP C18 silica gel eluted with gradient H_2O/methanol (100:0-0:100) to afford 5 subfractions (Fr. 3.1–3.5). Fr. 3.3 (14.6 g) was purified by preparative HPLC using MeOH/H_2O (40:60, *v*/*v*) as the mobile phase (1.5 mL/min) to afford compound **1** (16.3 mg, $t_R = 24.5$ min). The extract B was subjected to column chromatography eluted

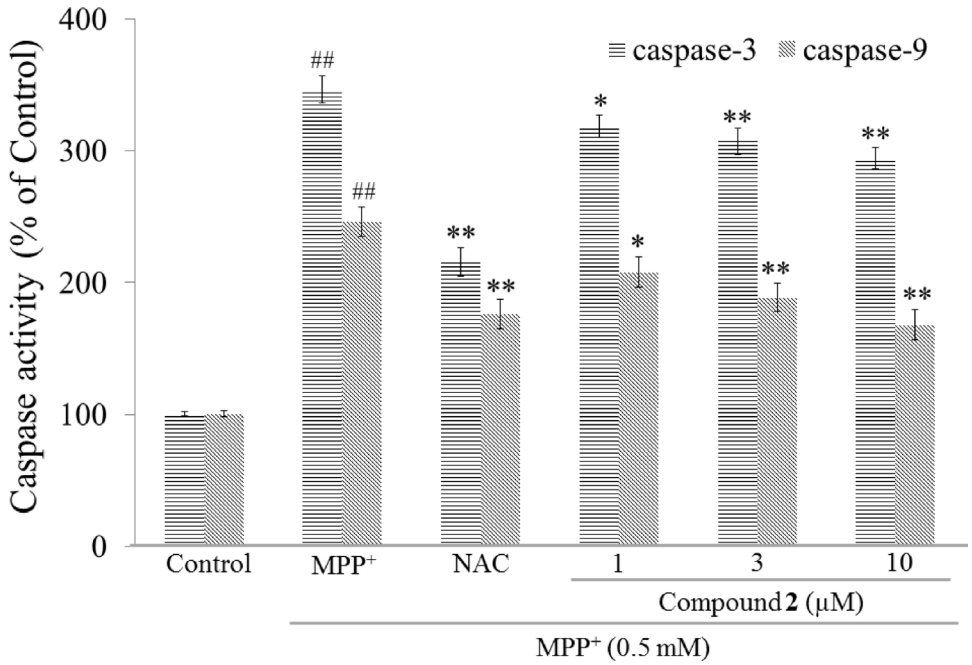

Fig. 5. Effect of compound **2** on caspase activity of MPP^+-damaged PC12 cells. Values were expressed as mean ± SD ($n = 5$). $^{\#\#}P < 0.01$ *vs* control group; $^{*}P < 0.05$, $^{**}P < 0.01$ *vs* MPP^+ group.

with petroleum ether, ethyl acetate, acetone and methanol. The ethyl acetate portion (77.3 g) was chromatographed over a silica gel with gradient elution of petroleum ether/ethyl acetate (100:0–0:100) to obtain 6 fractions (Fr. 1–Fr. 6). Fr. 4 (11.2 g) was subjected to Sephadex LH-20 column chromatography eluted with methanol to give 6 subfractions (Fr. 4.1–Fr. 4.6). Fr. 4.3 (1.8 g) was further purified on HPLC (MeOH/H_2O, 55:45, *v*/*v*, 1.5 mL/min) to give compound **2** (20.2 mg, t_R = 22 min).

4.4. Analytical acid hydrolysis

Compound **1** (1 mg) was hydrolyzed with 2 M HCl at 85 °C for 1 h and then extracted with ethyl acetate. The aqueous layer was evaporated and dissolved in pyridine (1 mL). After adding 1 mg L-cysteine methyl ester hydrochloride, the mixture was heated at 60 °C for 1 h. 20 μL O-tolyl isothiocyanate was then added and the mixture was heated at 60 °C for another 1 h. An aliquot (2 mL) of the supernatant was subjected to HPLC analysis. The mobile phase consisted of acetonitrile and 0.1% formic acid. The sugar residues of compounds **1** were identified by comparing the retention times of the corresponding derivative with the standard D-glucose (t_R = 25.65 min) and D-galactose (t_R = 31.86 min).

4.5. *Rhamnocitrin-3-O-β-D-glucopyranosyl-4′-O-β-D-galactosyl-(1→3)-O-β-D-glucopyranoside* (***1***)

UV (MeOH) λ_{max} (log ε) 338(3.12), 262(3.22) (nm). IR (KBr) ν_{max} 3365, 2878, 1675, 1553, 1462, 1232, 825 and 665 cm^{-1}. ^1H and ^{13}C NMR spectroscopic data see Table 1. HR-ESI–MS: *m*/*z* found 809.2116 $[M+Na]^+$ (cacld. for $C_{34}H_{42}O_{21}Na^+$, 809.2111).

4.6. *(3R)-5,6,7-trihydroxy-3-isopropyl-3-methylisochroman-1-one* (***2***)

[α]20 D = −55.7 (*c* 0.08, $CHCl_3$). UV (MeOH) λ_{max} (log ε): 233 (2.29), 281 (2.26). IR (KBr): ν_{max} 3361, 3283, 2992, 1711, 1633, 1451, 1342, 1015, 859 cm^{-1}. ^1H and ^{13}C NMR spectroscopic data see Table 1. HR-ESI–MS: *m*/*z* found 251.0922 $[M-H]^-$ (calcd. for $C_{13}H_{15}O_5$, 251.0927).

4.7. Cell culture and treatment

Differentiated PC12 cells were maintained in DMEM medium supplemented with 10% FBS, 100 U/mL penicillin G and 100 μg/mL streptomycin at 37 °C in humidified 95% air/5% CO_2. PC12 cells were treated as: cells were incubated for 24 h with compound **2** at 1, 3, 10 μM or *N*-acetyl-L-cysteine (NAC, 10 μM, positive control), respectively; cells treated by DMSO as the negative control. Consequently, PC12 cells were treated by 0.5 mM MPP^+ for 24 h.

4.8. Cell viability measurement

Cell viability was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. After treatment with compound **2** at 1, 3, 10 μM or NAC at 10 μM for 24 h and then 0.5 mM MPP^+ for 24 h, 0.5 mg/mL MTT solution was added in each well and incubated for another 4 h at 37 °C. Culture medium was removed and the formazan crystals were solubilized with DMSO. The absorbance was measured at 570 nm by a microplate reader. Cell viability was expressed as the percentage of the negative control, which was set to 100%.

4.9. Measurement of mitochondrial membrane potential

The mitochondrial membrane potential (MMP) was measured by the fluorescent probe JC-1. JC-1 is sensitive to MMP, and the changes in the ratio between aggregate (red) and monomer (green) fluorescence can indicate the condition of MMP. After treatment with compound **2** at 1, 3, 10 μM or NAC at 10 μM for 24 h and then 0.5 mM MPP^+ for 24 h, PC12 cells were incubated with JC-1 for 15 min at 37 °C in the dark. After two more rinses, the fluorescence intensity was determined on a fluorescence microplate reader at an excitation of 490 nm and emission of 530 nm (green fluorescent monomers) and 590 nm (red fluorescent aggregates) respectively.

The change of MMP was expressed as a percentage of the negative control, which was set to 100% (Wu et al., 2015).

4.10. Measurement of DNA fragmentation

Quantification of DNA fragmentation was measured by Cell Death Detection ELISA^{plus} kit. After treatment with compound **2** at 1, 3, 10 μM or NAC at 10 μM for 24 h and then 0.5 mM MPP^+ for 24 h, PC12 cells were washed and lyzed for 30 min. After a centrifugation at 1000 rpm for 10 min at 4 °C, 20 μL supernatant was transferred to a streptavidin-coated microplate and incubated with a mixture of anti-histone-biotin and anti-DNA-peroxidase. The peroxidase amount in the immunocomplex was quantified by adding 2,2′-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) as the substrate. The absorbance of the reaction mixture was measured by a microplate reader at 405 nm. The extent of DNA fragmentation was expressed as a percentage of the negative control, which was set to 100% (Wu et al., 2015).

4.11. Measurement of caspase activity

The caspase activity was measured by the assay kit. After treatment with compound **2** at 1, 3, 10 μM or NAC at 10 μM for 24 h and then 0.5 mM MPP^+ for 24 h, PC12 cells were washed twice and lyzed for 30 min on ice. After a centrifugation at 20,000 rpm for 10 min at 4 °C, aliquots of supernatants containing 25 mg protein were added to a reaction buffer supplemented with 0.1% CHAPS, 100 mM PMSF and 5 mM DTT. The reactions were initiated after addition of the following fluorescent substrates (50 mM at the final concentration): Ac-DEVD-Amc for caspase-3 activity, Ac-LEDH-Afc for caspase-9 activity. After incubation at 37 °C for 2 h, the cleavage of the substrates was measured (Amc: 390/475 nm; Afc: 400/505 nm) by a microplate reader. The activity of caspase was expressed as a percentage of the negative control (Wu et al., 2015).

4.12. Statistical analysis

Values were statistically analyzed by one-way analysis of variance (ANOVA) using the Sigma Stat statistical software (SPSS Inc., Chicago, IL). Differences were considered as significant at $P < 0.05$.

References

Adibhatla, R.M., Hatcher, J.F., 2010. Lipid oxidation and peroxidation in CNS health and disease: from molecular mechanisms to therapeutic opportunities. Antioxid. Redox. Signal. 12, 125–169.

Birben, E., Sahiner, U.M., Sackesen, C., Erzurum, S., Kalayci, O., 2012. Oxidative stress and antioxidant defense. World Allergy Organ J. 5, 9–19.

Grienke, U., Schmidtke, M., Kirchmair, J., Pfarr, K., Wutzler, P., Dürrwald, R., Wolber, G., Liedl, K.R., Stuppner, H., Rollinger, J.M., 2010. Antiviral potential and molecular insight into neuraminidase inhibiting diarylheptanoids from *Alpinia katsumadai*. J. Med. Chem. 53, 778–786.

Jeong, G.S., Li, B., Lee, D.S., Byun, E., Kang, D.G., Lee, H.S., Kim, Y.C., 2007. Cytoprotective constituents of *Alpinia katsumadai* seeds against glutamate-induced oxidative injury in HT22 cells. Nat. Prod. Sci. 13, 268–271.

Krohn, K., Bahramsari, R., Flörke, U., Ludewig, K., Kliche-Spory, C., Michel, A., Aust, H. J., Draeger, S., Schulz, B., Antus, S., 1997. Dihydroisocoumarins from fungi: isolation structure elucidation, circular dichroism and biological activity. Phytochemistry 45, 313–320.

Lee, C.S., Park, S.Y., Ko, H.H., Song, J.H., Shin, Y.K., Han, E.S., 2005. Inhibition of MPP^+-induced mitochondrial damage and cell death by trifluoperazine and W-7 in PC12 cells. Neurochem. Int. 46, 169–178.

Leuner, K., Pantel, J., Frey, C., Schindowski, K., Schulz, K., Wegat, T., Maurer, K., Eckert, A., Müller, W.E., 2007. Enhanced apoptosis, oxidative stress and mitochondrial dysfunction in lymphocytes as potential biomarkers for Alzheimer's disease. J. Neural. Transm. 72, 207–215.

Li, Y.Y., Chou, G.X., Wang, Z.T., 2010. New diarylheptanoids and kavalactone from *Alpinia katsumadai* Hayata. Helv. Chim. Acta 93, 382–388.

Li, H., Park, J.H., Yan, B., Yoo, K.Y., Lee, C.H., Choi, J.H., Hwang, I.K., Won, M.H., 2011. Neuroprotection of *Alpinia katsumadai* seed extract against neuronal damage in the ischemic gerbil hippocampus is linked to altered brain-derived neurotrophic factor. Lab. Anim. Res. 27, 67–71.

Luo, P., Chen, T., Zhao, Y., Xu, H., Huo, K., Zhao, M., Yang, Y., Fei, Z., 2012. Protective effect of Homer 1a against hydrogen peroxide induced oxidative stress in PC12 cells. Free Radic. Res. 46, 766–776.

Nam, J.W., Seo, E.K., 2012. Structural characterization and biological effects of constituents of the seeds of *Alpinia katsumadai* (Alpina Katsumadai Seed). Nat. Prod. Commun. 7, 795–798.

Ngo, K.S., Brown, G.D., 1998. Stilbenes monoterpenes, diarylheptanoids, labdanes and chalcones from *Alpinia katsumadai*. Phytochemistry 47, 1117–1123.

Surendran, S., Rajasankar, S., 2010. Parkinson's disease: oxidative stress and therapeutic approaches. Neurol. Sci. 31, 531–540.

Tanaka, T., Nakashima, T., Ueda, T., Tomii, K., Kouno, I., 2007. Facile discrimination of aldose enantiomers by reversed-phase HPLC. Chem. Pharm. Bull. 55, 899–901.

Wang, X., Michaelis, E.K., 2010. Selective neuronal vulnerability to oxidative stress in the brain. Front. Aging Neurosci. 2, 12–25.

Wu, Y.Z., Qiao, F., Xu, G.W., Zhao, J., Teng, J.F., Li, C., Deng, W.J., 2015. Neuroprotective metabolites from the endophytic fungus *Penicillium citrinum* of the mangrove *Bruguiera gymnorrhiza*. Phytochem. Lett. 12, 128–152.

Xin, B.R., Liu, J.F., Kang, J., Chan, W.P., 2014. (2*R* 3*S*)-pinobanksin-3-cinnamate, a new flavonone from seeds of *Alpinia galanga* willd., presents in vitro neuroprotective effects. Mol. Cell. Toxicol. 10, 165–172.

Xu, J.D., Zhang, L.W., Liu, Y.F., 2013. Synthesis and antioxidant activities of flavonoids derivatives troxerutin and 3′,4′,7-triacetoxyethoxyquercetin. Chin. Chem. Lett. 24, 223–226.

Yang, Y., Kinoshita, K., Koyama, K., Takahashi, K., 1999. Anti-emetic principles of *Alpinia katsumadai* hayata. Nat. Prod. Sci. 5, 20–24.