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New dibenzocyclooctadiene lignans from the *Kadsura ananosma*

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ABSTRACT

Fourteen new dibenzocyclooctadiene lignans, ananonins A–N (**1**–**14**), together with five known compounds, were isolated from the seeds of *Kadsura ananosma*. The structures and absolute configurations of **1**–**14** were established using a combination of MS, NMR, and CD techniques. The biological activity of these compounds was evaluated, and ananonin M (**13**) showed moderate neuroprotective effects in an in vitro assay.

1. Introduction

The genus of *Kadsura* belongs to the family Schisandraceae. Some species of this genus have been reported to contain dibenzocyclooctadiene lignans, lanostane and cycloartane triterpenoids.[1–4] Previous pharmacological studies have indicated that the principle bioactive constituents of *Kadsura* medicinal plant were lignans, especially the dibenzocyclooctadiene type, some of which possessed antitumor,[5] anti-HIV,[6,7] and cytotoxic[8] effects.

Kadsura ananosma Kerr is a liana indigenous to Yunnan Province, People's Republic of China.[9] Previous work mainly focus on the investigation of the stems of this species and led to the isolation of triterpenoids, sesquiterpenoids, and lignans.[10–16] For further comprehensive acquaint with the constituents of this plant and search for biologically active natural products, we reinvestigated the seeds of *K. ananosma*, and this paper reports on the isolation and structure elucidation of new dibenzocyclooctadiene lignans, ananonins A–N (**1**–**14**), and the known compounds tiegusanin I (**15**),[17] pre-gomisin (**16**),[18] kadangustin H (**17**),[19] kadangustin I (**18**),[19] and 10-hydroxyl-15-oxo-α-cadinol (**19**, Fig. 1).[20] The structures of these new compounds **1**–**14** were established by means of MS and extensive NMR spectra, and the absolute configurations of these new compounds were determined by CD and ROESY experiments. In this paper, the isolation, structure elucidation, and the anti-neurodegenerative activity are reported.

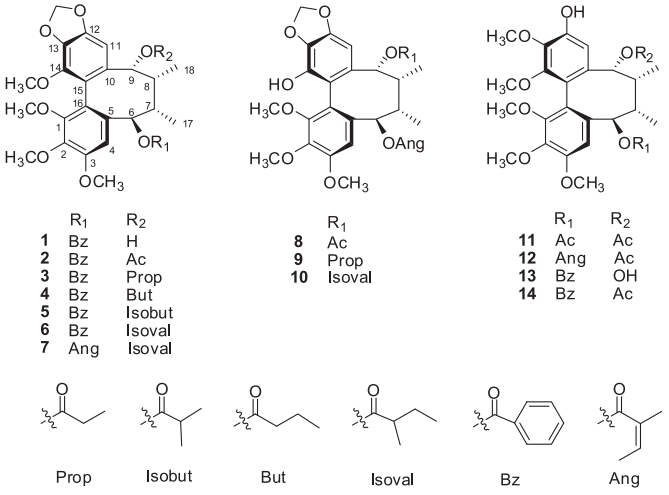

Fig. 1. Compounds isolated from *K. ananosma*.

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2. Results and discussion

Ananonin A (**1**) was assigned a molecular formula of $C_{30}H_{32}O_9$, according to HRESIMS *m*/*z* 559.1930 $[M+Na]^+$ (calcd 559.1944), which requires 15° of unsaturation. The UV spectrum showed λ_{max} ($CHCl_3$) values at 214, 221, and 241 nm. The IR spectrum showed the presence of a hydroxy group (3444 cm^{-1}) and a conjugated ester (1733 cm^{-1}). The 1D NMR spectra exhibited 18 carbon atoms (including 12 aromatic ones, four methines, and two secondary methyls) for the dibenzocyclooctadiene skeleton,[21] in addition to one benzoyloxy group (δ_C 165.4s, 130.1s, 129.6d, 127.9d, 132.8d, 127.9d, and 129.6d),[17] one methylenedioxy group, and four methoxy groups. HMBC correlations of the methylenedioxy protons with C-12 and C-13, and of the four methoxy group signals with C-1, C-2, C-3, and C-14, showed that the methylenedioxy group is connected to C-12 and C-13, and the four methoxy groups are located at C-1, C-2, C-3, and C-14, respectively. The presence of the benzoyloxy group at C-6 was deduced from the HMBC correlation of H-6 (δ_H 5.98) with the ester carbonyl (δ_C 165.4, C-1′). According to the molecular formula, the benzylic oxymethine at C-9 should be substituted by a hydroxy group (Fig. 1).

The CD spectrum of **1** exhibited a positive Cotton effect at λ_{max} 225 nm and a negative value at λ_{max} 248 nm, indicating a *S*-biphenyl configuration.[21] With the axial chirality defined, a ROESY experiment was used to establish the absolute configuration of the remaining stereocenters. The observed ROESY correlations of H-11 with H-8 and H-9, of H-4 with H-6 and H_3-17, and of H_3-17 with H_3-18 indicated that H-6, CH_3-17, and CH_3-18 are α-oriented, and that H-9 is β-oriented (Fig. 2). The proton coupling constants of H-6 (δ_H 5.98, d, *J*=7.0 Hz) and H-9 (δ_H 4.71, s) further confirmed the above deductions. These conclusions were consistent with **1** being a cyclooctadiene lignan with a twisted boat/chair conformation having C-7 (*R*), C-8 (*R*), and C-9 (*R*) absolute configurations. Thus, the structure of **1** was established as shown, and named ananonin A.

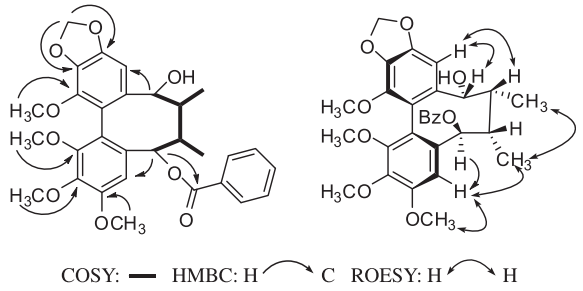

Fig. 2. Key HMBC and ROESY correlations of **1**.

Comparison of the NMR data of **1** with those of compounds **2**–**6** disclosed that the main structural differences between these compounds concerned the substituent at C-9. Ananonin B (**2**) gave a molecular formula of $C_{32}H_{34}O_{10}$ by HRESIMS at *m*/*z* 601.2059 $[M+Na]^+$ (calcd 601.2049). The ^{13}C NMR and DEPT data of **2** indicated the presence of an acetyl group (δ_C 170.1s, and 20.7q). The HMBC correlation of H-9 (δ_H 5.75) with the acetate carbonyl carbon (δ_C 170.1) led to the positioning of the acetyl group at C-9. The molecular formula of **3** was determined as $C_{33}H_{36}O_{10}$ by HRESIMS at *m*/*z* 615.2190 $[M+Na]^+$ (calcd 615.2206), and the NMR data of **3** also showed similarities with the analogous values for **1**. However, a signal for a propionyl group (δ_C 173.5s, 27.1t, and 8.6q) was evident at C-9 in **3**, which was confirmed by the HMBC correlations from H-9 (δ_H 5.76) to the ester carbonyl (δ_C 173.5, C-1″). Ananonins D (**4**), E (**5**), and F (**6**) showed molecular ions at *m*/*z* 629.2366, 629.2367, and 643.2504 in their HRESIMS, corresponding to the molecular formulas, $C_{34}H_{38}O_{10}$, $C_{34}H_{38}O_{10}$, and $C_{35}H_{40}O_{10}$, respectively. The major differences were in the replacement of a hydroxy group at C-9 in **1** by a butyryl group (δ_C 172.8s, 35.8t, 17.9t, and 13.6q) in **4**, by a isobutyryl group (δ_C 176.5s, 33.7d, 18.1q, and 19.3q) in **5**, and by a isovaleryl group (δ_C 176.0s, 40.2d, 26.6t, 11.2q, and 15.2q) in **6**.[17]

The CD, UV, and IR data suggested that **2**–**6** are *S*-biphenyl-configured dibenzocyclooctadiene lignans. ROESY correlations obtained for **2**–**6** were shown from H-11 to H-8 and H-9, from H-4 to H-6 and H_3-17, and from H_3-17 to H_3-18 indicated that H-6, CH_3-17 and CH_3-18 are α-oriented, and that H-9 is β-oriented. Thus, the structures of ananonins B–F (**2**–**6**) were established as shown.

Ananonin G (**7**) was assigned as $C_{33}H_{42}O_{10}$, as deduced from the HRESIMS (*m*/*z* 621.2660 $[M+Na]^+$) and in accordance with its NMR data. The UV, IR, CD, and NMR spectra of **7** suggested the presence of a *S*-biphenyl-configured dibenzocyclooctadiene lignan with almost identical data to **6**, except for the substituent at C-6. Analysis of the 1D NMR data showed an angeloyloxy group (δ_C 166.7s, 127.9s, 138.5d, 15.5q, and 19.9q) in **7** instead of a benzoyloxy group in **6**, which was deduced from an HMBC correlation of H-6 (δ_H 5.83) with ester carbonyl (δ_C 166.7, C-1′). The configuration of **7** was determined through ROESY correlations of H-11/H-8, H-9; H-4/H-6, H_3-17; and H_3-18/H_3-17, as well as the proton coupling constants of H-6 (d, *J*=8.1 Hz) and H-9 (br s), which were in agreement with a cyclooctadiene lignan with a twisted boat/chair conformation having C-6 (*R*), C-7 (*S*), C-8 (*R*), and C-9 (*R*) absolute configurations. Therefore, the structure of ananonin G (**7**) was determined as shown.

The molecular formula of ananonin H (**8**) was assigned as $C_{29}H_{34}O_{10}$, on the basis of the HRESIMS (*m*/*z* 565.2034 $[M+Na]^+$). The 1D NMR spectra exhibited 18 carbon atoms (including 12 aromatic ones, four methines, and two secondary methyls) for the dibenzocyclooctadiene skeleton, in addition to an angeloyloxy group (δ_C 166.7s, 127.4s, 139.0d, 15.6q, and 20.0q), a methylenedioxy group, three methoxy groups, and an acetyl group. The existence of the acetyl group and the angeloyloxy group at C-9 and C-6, respectively, was deduced from the HMBC correlations of H-6 (δ_H 5.75) with ester carbonyl (δ_C 166.7, C-1′), and of H-9 (δ_H 5.72) with acetate carbonyl (δ_C 169.9) (Fig. 3). Further analysis of the HMBC spectrum showed that the methylenedioxy group was located at C-12 and C-13, and the three methoxy groups were located at C-1, C-2, and C-3, respectively. According to the molecular formula, the quaternary carbon at C-14 should be substituted by a hydroxy group. The biphenyl group in **8** was determined to have a *S*-biphenyl configuration from its CD spectrum. The observed ROESY correlations of H-11 with H-8 and H-9, of H-4 with H-6 and H_3-17, and of H_3-17 with H_3-18 indicated that H-6, CH_3-17 and CH_3-18 are α-oriented, and that H-9 is β-oriented. Therefore, the structure of ananonin H (**8**) was determined as shown.

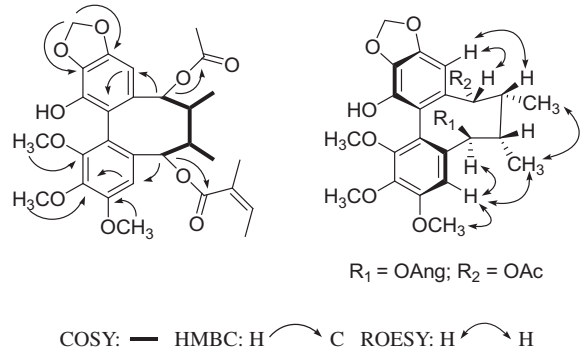

Fig. 3. Key HMBC and ROESY correlations of **8**.

Ananonins I (**9**) and J (**10**) were assigned with the molecular formula, $C_{30}H_{36}O_{10}$ and $C_{32}H_{40}O_{10}$, as determined by HRESIMS (*m*/*z* 579.2212 $[M+Na]^+$, and 607.2504 $[M+Na]^+$, respectively). The main difference found between **8**, **9**, and **10** concerned the substituent

group located at C-9. Analysis of the NMR data showed a propionyl group at C-9 in **9**, and a isovaleryl group in **10**, instead of an acetyl group in **8**, which was deduced from an HMBC correlation of H-9 (δ_H 5.76) with ester carbonyl (δ_C 173.4, C-1″) in **9**, and of H-9 (δ_H 5.75) with ester carbonyl (δ_C 176.2, C-1″) in **10**. By comparison of CD, UV, and ROESY spectra with those of **8**, compounds **9** and **10** were also assigned *S*-biphenyl-configured dibenzocyclooctadiene lignans with twisted boat/chair conformation of the cyclooctadiene ring and quasi-axial CH_3-17 and quasi-equatorial CH_3-18.

Ananonins K (**11**) showed the molecular ion peak $[M+Na]^+$ at *m*/*z* 541.2059 (calcd 541.2049) in the HRESIMS, corresponding to the molecular formula $C_{27}H_{34}O_{10}$. The 1D NMR of **11** indicated the presence of 12 aromatic carbons, two aromatic protons, five methoxy, and two acetyl groups, implied that **11** is a dibenzocyclooctadiene lignan possessing a hydroy group. HMBC correlations of H-11 with C-9, C-10, C-12, C-13, and C-15, and H-4 with C-2, C-3, C-5, C-6, and C-16, and $^1H-^1H$ COSY correlations of H-6/H-7/H-8/H-9, H-7/H_3-17, and H-8/H_3-18 (Fig. 3) confirmed the above conclusion. Further analysis of the HMBC spectrum showed five methoxy groups to be located at C-1, C-2, C-3, C-13, and C-14, respectively, indicated that a hydroxy group was located at C-12 according to the molecular formula. The two acetyl groups assigned to C-6 and C-9, according to the HMBC correlations of H-6 and H-9 with acetate carbonyl.

The CD curve of **11** showed a negative Cotton effect around 250 nm and a positive value around 210 nm, suggesting that **11** possesses a *S*-biphenyl configuration. ROESY correlations of H-11 with H-8 and H-9, and of H-4 with H-6 and H_3-17, suggested a cyclooctadiene lignan with a twisted boat/chair conformation of **11**, consistent with the absolute configurations C-6 (*R*), C-7 (*S*), C-8 (*R*), and C-9 (*R*). Therefore, the structure of ananonin K (**11**) was determined as shown.

Analysis of the 2D NMR data of ananonins L–N (**12**–**14**) and comparison of their 1D NMR data with those of **11** revealed that the main structure differences between these compounds were the substituent patterns at C-6 and C-9. The 1D NMR spectra of **12** indicated the characteristic signals for an angeloyloxy group (δ_C 166.7s, 127.7s, 138.8d, 15.6q, and 20.7q), which was determined to be located at C-6 by HMBC correlations of H-6 with C-1′ (δ_C 166.7). Similarly, the 1D NMR spectra of **13** were very close to those of **11** except for the characteristic signals for a benzoyloxy group (δ_C 165.4s, 130.0s, 129.6d, 128.1d, 132.9d, 128.1d, and 129.6d) at C-6. HMBC correlations of H-6 with C-1′ (δ_C 165.4) in **13** established the location of the benzoyloxy moiety at C-6. By contrast, the only difference between **13** and **14** was the substituent at C-9. There was a hydroxy group at C-9 in **13** while an acetyl group substituent in **14**, which was in accord with the observation of remarkable downfield shift of the signal of H-9 from δ_H 4.73 in **13** to δ_H 5.78 in **14**. By comparison of CD, UV, and ROESY spectra with those of **11**, compounds **12**–**14** were assigned as *S*-biphenyl-configured dibenzocyclooctadiene ring. Therefore, the structures of compounds **12**–**14** were determined as shown.

The neuroprotective effects of all dibenzocyclooctadiene lignans were evaluated according to a reported in vitro protocol[22] using SH-SY5Y neuroblastoma cells, a widely used neuroblastoma cell line for study of neurodegenerative disease.[23,24] As may be seen from Table 5, ananonin M (**13**) showed moderate neuroprotective effect against oxidative stress-induced neurotoxicity of these compounds tested. Due to limitations in the amounts available, compound **4** was not tested in the bioassay used.

3. Conclusion

In summary, this paper describes the isolation and structure elucidation of 14 new dibenzocyclooctadiene lignans, ananonins A–N (**1**–**14**), as well as five known compounds from the seeds of *K. ananosma*, while our previous researches exhibited that the remarkably varied triterpenoids are mainly constituents of the stems of *K. ananosma*.[15,16] Our present research expanded considerably the library for dibenzocyclooctadiene lignans and also illuminated the variety of the metabolites in different parts of *K. ananosma*. Particularly, ananonin M (**13**) showed moderate neuroprotective effect in an in vitro cell survival assay.

4. Experimental

4.1. General procedures

Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectrophotometer was used for scanning IR spectroscopy with KBr pellets. 1D and 2D NMR spectra were recorded on Bruker AM-400 and DRX-500 spectrometers with TMS as internal standard. Chemical shifts (δ) are expressed in parts per million with reference to the solvent signals. Mass spectra were performed on an API QSTAR time-of-flight spectrometer and a VG Autospec-3000 spectrometer, respectively. Semipreparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C18 (9.4 mm×25 cm) column. Column chromatography was performed with silica gel (200–300 mesh, Qingdao Marine Chemical, Inc., Qingdao, People's Republic of China), and MCI gel (75–150 μM, Mitsubishi Chemical Corporation, Tokyo, Japan). Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

4.2. Collection and identification

The seeds of *K. ananosma* were collected in Simao Country of Yunnan Province, People's Republic of China, in October 2008, and identified by Prof. Xi-Wen Li, Kunming Institute of Botany. A voucher specimen (KIB 08102010) has been deposited in the Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences.

4.3. Extraction and isolation

The air-dried and powdered seeds of *K. ananosma* (250 g) were extracted with 70% aqueous Me_2CO (3×500 mL) at room temperature and concentrated in vacuo to yield a residue, which was partitioned between H_2O and EtOAc. The EtOAc extract (6.5 g) was chromatographed on MCI CHP 20P gel eluting with 90% CH_3OH-H_2O. The 90% CH_3OH-H_2O fraction (5.0 g) was subjected to silica gel (200–300 mesh) column chromatography, using a $CHCl_3-Me_2CO$ gradient system (9:1, 8:2, 2:1, 1:1, 0:1) to yield 4 fractions. Semipreparative HPLC separation of the subfraction 1 (68% CH_3CN-H_2O, 3 mL min^{-1}) afforded compounds **2** (120 mg), **3** (8 mg), **4** (1.5 mg), **5** (3 mg), **6** (10 mg), **7** (69 mg), and **15** (64 mg). Semipreparative HPLC separation of the subfraction 2 (63% CH_3CN-H_2O, 3 mL min^{-1}) gave compounds **1** (2 mg), **8** (22 mg), **9** (4 mg), **10** (32 mg), **11** (5 mg), **12** (11 mg), and **14** (21 mg). Semipreparative HPLC separation of the subfraction 3 (60% CH_3CN-H_2O, 3 mL min^{-1}) produced compounds **13** (3 mg) and **19** (25 mg). Semipreparative HPLC separation of the subfraction 4 (50% CH_3CN-H_2O, 3 mL min^{-1}) yielded **16** (9 mg), **17** (12 mg), and **18** (3 mg).

*4.3.1. Ananonin A (**1**).* White solid; $[\alpha]_{D^{27}}$ −40.3 (*c* 0.24, $CHCl_3$); CD λ_{max} (CH_3OH)/nm ($\Delta\varepsilon$) 225 (+25), 248 (−30); UV λ_{max} ($CHCl_3$)/nm (log ε) 241 (4.14), 221 (3.77), 214 (3.75); IR ν_{max} (KBr)/cm^{-1} 3444, 2924, 1733, 1268; ^1H and ^{13}C NMR data (see Tables 1 and 3); (+)-ESIMS *m*/*z* 559 (100) $[M+Na]^+$; (+)-HRESIMS *m*/*z* 559.1930 $[M+Na]^+$ (calcd for $C_{30}H_{32}O_9Na$, 559.1944).

*4.3.2. Ananonin B (**2**).* white solid; $[\alpha]_{D^{29}}$ −48.8 (*c* 0.18, $CHCl_3$); CD λ_{max} (CH_3OH)/nm ($\Delta\varepsilon$) 225 (+10), 247 (−11); UV λ_{max} ($CHCl_3$)/nm (log ε) 241 (4.15), 225 (3.58), 203 (3.70), 199 (3.71); IR ν_{max} (KBr)/cm^{-1}

Table 1
^{1}H NMR data of **1**–**7** in $CDCl_3$, δ in ppm (J in Hz)

H	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[a]	**6**[b]	**7**[a]
4	6.77 (s)	6.71 (s)	6.70 (s)	6.72 (s)	6.73 (s)	6.72 (s)	6.71 (s)
6	5.98 (d, 7.0)	6.01 (d, 7.1)	6.01 (d, 7.1)	6.02 (d, 7.1)	6.02 (d, 7.2)	6.02 (d, 7.3)	5.83 (d, 8.1)
7	2.27 (m)	2.28 (m)	2.27 (m)	2.29 (m)	2.29 (m)	2.29 (m)	2.17 (m)
8	2.27 (m)	2.37 (m)	2.36 (m)	2.38 (m)	2.39 (m)	2.39 (m)	2.22 (m)
9	4.71 (s)	5.75 (d, 1.7)	5.76 (d, 1.7)	5.80 (s)	5.78 (s)	5.79 (d, 2.2)	5.77 (br s)
11	6.39 (s)	6.51 (s)	6.52 (s)	6.53 (s)	6.54 (s)	6.54 (s)	6.45 (s)
17	1.01 (d, 6.9)	0.98 (d, 7.2)	0.97 (d, 7.2)	0.98 (d, 7.2)	1.00 (d, 7.2)	0.99 (d, 7.2)	1.02 (d, 8.0)
18	1.19 (d, 6.6)	1.10 (d, 7.1)	1.09 (d, 7.1)	1.09 (d, 6.7)	1.10 (d, 7.0)	1.10 (d, 7.1)	1.15 (d, 8.0)
3′	7.59 (m)	7.53 (m)	7.52 (d, 5.8)	7.55 (br s)	7.56 (m)	7.57 (m)	5.98 (overlap)
4′	7.32 (t, 7.7)	7.30 (t, 7.7)	7.30 (dd, 7.4, 5.8)	7.32 (t, 7.7)	7.32 (t, 7.7)	7.34 (t, 7.7)	1.87 (d, 7.2)
5′	7.51 (t, 7.4)	7.47 (t, 7.4)	7.47 (t, 7.4)	7.49 (t, 7.4)	7.49 (t, 7.3)	7.49 (t, 7.4)	1.54 (s)
6′	7.32 (t, 7.7)	7.30 (t, 7.7)	7.30 (dd, 7.4, 5.8)	7.32 (t, 7.7)	7.32 (t, 7.7)	7.34 (t, 7.7)	
7′	7.59 (m)	7.53 (m)	7.52 (d, 5.8)	7.55 (br s)	7.56 (m)	7.57 (m)	
2″			1.85 (m)	1.79 (m)	1.98 (m)	1.78 (m)	1.75 (m)
			1.79 (m)				
3″			0.86 (t, 7.6)	1.37 (m)	0.90 (d, 7.0)	1.38 (m)	1.38 (m)
						1.25 (m)	1.25 (m)
4″				0.80 (t, 7.4)	0.90 (d, 7.0)	0.76 (t, 7.4)	0.74 (t, 7.4)
5″						0.89 (d, 7.1)	0.87 (d, 6.9)
AcO-9		1.60 (s)					
MeO-1	3.65 (s)	3.58 (s)	3.57 (s)	3.59 (s)	3.62 (s)	3.65 (s)	3.62 (s)
MeO-2	3.54 (s)	3.47 (s)	3.46 (s)	3.48 (s)	3.49 (s)	3.49 (s)	3.90 (s)
MeO-3	3.92 (s)	3.95 (s)	3.90 (s)	3.92 (s)	3.92 (s)	3.94 (s)	3.93 (s)
MeO-14	3.88 (s)	3.85 (s)	3.84 (s)	3.84 (s)	3.85 (s)	3.85 (s)	3.77 (s)
OCH_2O	5.91 (s)	5.86 (s)	5.86 (s)	5.88 (s)	5.88 (s)	5.88 (s)	5.98 (s)
	5.85 (s)	5.83 (s)	5.83 (s)	5.86 (s)	5.86 (s)	5.86 (s)	5.94 (s)

[a] Recorded at 500 MHz.
[b] Recorded at 400 MHz.

Table 2
^{13}C NMR data of **1**–**7** in $CDCl_3$, δ in ppm

C	**1**[a]	**2**[a]	**3**[b]	**4**[a]	**5**[a]	**6**[b]	**7**[a]
1	152.6 (s)	151.7 (s)	151.6 (s)	151.8 (s)	151.8 (s)	151.7 (s)	151.8 (s)
2	142.1 (s)	141.1 (s)	141.0 (s)	142.0 (s)	141.2 (s)	141.0 (s)	141.1 (s)
3	152.4 (s)	151.7 (s)	151.7 (s)	151.8 (s)	151.8 (s)	151.7 (s)	151.5 (s)
4	110.6 (d)	109.9 (d)	109.8 (d)	110.1 (d)	110.1 (d)	109.9 (d)	110.0 (d)
5	131.0 (s)	130.9 (s)	130.9 (s)	130.9 (s)	130.8 (s)	130.8 (s)	131.1 (s)
6	81.5 (d)	81.4 (d)	81.4 (d)	81.4 (d)	81.5 (d)	81.4 (d)	80.6 (d)
7	39.3 (d)	38.2 (d)	39.1 (d)	39.1 (d)	39.0 (d)	38.9 (d)	38.4 (d)
8	39.3 (d)	39.1 (d)	38.1 (d)	39.1 (d)	39.0 (d)	39.0 (d)	38.3 (d)
9	81.7 (d)	81.4 (d)	81.4 (d)	81.0 (d)	81.5 (d)	81.0 (d)	80.5 (d)
10	136.9 (s)	133.4 (s)	133.6 (s)	133.6 (s)	133.6 (s)	133.6 (s)	133.1 (s)
11	101.9 (d)	102.1 (d)	102.2 (d)	102.3 (d)	102.5 (d)	102.4 (d)	102.4 (d)
12	148.8 (s)	148.6 (s)	148.5 (s)	148.6 (s)	148.6 (s)	148.5 (s)	148.5 (s)
13	135.4 (s)	135.8 (s)	135.8 (s)	136.0 (s)	135.9 (s)	135.8 (s)	135.8 (s)
14	141.4 (s)	141.3 (s)	141.0 (s)	142.0 (s)	141.3 (s)	141.5 (s)	141.3 (s)
15	119.7 (s)	121.1 (s)	121.0 (s)	121.3 (s)	121.3 (s)	121.3 (s)	121.2 (s)
16	121.9 (s)	123.0 (s)	122.2 (s)	122.5 (s)	123.0 (s)	123.0 (s)	122.5 (s)
17	15.8 (q)	17.7 (q)	14.8 (q)	14.8 (q)	15.2 (q)	15.2 (q)	15.8 (q)
18	17.5 (q)	17.8 (q)	14.8 (q)	14.8 (q)	15.2 (q)	15.2 (q)	15.8 (q)
1′	165.4 (s)	165.3 (s)	165.3 (s)	165.3 (s)	165.3 (s)	165.3 (s)	166.7 (s)
2′	130.1 (s)	130.0 (s)	130.0 (s)	130.1 (s)	130.1 (s)	130.0 (s)	127.9 (s)
3′	129.6 (d)	129.5 (d)	129.5 (d)	129.6 (d)	129.6 (d)	129.5 (d)	138.5 (d)
4′	127.9 (d)	127.9 (d)	127.9 (d)	127.9 (d)	127.9 (d)	127.9 (d)	15.5 (q)
5′	132.8 (d)	132.8 (d)	132.8 (d)	132.7 (d)	132.7 (d)	132.7 (d)	19.9 (q)
6′	127.9 (d)	127.9 (d)	127.9 (d)	127.9 (d)	127.9 (d)	127.9 (d)	
7′	129.6 (d)	129.5 (d)	129.5 (d)	129.6 (d)	129.6 (d)	129.5 (d)	
1″			173.5 (s)	172.8 (s)	176.5 (s)	176.0 (s)	176.0 (s)
2″			27.1 (t)	35.8 (t)	33.7 (d)	40.2 (d)	40.1 (d)
3″			8.6 (q)	17.9 (t)	18.1 (q)	26.6 (t)	26.5 (t)
4″				13.6 (q)	19.3 (q)	11.2 (q)	11.1 (q)
5″						15.2 (q)	15.1 (q)
AcO-9		170.1 (s)					
		20.7 (q)					
MeO-1	60.6 (q)	60.3 (q)	60.4 (q)	60.4 (q)	60.6 (q)	60.4 (q)	60.4 (q)
MeO-2	59.1 (q)	59.0 (q)	60.4 (q)	59.0 (q)	59.0 (q)	60.6 (q)	60.4 (q)
MeO-3	56.0 (q)	56.0 (q)	55.9 (q)	56.0 (q)	56.0 (q)	55.9 (q)	55.8 (q)
MeO-14	60.8 (q)	60.5 (q)	59.0 (q)	60.5 (q)	60.4 (q)	58.9 (q)	59.2 (q)
OCH_2O	100.9 (t)	101.0 (t)	100.9 (t)	101.0 (t)	101.0 (t)	100.9 (t)	100.9 (t)

[a] Recorded at 125 MHz.
[b] Recorded at 100 MHz.

Table 3
^{1}H NMR data of **8**–**14** in $CDCl_3$, δ in ppm (500 MHz, J in Hz)

H	**8**[b]	**9**[b]	**10**[a]	**11**[a]	**12**[a]	**13**[a]	**14**[a]
4	6.80 (s)	6.80 (s)	6.78 (s)	6.70 (s)	6.70 (s)	6.79 (s)	6.70 (s)
6	5.75 (d, 8.9)	5.75 (d, 7.2)	5.72 (d, 8.2)	5.75 (d, 8.8)	5.87 (d, 7.1)	6.08 (br s)	6.10 (br s)
7	2.11 (m)	2.13 (m)	2.08 (m)	2.05 (m)	2.07 (m)	2.27 (m)	2.24 (m)
8	2.24 (m)	2.27 (m)	2.23 (m)	2.11 (m)	2.18 (m)	2.27 (m)	2.34 (m)
9	5.72 (br s)	5.76 (d, 4.3)	5.75 (d, 3.6)	5.78 (d, 4.7)	5.72 (s)	4.73 (s)	5.78 (s)
11	6.38 (s)	6.40 (s)	6.48 (s)	6.58 (s)	6.56 (s)	6.59 (s)	6.68 (s)
17	0.96 (d, 7.1)	0.94 (d, 7.2)	0.94 (d, 7.1)	0.89 (d, 7.0)	0.88 (d, 7.0)	0.70 (d, 6.9)	0.83 (d, 7.0)
18	1.02 (d, 7.1)	1.03 (d, 7.1)	0.99 (d, 6.9)	0.96 (br s)	0.93 (br s)	0.99 (br s)	0.93 (br s)
3′	5.93 (overlap)	5.83 (overlap)	5.93 (overlap)		5.88 (overlap)	7.58 (m)	7.43 (overlap)
4′	1.84 (d, 7.2)	1.85 (dd, 7.2, 1.4)	1.82 (dd, 7.2, 1.4)		1.80 (d, 7.0)	7.29 (t, 7.6)	7.24 (t, 6.6)
5′	1.52 (s)	1.53 (s)	1.53 (s)		1.56 (s)	7.48 (t, 7.4)	7.42 (overlap)
6′						7.29 (t, 7.6)	7.24 (t, 6.6)
7′						7.58 (m)	7.43 (overlap)
2″		1.81 (m)	1.69 (m)				
3″		0.86 (t, 6.7)	1.34 (m), 1.19 (m)				
4″			0.72 (t, 7.4)				
5″			0.83 (d, 6.9)				
AcO-6				1.56 (s)			
AcO-9	1.56 (s)			1.56 (s)			1.58 (s)
MeO-1	3.55 (s)	3.55 (s)	3.56 (s)	3.60 (s)	3.53 (s)	3.66 (s)	3.58 (s)
MeO-2	3.89 (s)	3.90 (s)	3.86 (s)	3.87 (s)	3.83 (s)	3.88 (s)	3.82 (s)
MeO-3	3.92 (s)	3.93 (s)	3.89 (s)	3.89 (s)	3.87 (s)	3.93 (s)	3.89 (s)
MeO-13				4.01 (s)	3.86 (s)	3.93 (s)	3.89 (s)
MeO-14				3.65 (s)	3.48 (s)	3.34 (s)	3.64 (s)
OCH_2O	6.00 (s), 5.98 (s)	6.01 (s), 5.98 (s)	5.97 (s), 5.96 (s)				

[a] Recorded at 500 MHz.
[b] Recorded at 400 MHz.

2937, 1740, 1716, 1106; ^{1}H and ^{13}C NMR data (see Tables 1 and 3); (+)-ESIMS m/z 601 (100) $[M+Na]^+$; (+)-HRESIMS m/z 601.2059 $[M+Na]^+$ (calcd for $C_{32}H_{34}O_{10}Na$, 601.2049).

*4.3.3. Ananonin C (**3**).* White solid; $[\alpha]_{D^{28}}$ −41.0 (c 0.16, $CHCl_3$); CD λ_{max} (CH_3OH)/nm ($\Delta\varepsilon$) 225 (+12), 247 (−10); UV λ_{max} ($CHCl_3$)/nm (log ε) 241 (4.10), 219 (3.58), 199 (3.72); IR ν_{max} (KBr)/cm^{-1} 2940, 1716, 1270; ^{1}H and ^{13}C NMR data (see Tables 1 and 3); (+)-ESIMS m/z 615 (100) $[M+Na]^+$; (+)-HRESIMS m/z 615.2190 $[M+Na]^+$ (calcd for $C_{33}H_{36}O_{10}Na$, 615.2206).

*4.3.4. Ananonin D (**4**).* White solid; $[\alpha]_{D^{26}}$ −18.1 (c 0.26, $CHCl_3$); CD λ_{max} (CH_3OH)/nm ($\Delta\varepsilon$) 225 (+5), 248 (−5); UV λ_{max} ($CHCl_3$)/nm (log ε) 241 (3.95), 222 (3.57), 195 (3.56); IR ν_{max} (KBr)/cm^{-1} 2964, 2935, 1734, 1720, 1270; ^{1}H and ^{13}C NMR data (see Tables 1 and 3); (+)-ESIMS m/z 629 $[M+Na]^+$; (+)-HRESIMS m/z 629.2366 $[M+Na]^+$ (calcd for $C_{34}H_{38}O_{10}Na$, 629.2362).

*4.3.5. Ananonin E (**5**).* White solid; $[\alpha]_{D^{27}}$ −21.7 (c 0.36, $CHCl_3$); CD λ_{max} (CH_3OH)/nm ($\Delta\varepsilon$) 225 (+20), 248 (−18); UV λ_{max} ($CHCl_3$)/nm (log ε) 241 (3.95), 226 (3.58), 215 (3.55), 202 (3.55); IR ν_{max} (KBr)/cm^{-1} 2925, 1733, 1716, 1270; ^{1}H and ^{13}C NMR data (see Tables 1 and 3); (+)-FAB m/z 607 (100) $[M+H]^+$; (+)-HRESIMS m/z 629.2367 $[M+Na]^+$ (calcd for $C_{34}H_{38}O_{10}Na$, 629.2362).

*4.3.6. Ananonin F (**6**).* White solid; $[\alpha]_{D^{27}}$ −5.5 (c 0.18, $CHCl_3$); CD λ_{max} (CH_3OH)/nm ($\Delta\varepsilon$) 225 (+15), 245 (−15); UV λ_{max} ($CHCl_3$)/nm (log ε) 241 (4.10), 220 (3.75), 197 (3.72); IR ν_{max} (KBr)/cm^{-1} 2967, 2936, 1722, 1107; ^{1}H and ^{13}C NMR data (see Tables 1 and 3); (+)-ESIMS m/z 643 (12) $[M+Na]^+$; (+)-HRESIMS m/z 643.2504 $[M+Na]^+$ (calcd for $C_{35}H_{40}O_{10}Na$, 643.2519).

*4.3.7. Ananonin G (**7**).* White solid; $[\alpha]_{D^{29}}$ +75.1 (c 0.19, $CHCl_3$); CD λ_{max} (CH_3OH)/nm ($\Delta\varepsilon$) 230 (+13), 250 (−5); UV λ_{max} ($CHCl_3$)/nm (log ε) 241 (4.00), 231 (3.49), 223 (3.46), 200 (3.62); IR ν_{max} (KBr)/cm^{-1} 2974, 2941, 1730, 1710, 1102; ^{1}H and ^{13}C NMR data (see Tables 1 and 3); (+)-ESIMS m/z 621 (40) $[M+Na]^+$; (+)-HRESIMS m/z 621.2660 $[M+Na]^+$ (calcd for $C_{33}H_{42}O_{10}Na$, 621.2675).

*4.3.8. Ananonin H (**8**).* White solid; $[\alpha]_{D^{27}}$ +49.3 (c 0.24, $CHCl_3$); CD λ_{max} (CH_3OH)/nm ($\Delta\varepsilon$) 225 (+20), 248 (−10); UV λ_{max} ($CHCl_3$)/nm (log ε) 241 (4.10), 220 (3.78), 194 (3.78); IR ν_{max} (KBr)/cm^{-1} 3435, 2968, 2938, 1738, 1715, 1235; ^{1}H and ^{13}C NMR data (see Tables 2 and 4); (+)-ESIMS m/z 565 (30) $[M+Na]^+$; (+)-HRESIMS m/z 565.2034 $[M+Na]^+$ (calcd for $C_{29}H_{34}O_{10}Na$, 565.2049).

*4.3.9. Ananonin I (**9**).* White solid; $[\alpha]_{D^{28}}$ +35.7 (c 0.18, $CHCl_3$); CD λ_{max} (CH_3OH)/nm ($\Delta\varepsilon$) 230 (+35), 248 (−18); UV λ_{max} ($CHCl_3$)/nm (log ε) 241 (4.05), 200 (3.69), 198 (3.70); IR ν_{max} (KBr)/cm^{-1} 3439, 2940, 1733, 1734, 1106; ^{1}H and ^{13}C NMR data (see Tables 2 and 4); (+)-ESIMS m/z 579 (10) $[M+Na]^+$; (+)-HRESIMS m/z 579.2212 $[M+Na]^+$ (calcd for $C_{30}H_{36}O_{10}Na$, 579.2206).

*4.3.10. Ananonin J (**10**).* White solid; $[\alpha]_{D^{27}}$ +69.9 (c 0.19, $CHCl_3$); CD λ_{max} (CH_3OH)/nm ($\Delta\varepsilon$) 230 (+25), 248 (−15); UV λ_{max} ($CHCl_3$)/nm (log ε) 241 (4.06), 223 (3.71), 198 (3.70); IR ν_{max} (KBr)/cm^{-1} 3434, 2936, 1733, 1729, 1714, 1106 cm^{-1}; ^{1}H and ^{13}C NMR data (see Tables 2 and 4); (+)-ESIMS m/z 607 (100) $[M+Na]^+$; (+)-HRESIMS m/z 607.2504 $[M+Na]^+$ (calcd for $C_{32}H_{40}O_{10}Na$, 607.2519).

*4.3.11. Ananonin K (**11**).* White solid; $[\alpha]_{D^{27}}$ +60.6 (c 0.19, $CHCl_3$); CD λ_{max} (CH_3OH)/nm ($\Delta\varepsilon$) 210 (+50), 250 (−25); UV λ_{max} ($CHCl_3$)/nm (log ε) 241 (3.93), 221 (3.64), 215 (3.60); IR ν_{max} (KBr)/cm^{-1} 3432, 2939, 1736, 1236; ^{1}H and ^{13}C NMR data (see Tables 2 and 4); (+)-ESIMS m/z 541 (100) $[M+Na]^+$; (+)-HRESIMS m/z 541.2059 $[M+Na]^+$ (calcd for $C_{27}H_{34}O_{10}Na$, 541.2049).

*4.3.12. Ananonin L (**12**).* White solid; $[\alpha]_{D^{27}}$ +58.3 (c 0.18, $CHCl_3$); CD λ_{max} (CH_3OH)/nm ($\Delta\varepsilon$) 210 (+19), 250 (−7); UV λ_{max} ($CHCl_3$)/nm (log ε) 241 (3.92), 228 (3.62), 224 (3.62), 201 (3.58), 194 (3.59); IR ν_{max} (KBr)/cm^{-1} 3429, 2939, 1741, 1712, 1231; ^{1}H and ^{13}C NMR data

Table 4
^{13}C NMR data of **8**–**14** in $CDCl_3$, δ in ppm

C	**8**[a]	**9**[a]	**10**[a]	**11**[a]	**12**[a]	**13**[b]	**14**[a]
1	150.6 (s)	150.6 (s)	151.0 (s)	151.9 (s)	151.6 (s)	152.6 (s)	151.7 (s)
2	141.8 (s)	141.5 (s)	141.9 (s)	141.6 (s)	140.7 (s)	142.5 (s)	141.5 (s)
3	152.5 (s)	152.5 (s)	152.9 (s)	151.5 (s)	153.6 (s)	152.3 (s)	151.7 (s)
4	111.7 (d)	111.7 (d)	111.0 (d)	110.6 (d)	110.5 (d)	111.3 (d)	109.8 (d)
5	133.3 (s)	133.5 (s)	133.7 (s)	131.1 (s)	130.3 (s)	130.9 (s)	130.7 (s)
6	80.7 (d)	80.8 (d)	81.1 (d)	81.1 (d)	80.5 (d)	81.3 (d)	81.1 (d)
7	38.5 (d)	38.5 (d)	38.7 (d)	37.7 (d)	39.0 (d)	39.4 (d)	39.8 (d)
8	38.6 (d)	38.8 (d)	39.1 (d)	37.7 (d)	39.0 (d)	38.9 (d)	39.8 (d)
9	80.6 (d)	80.4 (d)	80.6 (d)	80.1 (d)	80.5 (d)	81.6 (d)	80.9 (d)
10	133.4 (s)	133.5 (s)	133.9 (s)	134.8 (s)	134.8 (s)	135.0 (s)	135.3 (s)
11	101.2 (d)	101.3 (d)	101.7 (d)	109.4 (d)	109.5 (d)	109.2 (d)	109.6 (d)
12	148.3 (s)	148.3 (s)	148.7 (s)	148.7 (s)	148.7 (s)	148.9 (s)	148.7 (s)
13	134.5 (s)	134.5 (s)	134.7 (s)	138.9 (s)	138.8 (s)	138.8 (s)	139.0 (s)
14	137.7 (s)	137.6 (s)	138.0 (s)	151.0 (s)	151.5 (s)	150.6 (s)	150.6 (s)
15	117.5 (s)	117.5 (s)	118.0 (s)	121.1 (s)	120.9 (s)	119.8 (s)	121.2 (s)
16	120.5 (s)	120.8 (s)	120.5 (s)	123.8 (s)	122.9 (s)	122.3 (s)	122.9 (s)
17	15.8 (q)	15.7 (q)	15.4 (q)	17.8 (q)	15.4 (q)	15.2 (q)	15.8 (q)
18	15.6 (q)	15.7 (q)	15.4 (q)	17.2 (q)	15.4 (q)	15.2 (q)	15.8 (q)
1′	166.7 (s)	166.8 (s)	167.1 (s)		166.7 (s)	165.4 (s)	165.3 (s)
2′	127.4 (s)	127.4 (s)	127.7 (s)		127.7 (s)	130.0 (s)	129.8 (s)
3′	139.0 (d)	139.1 (d)	139.3 (d)		138.8 (d)	129.6 (d)	129.5 (d)
4′	15.6 (q)	15.7 (q)	16.0 (q)		15.6 (q)	128.1 (d)	128.1 (d)
5′	20.0 (q)	20.0 (q)	20.4 (q)		20.7 (q)	132.9 (d)	133.0 (d)
6′						128.1 (d)	128.1 (d)
7′						129.6 (d)	129.5 (d)
1″		173.4 (s)	176.2 (s)				
2″		27.1 (t)	40.5 (d)				
3″		8.6 (q)	26.8 (t)				
4″			11.4 (q)				
5″			15.3 (q)				
AcO-6				170.0 (s), 21.0 (q)			
AcO-9	169.9 (s) 20.6 (q)			170.0 (s), 20.6 (q)	170.0 (s), 20.7 (q)		170.0 (s), 20.7 (q)
MeO-1	60.8 (q)	60.8 (q)	61.1 (q)	60.2 (q)	60.1 (q)	60.3 (q)	60.3 (q)
MeO-2	60.7 (q)	60.8 (q)	61.2 (q)	60.5 (q)	60.6 (q)	60.6 (q)	60.4 (q)
MeO-3	56.1 (q)	56.1(q)	56.3 (q)	56.0 (q)	55.9 (q)	56.0 (q)	56.0 (q)
MeO-13				60.8 (q)	60.6 (q)	60.9 (q)	60.6 (q)
MeO-14				59.9 (q)	59.9 (q)	59.7 (q)	59.5 (q)
OCH_2O	101.7 (t)	101.7 (t)	102.0 (t)				

[a] Recorded at 100 MHz.
[b] Recorded at 125 MHz.

Table 5
Neuroprotective effects of compounds **1**–**3** and **4**–**19** on SH-SY5Y cells

Compound	H_2O_2 (100 μM)			Compound	H_2O_2 (100 μM)		
	Test concentration (μM)		Vehicle		Test concentration (μM)		Vehicle
	1	10			1	10	
1	58.0±1.4	59.2±1.0	58.6±1.9	**11**	65.3±1.5	66.7±0.5	60.3±1.1
2	48.4±2.2	49.52±2.0	52.9±3.8	**12**	56.7±1.5[a]	53.5±0.8	51.34±1.2
3	61.5±1.3	65.8±0.2[b]	60.31±1.1	**13**	68.4±2.0[b]	66.2±1.4[a]	59.4±0.7
5	60.5±1.5	55.6±0.6	58.6±1.9	**14**	59.3±1.1[a]	55.2±1.1[b]	64.8±1.9
6	53.4±1.2	56.1±3.9	53.7±1.6	**15**	53.6±1.0	45.7±1.1[a]	50.9±1.0
7	58.2±2.9[a]	68.2±2.8	65.4±3.0	**16**	68.7±2.0	65.0±2.3[b]	73.0±1.6
8	61.3±3.6	64.8±3.8	63.14±1.7	**17**	62.7±1.3	64.0±1.1	64.8±1.9
9	67.3±1.3[a]	69.0±2.1	73.0±1.6	**18**	56.5±1.6[a]	53.7±1.0	51.3±1.2
10	61.0±1.2	55.9±1.1	60.31±1.1	**19**	61.3±1.0	65.1±2.1	66.6±0.8

[a] $p<0.05$ versus H_2O_2 group.
[b] $p<0.01$ versus H_2O_2 group.

(see Tables 2 and 4); (+)-ESIMS *m*/*z* 581 (10) $[M+Na]^+$; (+)-HRESIMS *m*/*z* 581.2352 $[M+Na]^+$ (calcd for $C_{30}H_{38}O_{10}Na$, 581.2362).

*4.3.13. Ananonin M (**13**).* White solid; $[\alpha]_{D^{26}}$ −50.3 (*c* 0.22, $CHCl_3$); CD λ_{max} (CH_3OH)/nm ($\Delta\varepsilon$) 225 (+23), 240 (−30); UV λ_{max} ($CHCl_3$)/nm (log ε) 241 (4.13), 230 (3.70), 224 (3.69), 204 (3.74); IR ν_{max} (KBr)/cm^{-1} 3424, 2932, 1714, 1597, 1270; ^1H and ^{13}C NMR data (see Tables 2 and 4); (+)-ESIMS *m*/*z* 561 (80) $[M+Na]^+$; (+)-HRESIMS *m*/*z* 561.2092 $[M+Na]^+$ (calcd for $C_{30}H_{34}O_9Na$, 561.2100).

*4.3.14. Ananonin N (**14**).* White solid; $[\alpha]_{D^{28}}$ −52.1 (*c* 0.18, $CHCl_3$); CD λ_{max} (CH_3OH)/nm ($\Delta\varepsilon$) 220 (+7), 240 (−6); UV λ_{max} ($CHCl_3$)/nm (log ε) 241 (4.08), 219 (3.62), 198 (3.72); IR ν_{max} (KBr)/cm^{-1} 3432, 2939, 1740, 1716, 1257; ^1H and ^{13}C NMR data (see Tables 2 and 4);

(+)-ESIMS m/z 603 (100) $[M+Na]^+$; (+)-HRESIMS m/z 603.2207 $[M+Na]^+$ (calcd for $C_{32}H_{36}O_{10}Na$, 603.2208).

4.4. Cell survival assay

SH-SY5Y neuroblastoma cells were obtained from ATCC (American Type Culture Collection) and maintained at 37 °C in a humidified atmosphere containing 5% CO_2. Cells were seeded into 96-well plates (Greiner) at a density of 5×10^4 cells per mL in DMEM/F12 (Gibco), supplemented with 10% heat-inactivated bovine calf serum, 100 units/mL penicillin, and 100 mg/mL streptomycin. All experiments were carried out 24 h after cells were seeded. Appropriate concentrations of hydrogen peroxide (H_2O_2) were prepared in deionized water on the day of application to cultures. The SH-SY5Y cells were preincubated with different compounds 2 h before H_2O_2 (1 mM) was added, and the assay for cell viability was performed 24 h after H_2O_2 was added. Cell survival was evaluated by reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma).[25] The values of cell survival were normalized against the values for control group, which was set to 100%. Data were evaluated for statistical significance with one-way ANOVA followed by LSD test by using a computerized statistical package. Differences were considered significant at $p<0.05$.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2011.04.105. These data include MOL files and InChIKeys of the most important compounds described in this article.

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