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Discovery of Natural Products from *Curcuma longa* that Protect Cells from Beta-Amyloid Insult: A Drug Discovery Effort against Alzheimer's Disease

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From *Curcuma longa*, two novel compounds, 4″-(3‴-methoxy-4‴-hydroxyphenyl)-2″-oxo-3″-enebutanyl 3-(3′-methoxy-4′hydroxyphenyl)propenoate (calebin-A, **1**) and 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,4,6-heptatrien-3-one (**2**), and seven known compounds, 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione (curcumin, **3**), 1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-1,6-heptadiene-3,5-dione (demethoxycurcumin, **4**), 1,7-bis(4-hydroxyphenyl)-1,6-heptadiene-3,5-dione (bisdemethoxycurcumin, **5**), 1-hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)-6-heptene-3,5-dione (**6**), 1,7-bis(4-hydroxyphenyl)-1-heptene-3,5-dione (**7**), 1,7-bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one (**8**), and 1,5-bis(4-hydroxy-3-methoxyphenyl)-1,4-pentadien-3-one (**9**), were isolated following a bioassay-guided fractionation scheme utilizing an assay to detect protection of PC12 cells from β-amyloid insult. Compounds **1**, **3**−**5**, and **7** were found to more effectively protect PC12 cells from βA insult (ED_{50} = 0.5−10 μg/mL) than Congo red (**10**) (ED_{50} = 37−39 μg/mL).

Alzheimer's disease (AD) is the most common cause of progressive cognitive dysfunction. AD affects approximately four million Americans and causes more than 100 000 deaths each year with a total annual cost approaching $100 billion.[1,2] One of the principal pathological characteristics of AD is extracellular deposition of β-amyloid (βA) as senile plaques, composed of insoluble aggregates of βA, infiltrated by reactive microglia and astrocytes.[3,4] Senile plaques appear to be involved in cerebral amyloid angiopathy, consequent neuronal loss, and cerebral atrophy leading to dementia.[3,4] Since βA accumulation in the form of senile plaques has been recognized as one of the major potential causes of AD pathology, modulation of βA toxicity has been speculated to be an important therapeutic approach to control the onset of AD.[5,6]

Current pharmacological approaches related to AD treatment include antioxidant therapy,[7,8] acetylcholinesterase inhibitors,[9,10] nicotinic and muscarinic agonists,[11,12] estrogen,[13] nerve growth factor (NGF),[14,15] low molecular lipophilic compounds that can activate neurotrophic factor signaling pathway,[16] nonsteroidal antiinflammatory drugs such as ibuprofen and COX-2 inhibitors,[17] drugs that interfere with βA formation and deposition,[18] and drugs that attenuate βA toxicity.[19]

In the area of AD research, plants have attracted relatively little attention as a potentially valuable resource for drug discovery. Only *Ginkgo biloba* L. (Ginkgoaceae)[20,21] and *Huperzia serrata* (Thunb. ex Murray) Trevis. (Pteridophyta)[22,23] have been extensively investigated as natural therapeutic agents to treat AD patients. Thus it was envisioned that plants may produce natural products that may protect neuronal cells from βA insult.

During a preliminary study, we found a methanol extract of turmeric (*Curcuma longa* L., Zingiberaceae), a tropical herb indigenous to southern Asia, to protect PC12 cells from βA insult.[24] The dried rhizome of *C. longa* has been used as an aromatic stomachic, carminative, anthelmintic, laxative, and condiment in foods and for liver ailment.[25]

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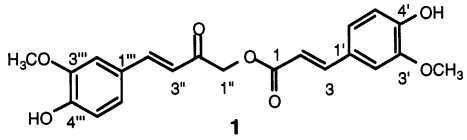
Results and Discussion

Following a bioassay-guided fractionation scheme utilizing an assay to detect protection of PC12 cells from β-amyloid insult, we isolated two new compounds, 4″-(3‴-methoxy-4‴-hydroxyphenyl)-2″-oxo-3″-enebutanyl 3-(3′-methoxy-4′-hydroxyphenyl)propenoate (calebin-A, **1**) and 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,4,6-heptatrien-3-one (**2**), in addition to seven known compounds, 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione (curcumin, **3**),[26,27] 1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-1,6-heptadiene-3,5-dione (demethoxycurcumin, **4**),[26,27] 1,7-bis(4-hydroxyphenyl)-1,6-heptadiene-3,5-dione (bisdemethoxycurcumin, **5**),[28] 1-hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)-6-heptene-3,5-dione (**6**),[28,29] 1,7-bis(4-hydroxyphenyl)-1-heptene-3,5-dione (**7**),[30] 1,7-bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one (**8**),[31] and 1,5-bis(4-hydroxy-3-methoxyphenyl)-1,4-pentadien-3-one (**9**).[32]



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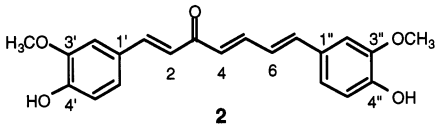

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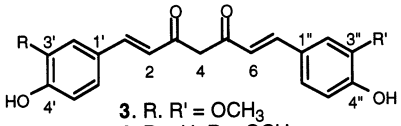
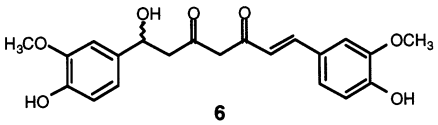


3. R. R′ = OCH_3
4. R = H, R = OCH_3
5. R, R′ = H



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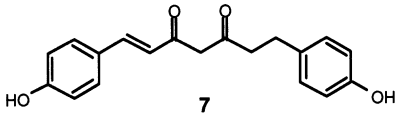

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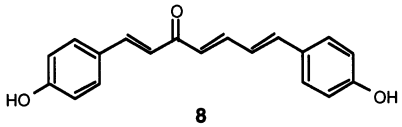

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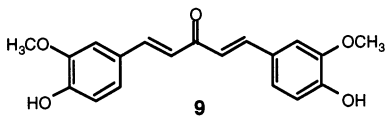

9

The structures of the known compounds were identified and confirmed by comparing their MS and ^{1}H and ^{13}C NMR spectral data with those reported in the literature and with authentic samples. Compound **7** was recently isolated from *Alpinia bleppharocalyx* K. Schum. (Zingiberaceae) as an antiplatelet agent,[30] but is reported here for the first time as a constituent of *C. longa*.

Compound **1** (calebin-A) was obtained as an amorphous yellow powder, mp 138−139 °C. The molecular formula was determined to be $C_{21}H_{20}O_7$ ([M]$^+$ at *m*/z 384.1215) by HREIMS. The IR absorption bands at 3393 and 1697 cm^{-1} indicated the presence of hydroxyl and ketone functionalities. The ^{13}C NMR spectrum of **1** shows signals for 20 carbons, including two carbonyl groups (C-2″, δ 192.9; C-1, δ 166.9) and three phenolic carbons (C-3′and C-3‴, δ_C 148.8; C-4′, δ_C 150.2; C-4‴, δ_C 150.5). The ^{1}H NMR spectrum of **1** shows the presence of four doublets, each with J = 15.9 Hz, at δ_H 7.68, δ_H 7.67, δ_H 6.86, and δ_H 6.52 attributable to two pairs of *trans* olefinic protons (H-3, H-4″, H-3″, and H-2, respectively), one pair of *ortho* coupled protons at δ_H 6.90 (H-5‴, d, J = 8.1 Hz) and δ_H 6.89 (H-5′, d, J = 8.1 Hz), one pair of *meta* coupled protons at δ_H 7.38 (H-2‴, d, J = 1.8 Hz) and δ_H 7.40 (H-2′, d, J = 1.8 Hz), and one pair of *ortho* and *meta* coupled protons at δ_H 7.19 (H-6‴, dd, J = 8.1, 1.8 Hz) and δ_H 7.22 (H-6′, dd, J = 8.1, 1.8 Hz). The chemical shift of the H-1″ protons at δ_H 5.10 (2H, s) and the ^{13}C NMR chemical shift of the C-1″ at δ_C 67.9 (t) suggest that it is a methylene group between a carbonyl carbon and an ester oxygen. The placement of methylene carbon at the 1″ position was established by ^{1}H−^{13}C long-range correlation of H-3″ (δ_H 6.86) with methylene carbon C-1″ (δ_C 67.9) in the HMBC NMR spectrum. This assignment was confirmed by HMBC correlation of H-1″ (δ_H 5.10) with C-1 (δ_C 166.9) and C-2″ (δ_C 196.9). On the basis of the spectral data, the structure of compound **1** (calebin-A) was determined as 4″-(3‴-methoxy-4‴-hydroxyphenyl)-2″-oxo-3″-enebutanyl 3-(3′-methoxy-4′-hydroxyphenyl)propenoate. The carbons and protons of compound **1** were assigned using APT, ^{1}H−^{1}H COSY, HMQC, and HMBC NMR techniques. HMBC NMR data were obtained using three different NMR solvent systems (acetone-d_6, DMSO-d_6, and $CDCl_3$), and the results from acetone-d_6 solvent system are presented in Table 1.

Table 1. ^{1}H and ^{13}C NMR Data and HMBC Correlation of Compound **1** in Acetone-d_6

position	^{1}H	^{13}C	HMBC
1		166.9	
2	6.52 (1H, d, 15.9)	115.1	C-1, C-1′
3	7.68 (1H, d, 15.9)	146.5	C-1, C-2, C-2′, C-6′
1′		127.3	
2′	7.40 (1H, d, 1.8)	111.4	C-3, C-4′, C-6′
3′		148.8	
4′		150.2	
5′	6.89 (1H, d, 8.1)	116.1	C-1′, C-3′
6′	7.22 (1H, dd, 8.1, 1.8)	124.2	C-2′, C-4′, C-5′
1″	5.10 (2H, s)	67.9	C-1, C-2″
2″		192.9	
3″	6.86 (1H, d, 15.9)	120.4	C-1″, C-2″, C-1‴
4″	7.67 (1H, d, 15.9)	144.4	C-2″, C-2‴, C-6‴
1‴		127.4	
2‴	7.38 (1H, d, 1.8)	111.6	C-4″, C-4‴, C-6‴
3‴		148.8	
4‴		150.5	
5‴	6.90 (1H, d, 8.1)	116.2	C-1‴, C-3‴
6‴	7.19 (1H, dd, 8.1, 1.8)	124.5	C-4″, C-2‴, C-4‴
3′OMe[a]	3.94 (3H, s)	56.34	C-3‴
3‴OMe[a]	3.91 (3H, s)	56.31	C-3‴

[a] The assignment can be interchanged.

The molecular formula of compound **2** was established as $C_{21}H_{20}O_5$ by HREIMS ([M]$^+$ at *m*/z 352.1324). The IR absorption bands at 3345 and 1635 cm^{-1} indicated the presence of hydroxyl and ketone functionalities. The ^{1}H NMR spectrum of compound **2** indicated the presence of two trisubstituted benzene rings (δ_H 6.85, 1H, d, J = 7.9 Hz, H-5″; δ_H 6.89, 1H, d, J = 7.9 Hz, H-5′; δ_H 7.07, 1H, dd, J = 1.8 and 7.9 Hz, H-6″; δ_H 7.22, 1H, dd, J = 1.8 and 7.9 Hz, H-6′; δ_H 7.24, 1H, d, J = 1.8 Hz, H-2″; δ_H 7.38, 1H, d, J = 1.8 Hz, H-2′), three sets of olefinic protons (δ_H 6.64, 1H, d, J = 15.2 Hz, H-4; δ_H 7.02, 1H, d, overlapped, H-7;

Table 2. ^{1}H and ^{13}C NMR Data and HMBC Correlation of Compound **2** in Acetone-d_6

position	^{1}H	^{13}C	HMBC
1	7.62 (1H, d, 15.9)	143.3	C-3, C-2′, C-6′
2	7.09 (1H, d, 15.9)	123.9	C-3, C-1′
3		188.8	
4	6.64 (1H, d, 15.2)	129.1	C-2, C-3, C-6
5	7.52 (1H, m)	143.8	C-3, C-6, C-7
6	7.03 (1H, overlapped)	125.6	C-5, C-1″
7	7.02 (1H, d, 3.5)	142.3	C-2″, C-6″
1′		128.1	–
2′	7.38 (1H, d, 1.8)	111.6	C-1, C-3′, C-4′, C-6′
3′		148.8	
4′		150.2	
5′	6.89 (1H, d, 8.1)	116.2	C-1′, C-2′, C-3′
6′	7.22 (1H, dd, 8.1, 1.8)	124.1	C-1, C-2′, C-4′
1″		129.6	
2″	7.24 (1H, d, 1.8)	111.7	C-7, C-1″, C-4″, C-6″
3″		148.8	
4″		149.1	
5″	6.85 (1H, d, 8.1)	116.1	C-1″, C-2″, C-3″
6″	7.07 (1H, dd, 8.1, 1.8)	122.6	C-7, C-2″, C-4″
3′OMe[a]	3.93 (3H, s)	56.4	C-3″
3″OMe[a]	3.91 (3H, s)	56.3	C-3″

[a] The assignments can be interchanged.

δ_H 7.03, 1H, overlapped, H-6; δ_H 7.09, 1H, d, J = 15.9 Hz, H-2; δ_H 7.52, 1H, m, H-5; δ_H 7.62, 1H, d, J = 15.9 Hz, H-1), and two methoxyl groups (δ_H 3.93, OCH_3-3′; δ_H 3.91, OCH_3-3″). The ^{13}C NMR spectrum shows signals for a carbonyl carbon (δ_C 188.8, C-3) and two methoxyl carbons (δ_C 56.3, OCH_3-3″; δ_C 56.4, OCH_3-3′). These data indicated that compound **2** has only one ketone group. The HMBC correlation of H-1 (δ_H 7.62), H-2 (δ_H 7.09), H-4 (δ_H 6.64), and H-5 (δ_H 7.52) with the C-3 (δ_C 188.8) carbonyl suggested that H-1 and H-5 are three bonds away from the C-3 carbonyl group, while H-2 and H-4 are adjacent to the carbonyl group. The HMBC spectrum revealed H-6 (δ_H 7.03) to be correlated with C-1″ (δ_C 129.6) and C-5 (δ_C 143.8), and H-7 (δ_H 7.02) correlated with C-2″ (δ_C 110.6), C-6″ (δ_C 122.6), and C-5 (δ_C 143.8). The assignment of adjacent protons H-4, H-5, H-6, and H-7 was confirmed by the TOCSY NMR experiment. Thus, compound **2** was identified as 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,4,6-heptatrien-3-one. The carbons and protons of compound **2** were assigned using APT, COSY, TOCSY, HMQC, and HMBC NMR spectral data (Table 2).

Protection of neuronal cells from βA insult by compounds **1**−**9** was tested using PC12 cells (Table 3). Congo red (**10**), which has been reported to protect neuronal cells from βA insult,[33−35] was used as a positive control reference compound. Congo red's ability to protect neuronal cells from βA insult was discovered during a mechanism study of Congo red's interaction with βA fibrils, and it has been suggested as a useful prototype for drugs targeted to the amyloid pathology of AD.[35] Compounds **1**, **3**−**5**, and **7** were found to protect PC12 cells against βA insult, with ED_{50} values ranging between 0.5 and 10 μg/mL. With respect to compounds **1**, **3**−**5**, and **7**, compound **6** showed relatively weak protection against βA(25−35) and βA(1−42) insult with ED_{50} values of 30.7 and 44.3 μg/mL, respectively. Compounds **2**, **8**, and **9** were inactive. Compounds **1**−**9** were not toxic to cells at the concentration they were tested. Congo red (**10**) protected PC12 cells from βA(25−35) and βA(1−42) insult with ED_{50} values of 37.5 and 39.2 μg/mL, respectively, suggesting that compounds **1**, **3**−**5**, and **7** are better in protecting cells from βA insult than Congo red (**10**). Compounds **1** and **7** were most effective in protecting the cells from βA insult, with ED_{50} values < 2 μg/mL. Analyses of the activity of the isolated compounds suggested that neither the degree of conjugation nor the 3-methoxy group in the phenyl ring is important for the protection of PC12 cells from βA insult. Instead, the β-diketone moiety appeared to be important for the activity, since compounds **2**, **8**, and **9**, which have only one ketone group in their structures, did not protect PC12 cells from βA insult.

Table 3. Protection of PC12 Cells from βA Insult by Natural Products (**1**−**9**) Isolated from *C. longa*[a]

	PC12 cells	
compound	anti-βA(25−35) ED_{50}[b] (μg/mL)	anti-βA(1−42) ED_{50}[b] (μg/mL)
1	1.0 ± 0.3	2.0 ± 0.4
2	>50	>50
3	7.0 ± 1.1	10.0 ± 0.9
4	4.0 ± 0.5	5.0 ± 0.5
5	2.0 ± 0.6	3.5 ± 0.7
6	30.7 ± 3.3	44.3 ± 3.1
7	0.5 ± 0.2	1.0 ± 0.3
8	>50	>50
9	>50	>50
10	37.5 ± 5.4	39.2 ± 5.2

[a] Tests were performed in triplets on three different dates. Data are mean ± SEM from nine determinations. $P < 0.05$ (student's t-test). [b] ED_{50} represents the sample concentration that is required to achieve 50% cell viability, a midpoint between the treatment with 1% DMSO only and treatment with βA and 1% DMSO without the presence of test compound. Under βA and 1% DMSO treatment of cells, in the absence of test compound, >95% of cells were considered dead.

Experimental Section

General Experimental Procedures. Melting points were determined with a Fisher-Johns melting point apparatus (Pittsburgh, PA) and are uncorrected. Ultraviolet absorption spectra were recorded using a Beckman DU-7 spectrophotometer (Beckman Instrument Inc., Fullerton, CA). Infrared spectra were obtained using a Jasco FT/IR-410 Fourier transform infrared spectrometer (Jasco Corporation, Tokyo, Japan). Nuclear magnetic resonance (NMR) spectra were obtained on a Bruker Avance DPX-300 (300 MHz for ^{1}H NMR; 75 MHz for ^{13}C NMR). δ values are expressed in ppm and J values are expressed in Hz. Low- and high-resolution mass spectra were recorded on Finnigan MAT 90 mass spectrometer (Finnigan A.G., Bremen, Germany). High-performance liquid chromatography (HPLC, Waters, Waters Co, Milford, MA) was performed on analytical and semipreparative scales using YMC ODC-AQ Pack columns [250 × 4.6 mm i.d., S-5 μm, 120 Å (analytical); 250 × 20 mm i.d., S-5 μm, 120 Å (semipreparative); YMC, Inc., Wilmington, NC] with YMC ODC-AQ Guard Pack guard columns [10 × 2.1 mm i.d., S-5 μm, 120 Å (analytical); 50 × 20 mm i.d., S-5 μm, 120 Å (semipreparative)]. Samples were injected onto analytical and semipreparative columns using a Waters 717 Plus autosampler.

Chemicals. The chemicals were purchased from Aldrich chemical company. MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide), SDS (electrophoresis grade), dimethyl sulfoxide (DMSO), and *N*,*N*-dimethyl formamide (DMF) were purchased from Sigma (St Louis, MO). βA(25−35) and βA(1−42) were purchased from BaChem California Inc. (Torrance CA). Culture media and supplements were obtained from Life Technologies (Grand Island, NY). Other general supplies needed for bioassay were purchased from Fisher Scientific (Itasca, IL). MTT was dissolved in sterile PBS (1 mg/mL) and further sterilized by filtration through a 0.2 μm filter. Sterilized MTT solution was stored at 4 °C in an amber bottle until use. Lysis buffer was prepared by dissolving 20% w/v of SDS in 50% DMF in distilled water; the pH was adjusted to 4.7 using 80% acetic acid and 2.5% HCl.

Plant Material. Turmeric powder (*Curcuma longa* L., Zingiberaceae) was provided by Swagger Foods Corporation

(Vernon Hills, IL). A voucher sample (SP-99-017) was deposited at the John G. Searle Herbarium, Field Museum of Natural History, Chicago, IL.

Extraction and Isolation. The natural products that protect PC12 cells from βA insult were isolated using a bioassay-guided fractionation scheme. Briefly, ground turmeric (886 g) was extracted with 90% methanol overnight (3×) by percolation, and the combined methanol extract was concentrated under vacuum to afford a residue of 150 g. The residue (110 g) was dissolved in a small amount of methanol and partitioned between petroleum ether/water, chloroform/water, and ethyl acetate/water, successively. After removing the solvents under vacuum, the residues from each partition (petroleum ether, 21.2 g; chloroform, 40.5 g; ethyl acetate, 30.2 g; aqueous, 14.6 g) were screened for their ability to protect the cells from βA(25−35) and βA(1−42) insult at 1.0 and 5.0 μg/mL, respectively. The biologically active chloroform residue (20 g) was subjected to normal-phase open column chromatography (Si gel, 1 kg, 70−230 mesh) using a chloroform/methanol gradient solvent system (100% chloroform to 100% methanol) to afford 10 fractions (F001−F010). Compound **3** (102 mg) was obtained as yellow crystals from fraction F002 (700 mg). The leftover residue of fraction F002 (540 mg) was further applied to normal-phase open column chromatography (Si gel, 1 kg, 70−230 mesh), eluting with chloroform to afford three fractions, F011 (105 mg), F012 (132 mg), and F013 (83 mg). Further purification of fraction F012 (120 mg) and F013 (70 mg) was performed using C_{18}-ODS reversed-phase semipreparative HPLC (70% methanol in water and 70% acetonitrile in water solvent systems; flow rate: 5 mL/min) and Sephadex LH-20 column chromatography (80% methanol in water) to afford compounds **1** (9.2 mg), **2** (0.9 mg), **6** (0.7 mg), and **9** (3.5 mg) from fraction F012 and compound **4** (10.4 mg) from fraction F013. Fractions F005 (1.9 g) and F006 (1.7 g) were combined and subjected to open column chromatography (Si gel as resin) using a chloroform/methanol gradient solvent system (100% chloroform to 100% methanol) to afford 10 fractions (F014−F023). Compound **5** was precipitated from fraction F018 (155 mg), and the precipitate was filtered and further purified using C_{18}-ODS reversed-phase semipreparative HPLC using 70% methanol in water (flow rate: 5 mL/min) as an elution system to afford 52.2 mg of compound **5**. The purification of fraction F017 (305 mg) by silica gel column chromatography using a chloroform/acetone gradient solvent system (100% chloroform to 100% acetone) and C_{18}-ODS reversed-phase semipreparative HPLC (80% methanol in water solvent system, flow rate: 5 mL/min) afforded compounds **7** (3.5 mg) and **8** (1.1 mg).

Cell Cultures. PC12 rat pheochromocytoma cells were obtained from the American Type Culture Collection (ATCC). Cells were routinely cultured on a tissue culture plate (Corning, New York, NY). Cells were grown in high glucose Dulbecco's Modified Eagle Medium (DMEM), 10% horse serum, 5% fetal calf serum, and 1% penicillin/streptomycin.

Determination of Ability to Protect PC12 Cells against βA Insult. In AD, βA(1−42) insult to neuronal cells has been identified as one of the major causes of the onset of the disease.[3−7] βA(25−35) has been identified as the toxic fragment of βA(1−42) which causes insult to neuronal cells.[7,35] Compounds were evaluated for neuronal cell protection against βA insult using both peptides, βA(25−35) or βA(1−42).

Bioassay was performed according to a previously published method,[24] which was adapted and modified from a system previously reported.[36] A sample's ability to protect PC12 cells from βA(25−35) or βA(1−42) insult was determined by measuring the cell's potential to reduce MTT to MTT formazan, which reflects the viability of cells.[24,36,37] Briefly, 90 μL of exponentially growing cells (2000 cells per mL) were plated in collagen-coated 96-well tissue culture plates overnight. Cells were incubated with βA(1−42) (2.0 μg/mL, prepared from a stock solution (1.0 mg/mL in DMSO)) and test compound at various concentrations (50.0, 10.0, 2.0, and 0.4 μg/mL) for 24 h. The final DMSO concentration was less than 1%. After the 24 h incubation of cells, MTT solution (25 μL per well, 1 mg/mL stock solution) was added for 1 h at 37 °C, 100 μL of Lysing buffer was added, and the cells were incubated overnight at 37 °C. Optical density of the resulting solutions was colorimetrically determined at 570 nm using a microplate reader. Dose−response curves were prepared, and the results are expressed as ED_{50} values in μg/mL (Table 1). ED_{50} values of samples were defined as the sample concentration (μg/mL) that is required to achieve 50% cell viability under βA insult, a midpoint value between the treatment with 1% DMSO alone and treatment with βA and 1% DMSO without the presence of test compound. In the absence of test compound, both βA-(1−42) and βA(25−35) were toxic to cells at 2.0 and 1.0 μg/mL concentration, respectively, as determined by an MTT reduction assay.[24,36,37] At these βA concentrations, >95% of cells were considered dead. Treatment of cells with test compound in the presence of 1% DMSO, in the absence of βA, did not show any change in cell viability with respect to 1% DMSO alone treated cells. Congo red (**10**), which has been reported to protect neuronal cells from βA insult, was tested as a positive control.[33−35] Bioassay using 1.0 μg/mL of βA(25−35) was similarly performed.

4″-(3‴-Methoxy-4‴-hydroxyphenyl)-2″-oxo-3″-enebutanyl-3-(3′-methoxy-4′-hydroxyphenyl)propenoate (calebin-A, 1): light yellow powder; mp 138−139 °C; IR ν_{max} (neat) 3393,1697, 1588, 1512, 1430, 1268, 1158, 1032, 980, 846, 812, 754 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 339 (3.73), 240 (3.44), 219 (3.42) nm; ^{1}H NMR (acetone-d_6, 300 MHz), see Table 1; ^{13}C NMR (acetone-d_6, 75 MHz), see Table 1; HREIMS *m*/*z* $[M]^{\bullet+}$ 384.1215 ($C_{21}H_{20}O_7$, calcd 384.1209), EIMS *m*/*z* 384 ($M^{\bullet+}$, 17), 194 (11), 178 (25), 177 (100), 145 (36), 117 (18).

1,7-Bis(4-hydroxy-3-methoxyphenyl)-1,4,6-heptatrien-3-one (2): yellow powder; mp 128−129 °C; IR ν_{max} (neat) 3345, 1635, 1510, 1278, 1088 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 406 (3.84), 267 (3.54) nm; ^{1}H NMR (acetone-d_6, 300 MHz), see Table 2; ^{13}C NMR (acetone-d_6, 75 MHz), see Table 2; HREIMS *m*/*z* $[M]^{\bullet+}$ 352.1324 ($C_{21}H_{20}O_5$, calcd 352.1310); EIMS *m*/*z* 352 ($M^{\bullet+}$, 59), 272 (100), 177 (42), 151 (37), 149 (58), 137 (97), 115 (44).

1,7-Bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione (curcumin, 3): yellow needles; mp 183−184 °C [lit.[27] mp 183 °C]; IR, UV, NMR, and MS data were in agreement with literature values.[26,27]

1-(4-Hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-1,6-heptadiene-3,5-dione (demethoxycurcumin, 4): yellow needles; mp 180−181 °C [lit.[27] mp 181−182 °C]; IR, UV, NMR, and MS data were in agreement with literature values.[26,27]

1,7-Bis(4-hydroxyphenyl)-1,6-heptadiene-3,5-dione (bisdemethoxycurcumin, 5): yellow needles; mp 232−233 °C [lit.[27] mp 232−234 °C]; IR, UV, NMR, and MS data were in agreements with literature values.[28]

1-Hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)-6-heptene-3,5-dione (6); yellow powder; mp 92−94 °C [lit.[28] mp 84−88 °C]; IR, UV, NMR, and MS data were in agreement with literature values.[28,29]

1,7-Bis(4-hydroxyphenyl)-1-heptene-3,5-one (7): yellow needles; mp 145−146 °C [lit.[30] mp 148−149 °C]; IR, UV, NMR, and MS data were in agreement with literature values.[30]

1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one (8): yellow powder; mp 147−148 °C; IR, UV, NMR, and MS data were in agreement with literature values.[31]

1,5-Bis(4-hydroxy-3-methoxyphenyl)-1,4-pentadien-3-one (9): yellow powder; mp 85−86 °C [lit.[32] mp 82−83 °C]; IR, UV, NMR, and MS data were in agreement with literature values.[32]

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