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Total Synthesis of Calebin-A, Preparation of Its Analogues, and Their Neuronal Cell Protectivity Against β-Amyloid Insult

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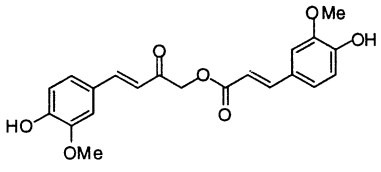
Abstract—A total synthesis of Calebin-A (**1**), a novel curcuminoid isolated from turmeric (*Curcuma longa*, Zingiberaceae) that has been demonstrated to protect neuronal cells from β-amyloid insult, was successfully achieved in four steps. Elaborating on this synthetic route, 13 analogues were prepared for a structure–activity relationship (SAR) study. It was found that the parent compound **1** and derivatives **21**, **28**, and **30** protect PC12 rat pheocromocytoma and IMR-32 human neuroblastoma cells from β-amyloid(25–35) insult. These results suggest that hydroxy group at *para*-position is most critical for the expression of biological activity.

Alzheimer's disease (AD) is the most common cause of progressive cognitive dysfunction that affects approximately four million Americans, causing more than 100,000 deaths each year with a total annual cost of treatment approaching $100 billion.[1] β-Amyloid (βA) insult to neuronal cells was recently found to be one of the major causes of AD pathology. Thus, modulation of βA insult has been speculated to be an important therapeutic approach to control the onset of AD.

Recently, we discovered a novel curcuminoid, Calebin-A (**1**), from turmeric (*Curcuma longa* L., Zingiberaceae) that protected PC12 rat pheocromocytoma and normal human umbilical vein endothelial HUVEC cells from βA(1–42) insult.[2] Turmeric has been used as curry spice and as a well-known constituent of Indonesian traditional medicine.[3]



Calebin-A, 1

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In the present study, we report the first total synthesis of Calebin-A (**1**), along with its derivatives for a structure–activity relationship (SAR) study using PC12 and IMR32 cells against βA(25–35) insult.

Melting points were determined with a Fisher–Johns Melting Point apparatus and were uncorrected. UV spectra were obtained on a Beckman DU-7 spectrophotometer. ^{1}H NMR (300 MHz) and ^{13}C NMR (75.6 MHz) spectra were run on a Bruker DPX-300 spectrometer with TMS as an internal standard. EI-MS was performed with a Finnigan MAT 90 instrument. The structures of the compounds were elucidated by using ^{1}H and ^{13}C NMR and mass spectral analysis.

Total Synthesis of Calebin-A (Scheme 1)

Alcohol functionality of acetol **2** and vanillin **4** was protected as THP ether using DHP in the presence of PPTS in THF in quantitative yield.[4] THP ether of acetol **3** was reacted with LDA in THF at −78 °C and reacted with THP ether of vanillin **5** to afford THP ether of β-hydroxy ketone **6** in 27% yield. THP protection groups were removed from β-hydroxy ketone THP ether **6** by stirring in methanol in the presence of PPTS at 50 °C that also caused the dehydration of β-hydroxyl group to afford compound **7** in 42% yield.[5] The ferulic acid **8** and the alcohol **7** were coupled in the presence of dicyclohexylcarbodiimide (DCC), dimethylamino-pyridine

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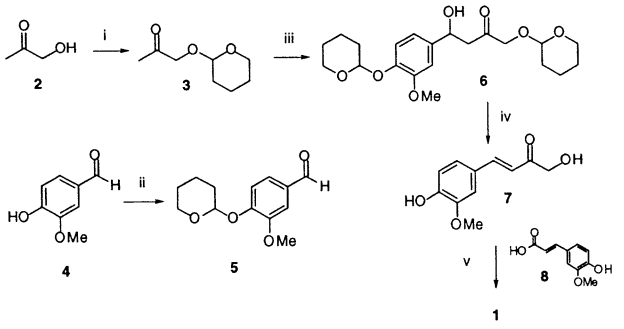
(DMAP), and DMAP–HCl in $CHCl_3$ at room temperature[6] to afford product **1** (48%). The ^1H NMR of the product was identical to that of the authentic sample.

Synthesis of Calebin-A Derivatives

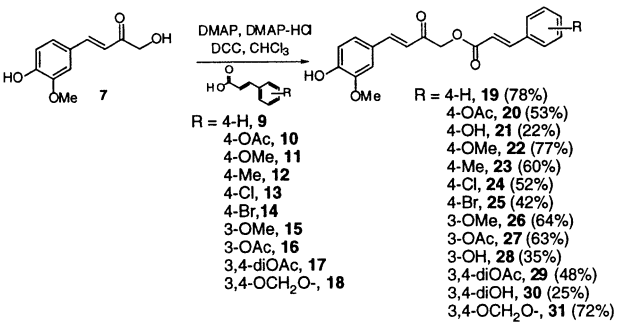
The alcohol **7** was coupled with cinnamic acid derivatives **9**–**18** in the presence of DCC, DMAP, and DMAP–HCl in $CHCl_3$ at room temperature to afford products **19**, **20**, **22**–**27**, **29**, and **31**. Compounds **21**, **28**, and **30** were prepared by removing the acetyl group from compound **20**, **27**, and **29**, respectively, using K_2CO_3/MeOH/H_2O system at room temperature (Scheme 2).

Bioassay

The bioassays were performed according to the published procedure.[2,7] PC12 rat pheochromocytoma and IMR-32 human neuroblastoma cells were obtained from the American Type Culture Collection (Rockville, MD, USA). Cells were routinely cultured on a polystyrene-coated Corning tissue culture plate (Corning, New York, USA). Culture media and supplements were obtained from Life Technologies (Grand Island, USA). PC12 cells were grown in high glucose Dulbecco's Modified Eagle Medium, 10% horse serum, 5% fetal calf serum, and 1% penicillin/streptomycin.[2,7] IMR-32 cells were cultured in Minimum Esssential Medium with Earle's salt with L-glutamine, 10% fetal bovine serum, and 1% penicillin/streptomycin.[8] For the bioassay using βA(25–35), 90 μL of exponentially growing cells (2000 cells per mL) were plated in 96-well tissue culture plates. βA(25–35), the cytotoxic fragment of βA(1–42),[9,10] was purchased from Bachem California (Torrance, CA, USA).



Scheme 1. Synthesis of Calebin-A: (i) DHP, acetol, PPTS, THP; (ii) THF, DHP, PPTS; (iii) LDA, THF (at −78 °C for 1 h) and then add compound **5**; (iv) PPTS, methanol; (v) compound **8**, DMAP, DMAP–HCl, DCC, $CHCl_3$, overnight.



Scheme 2. Synthesis of Calebin-A analogues.

βA(25–35) was shown to exert direct toxic effects on neurons and inhibit the neurite outgrowth in vitro in a dose dependent manner.[9,10] Although βA(25–35) did not appear to cause cell death at low concentration (1–5 μg/mL), it was found to cause a profound cell damage, such that, cell viability was undermined. This βA(25–35)-induced cell viability reduction was determined by observing the amount of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) reduction.[11,12] Under our experimental condition, βA(25–35) was found to attenuate the viability of PC12 and IMR-32 cells at $ED_{50} = 1.0$ μg/mL.

The compounds' ability to protect PC12 and IMR32 cells from βA(25–35) insult was investigated. PC12 cells (2000 cells per mL) were incubated with βA(25–35) (1.0 μg/mL, prepared from a stock solution, 1.0 mg/mL in DMSO) and the test compounds at various concentrations (25, 5, 1, 0.2, and 0.04 μg/mL) in a 96-well culture plate for 24 h. The final DMSO concentration was less than 1%. The compounds' ability to protect PC12 cells from βA(25–35) insult was determined by measuring the cell's potential to reduce MTT against positive control (1% DMSO only) and negative control [1.0 μg/mL βA(25–35) in 1% DMSO without the presence of the test compounds]. After an incubation of cells in MTT solution (25 μL per well, 1 mg/mL stock solution) for 2 h at 37 °C, 100 μL Lysing buffer was added and incubated overnight at 37 °C. The optical density of the resulting solutions was colorimetrically determined at 570 nm using ELISA microplate reader. Dose–response curves were prepared and the results were expressed as ED_{50} values in μg/mL (Table 1). The compounds' ability to protect IMR-32 cells (2000 cells per mL) from βA(25–35) insult was similarly evaluated using 1.0 μg/mL of βA(25–35). Curcumin (**32**) and (±)-α-tocopherol (vitamin E, **33**) were used as control compounds.

Discussion

Starting from acetol, Calebin-A (**1**) was synthesized in four steps. Using compound **7** as the key intermediate, Calebin-A derivatives were prepared in good to moderate yield in one step. The low yields in deacetylation of compounds **20**, **27**, and **29** are attributed to saponification of the ester functionality in the parent structure. Among the Calebin-A derivatives, only compounds **21**, **28**, and **30**, in addition to the parent compound Calebin-A (**1**), protected cells from βA(25–35) insult (Table 1). The cell protection from βA(25–35) insult by these compounds showed that 4-hydroxy group on cinnamate portion of the compound is important for the compounds' ability to protect cells from βA(25–35) insult. The result from compound **28** showed that 3-hydroxy

Table 1. Evaluation of Calebin-A analogues against β-amyloid(25–35) insult toward PC12 and IMR-32 cells

Compound	ED_{50} (μg/mL)[a]	
	PC12	IMR32
1	1.0±0.3	1.4±1.1
19	np[b]	np
20	np	np
21	0.7±0.4	1.2±0.6
22	np	np
23	np	np
24	np	np
25	np	np
26	np	np
27	np	np
28	13.2±0.9	15.8±1.2
29	np	np
30	7.3±0.5	7.9±0.6
31	np	np
32	7.1±0.4	7.4±0.7
33	np	np

The tests were performed in triplets on three different dates. Data are mean±SEM from nine determinations. $P<0.05$ (Student's *t*-test).
[a]ED_{50} represents the sample concentration that is required to achieve 50% cell viability, a mid-point between the positive control values and the negative control values.
[b]np denotes no protection from βA(25–35) insult.

group is less effective than 4-hydroxy group in protecting cells from βA(25–35) insult. The result from compound **30** suggested that 3-hydroxy group may be hindering the efficacy of 4-hydroxy group, probably through hydrogen bonding between them. Evidently, 3-methoxyl group is not critical for the expression of the biological activity. Under the same experimental condition, vitamin E (**33**) did not protect cells from βA(25–35) insult at concentrations >25 μg/mL, while curcumin (**32**) protected cells at $ED_{50}=7$ μg/mL.[2,7] It is noteworthy that compounds **1**, **21**, **28**, and **30** were not cytotoxic at concentrations >25 μg/mL. These results clearly demonstrated that compounds **1** and **21** warrant further investigation for the development as potentially valuable agents to treat AD patients. We are in the process of developing these compounds into therapeutic agents that may be used to treat AD.

Acknowledgements

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