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Side-chain length is important for shogaols in protecting neuronal cells from β-amyloid insult

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Abstract—Ten shogaols were synthesized to evaluate the importance of the side-chain length in protecting cells from βA(1-42) insult using PC12 rat pheochromocytoma and IMR-32 human neuroblastoma cells. The compounds cell protectivity against βA insult was demonstrated using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) reduction assay. The efficacy of cell protection from βA insult by these shogaols was shown to improve as the length of the side chain increase.

1. Introduction

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 approaching $100 billion.[1] β-Amyloid (βA) insult to neuronal cells was recently hypothesized to be one of the major potential causes of AD pathology.[2,3] Thus, modulation of βA insult has been speculated to be an important therapeutic approach to control the onset of AD. Recently, we reported the discovery of four shogaols from ginger, *Zingiber officinale* L. (Zingiberaceae), that effectively protect IMR-32 human neuroblastoma and HUVEC cells from βA(25-35) insult.[4] The efficacy of cell protection from βA insult by these shogaols was suggested to improve as the length of the side chain increase. Herein, we report synthetic preparation of shogaols of various lengths and their cell protectivity from βA insult using PC12 rat pheochromocytoma and IMR-32 human neuroblastoma cells.

Ginger is one of the worlds favorite spices, probably discovered in the tropics of Southeast Asia. Ginger has benefited humankind as a wonder drug since the beginning of recorded history.[5,6] It is used to treat various kind of diseases such as common flu, muscle ache, headache, lung infections, high fever, bacterial and fungal infections, etc. Gingerols and shogaols found in ginger were shown to exhibit significant antihepatotoxic actions against CCl_4- and galactosamine-induced cytotoxicity in primary cultured rat hepatocytes.[7] The antihepatotoxic activity of gingerols and shogaols was dependent on the length of the side chain, with the [7]- and [8]-homologues eliciting the strongest activity. Oral administration of [6]-gingerol or [6]-shogaol at doses of 70–140 mg/kg and iv administration of both compounds at doses of 1.75–3.7 mg/kg to rats produced an inhibition of spontaneous motor activity, showed antipyretic and analgesic effects, and prolonged hexobarbital-induced sleeping time.[8,9] [6]-Shogaol was more effective than [6]-gingerol. [6]-Shogaol was also found to display an intense antitussive effect in comparison with dihydrocodeine phosphate.

Melting points were determined with a Fisher–Johns Melting Point apparatus and were uncorrected. ^{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 using ^{1}H and ^{13}C NMR and mass spectral analysis.

2. Chemistry

Synthesis of shogaols is shown in Scheme 1. Vanillin (**1**) was condensed with acetone in the presence of 75% aqueous NaOH overnight at room temperature, followed by acidification of the reaction solution using concd HCl to

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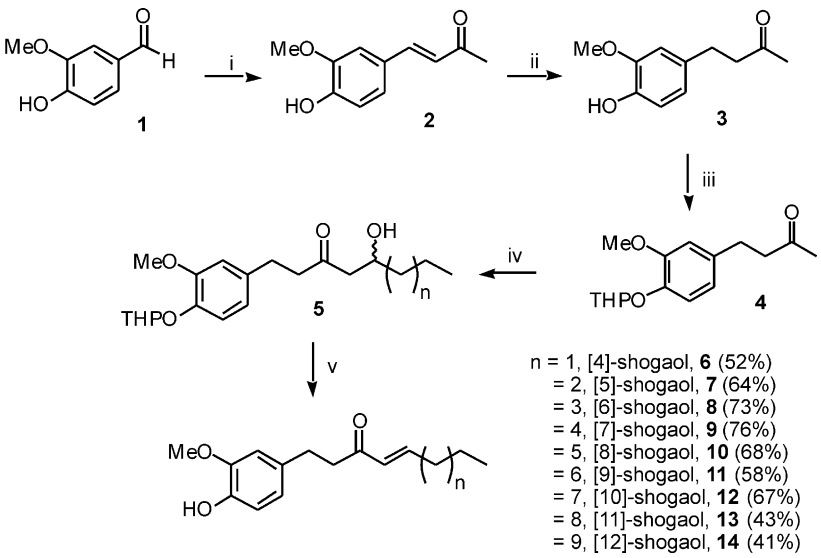


Scheme 1. Syntheis of shogaols: (i) acetone, 75% aqueous NaOH overnight followed by concd HCl; (ii) H_2, Pd–C, EtOH–HOAc; (iii) DHP, PPTS, CH_2Cl_2; (iv) LDA, −78 °C, 3 h followed by addition of alkyl aldehyde; (v) TsOH, benzene.

induce dehydration (overnight at room temperature) to afford vanillin acetone (**2**) in 95% yield. The double bond in vanillin acetone (**2**) was reduced using Raney nickel system[10] to zingerone (**3**) in 72% yield. The phenolic group of zingerone (**3**) was protected with THP group (DHP/PPTS/CH_2Cl_2).[11] The resulting THP protected zingerone (**4**) was reacted with lithium diisopropylamide (LDA) at −78 °C in THF under N_2 (2 h) to generate lithium enolate, which was reacted with various alkyl aldehydes at −78 °C for 2 h, slowly warming up to room temperature and stirring for an additional 3 h.[12,13] The residue from an aqueous work up that contains β-hydroxy ketone product (**5**) was refluxed overnight in benzene in the presence of TsOH to afford dehydration product with THP protecting group removed. The reaction mixture was diluted with EtOAc (100 mL) and washed with H_2O (50 mL×3). The water layer was back extracted using EtOAc (50 mL×2) and the organic layers were combined, dried ($MgSO_4$), filtered, and the solvent was removed in vacuo. The resulting residue was column chromatographed over silica gel (230–400 mesh) using petroleum ether/ ethyl acetate solvent system to afford the products (**6**–**14**) in various yield.

3. Cells

PC12 and IMR-32 cells were obtained from the American Type Culture Collection (Rockville, MD). Cells were routinely cultured on a polystyrene-coated Corning tissue culture plate (Corning, New York, NY). Culture media and supplements were obtained from Life Technologies (Grand Island, NY). PC12 cells were grown in high glucose Dulbecco's Modified Eagle Medium, 10% horse serum, 5% fetal calf serum, and 1% penicillin/streptomycin. IMR-32 cells were cultured in Minimum Essential Medium with Earle's salt with L-Glutamine, 10% fetal bovine serum, and 1% penicillin/ streptomycin. βA(1-42) was purchased from Bachem California (Torrance, CA).

4. Bioassay of shogaols against βA insult on PC12 and IMR-32 cells

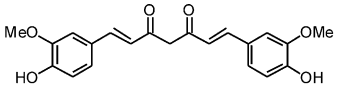
βA(1-42) was shown to exert direct toxic effects on neurons and inhibit the neurite outgrowth in vitro in a dose dependent manner.[14,15] Although βA(1-42) did not appear to cause cell death at concentrations (1–5 μg/mL), it was found to undermine cell viability as determined by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) reduction assay.[16–18] This βA(1-42)-induced cell viability reduction was determined by observing the amount of MTT reduction to MTT formazan.[16–18]

The compounds ability to protect PC12 cells from βA insult was investigated according to the published procedure.[15,17] For the bioassay, 90 μL of exponentially growing cells (2000 cells per mL) were plated in 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 (25, 5, 1, 0.2, and 0.04 μg/mL) for 24 h. The final DMSO concentration was less than 1%. The compounds ability to protect PC12 cells from βA(1-42) insult was determined by measuring the cells potential to reduce MTT to MTT formazan against cell treated with 1% DMSO only and cell treated with 1.0 μg/mL βA(1-42) and 1% DMSO without the presence of the test compound. After the incubation of cells in MTT solution (25 μL per well, 1 mg/mL stock solution) for 1 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 a microplate reader. Dose–response curves were prepared and the results were expressed as ED_{50} values in μM (Table 1). Curcumin (**15**) was used as a positive control. Bioassay on IMR-32 cells was similarly performed.

5. Results and discussion

In the present study shogaols were prepared in five steps. These shogaols were shown to protect PC12 and IMR-32 cells from βA insult (Table 1). Their efficacy to protect PC12 and IMR-32 cells from βA insult was shown to improve as the length of the side chain increase. The best result was obtained with [12]-shogaol (**14**). These shogaols did not show any cytotoxicity against PC12 and IMR-32 cells at concentrations >129 μM. These shogaols were shown to protect cells from βA(1-42) insult better than curcumin (**15**) (ED_{50} = 17.1–23.9 μM). Antioxidant property of these compounds[19] may be responsible for protecting cells from βA insult.

Table 1. Evaluation of zingerone **3**, shogaols (**6**–**14**), and curcumin (**15**) against β-amyloid(1-42) insult toward PC12 and IMR-32 cells



curcumin, **15**

Compd	PC12	IMR-32
	ED_{50} (μM)	
3	>129	>129
6	45.9±11.6	61.2±12.9
7	29.0±3.8	37.4±6.1
8	16.3±5.4	24.2±4.3
9	8.6±4.8	12.0±3.4
10	5.9±3.6	8.8±3.9
11	4.4±2.2	6.2±2.8
12	1.8±0.8	3.3±1.2
13	0.5±0.2	1.4±0.2
14	0.2±0.1	0.5±0.2
15	17.1±5.7	23.9±4.8

ED_{50} represent the sample concentration that is required to achieve 50% cell viability, a mid-point between the positive control values and the negative control values.

The tests were performed in triplets on three different dates. Data are mean±SEM from nine determinations. $P<0.05$ (student's *t*-test).

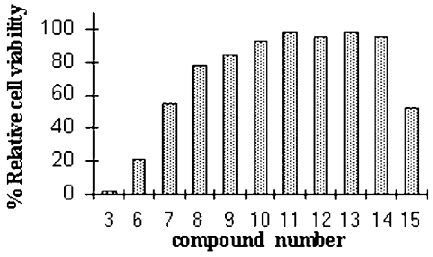


Figure 1. PC12 cells were treated overnight with test compounds (5 μg/mL) in the presence of βA(1-42) at 5.0 μg/mL, respectively. Cell viability was colorimetrically determined using MTT reduction assay. The tests were performed in triplets on three different dates. Data are mean±SEM from nine determinations. $P<0.05$ (student's *t*-test).

Figure 1 shows colorimetrically determined results of MTT reduction assay on PC12 cells, treated overnight with test compounds (5.0 μg/mL) in the presence of βA(1-42) at 5.0 μg/mL, respectively. The results further confirm that the efficacy of these shogaols in protecting cells from βA(1-42) insult improves as the side-chain length increases. The compounds increased cell protectivity from βA insult with respect to the increasing side chain length may be due to increased cell permeability of the compounds

In conclusion, our results suggest that shogaols may be potentially valuable natural therapeutic agents for the treatment of AD patients. We are in a process of developing these compounds into such therapeutic agents.

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