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New Stilbenoids Isolated from Fungus-Challenged Black Skin Peanut Seeds and Their Adipogenesis Inhibitory Activity in 3T3-L1 Cells

Zhongwei Liu,[†] Ji'en Wu,*[,‡] and Dejian Huang*[,†,§]

[†]Food Science and Technology Programme, [‡]Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore 117543, Singapore

[§]National University of Singapore (Suzhou) Research Institute, 377 Lin Quan Street, Suzhou Industrial Park, Jiangsu 215123, People's Republic of China

Supporting Information

ABSTRACT: One new stilbene derivative (3,5,3′-trihydroxy-4′-methoxy-5′-isopentenylstilbene, MIP) and two new stilbene dimers (arahypin-11 and arahypin-12) together with three known stilbenoids (arachidin-1, arachidin-3, and SB-1) were isolated from black skin peanut seeds challenged by the fungal strain *Rhizopus oligoporus*. The structures of the three new compounds were elucidated by analysis of HRESIMS, UV, 1D and 2D NMR spectra. The antiadipogenic and cytotoxic effects of the isolated compounds were investigated using 3T3-L1 cells at a concentration range of 1−10 μM. Among the compounds tested, arachidin-1 inhibited the 3T3-L1 adipocyte differentiation dose-dependently, whereas arahypin-11 and arahypin-12 exhibited significant cytotoxicity in 3T3-L1 preadipocytes.

KEYWORDS: *Arachis hypogaea, peanut seeds, stilbene, dimers, arahypin, arachidin, antiadipogenicity*

INTRODUCTION

Stilbene derivatives from peanuts (*Arachis hypogaea*) have attracted considerable interest of different research groups around the world[1−8] due to the diverse range of health-promoting properties they tend to display such as anti-inflammatory, antioxidant, and anticancer activities and possible therapeutic values for chronic diseases.[1,2] When challenged by a fungal strain, the peanut seeds produced a characteristic set of prenylated stilbene compounds including arachidin-1, arachidin-3, isopentadienylresveratrol (IPD), and SB-1,[2−7] (Figure 1), which were involved in a defense mechanism against physical injuries and fungal contamination. The relationship between the concentrations of these compounds and different processing methods of peanuts has been fully investigated.[2−7]

However, compared with major known stilbene derivative phytoalexins, other peanut stilbenoids in minor amounts were rarely reported except for a few previous studies by Sobolev et al.[5,6] in which arahypins and two stilbene dimers were isolated from challenged peanut seeds. The formation pathway of peanut stilbenoids suggests the possibility of the presence of more novel and complex stilbenoid derivatives. Until now, *Aspergillus* species are the main microbes used to challenge a few types of peanut seeds to produce stilbene phytoalexins. Combinations of new fungus species and different peanut types may have a potential to produce stilbenes of novel structures. In our previous study, a fungus strain, *Rhizopus oligoporus*, a starter for tempeh fermentation, was applied to challenge wounded Indian peanut seeds, from which two new dimeric stilbenoids, arachidin-8 and arachidin-9 (Figure 1), and one new monomeric stilbenoid, arachidin-10, were isolated. In this study, the same fungus strain was used to elicit black skin peanut seeds, a Chinese specialty peanut type, to produce novel stilbenoids we were able to isolate and characterize.

Obesity has become a worldwide public health concern and imposes significant risks on metabolic disorders including type 2 diabetes, hypertension, and coronary heart disease.[9] Inhibition of adipocyte differentiation has been an antiobesity strategy because adipose mass increase is caused not only by adipocyte hypertrophy but also by adipocyte hyperplasia (adipogenesis).[10] The entire process of adipogenesis consists of preadipocyte proliferation and their differentiation into mature adipocytes, which could be mimicked in vitro by the 3T3-L1 adipocyte differentiation system.[11] Recent studies revealed that some natural stilbenes such as piceatannol and vitisin A from peanuts and other plant sources could inhibit 3T3-L1 adipocyte differention.[12,13] These results also indicate the potential adipogenesis inhibitory activity of peanut stilbenoids. However, little is known about the effect of peanut prenylated stilbenoids on lipid metabolism in 3T3-L1 adipocytes. Therefore, in the present study, the antiadipogenic activity of the three new peanut stilbenoids arahypin-11 (**1**), arahypin-12 (**2**), and MIP (**3**) (Figure 2) together with resveratrol, arachidin-1, arachidin-3, and SB-1 was determined by 3T3-L1 adipocyte differentiation assay. At the same time, the cytotoxicity of these compounds in 3T3-L1 preadipocytes was evaluated in a MTT assay.

MATERIALS AND METHODS

Materials. Black skin peanut seeds were purchased from Beyond Organic food store (Beijing, China) and produced from Jianping in Liaoning province in China. Food grade *R. oligosporus* was obtained from PT Aneka Fermentasi Industri (Bandung, Indonesia). To obtain

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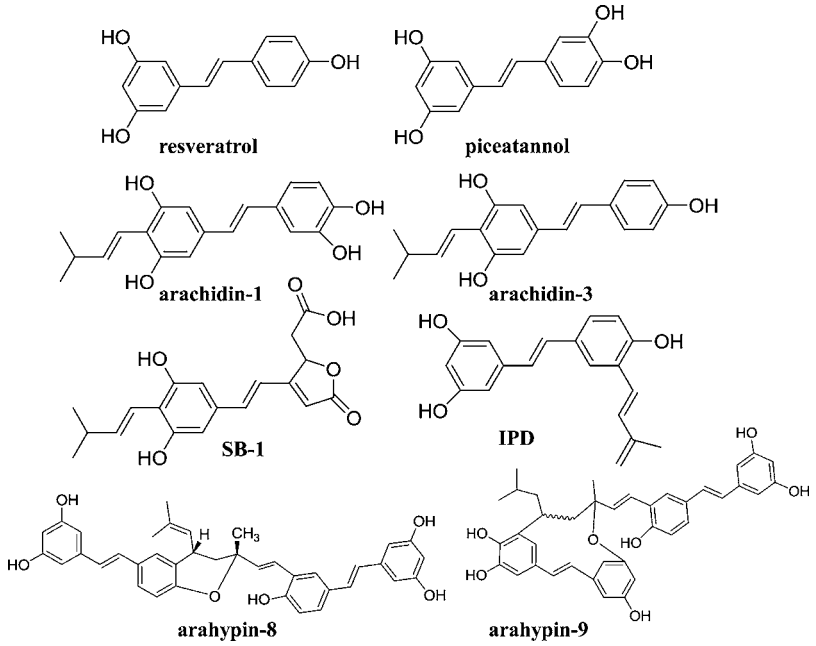

Figure 1. Structures of some known stilbenoids found in peanut seeds.

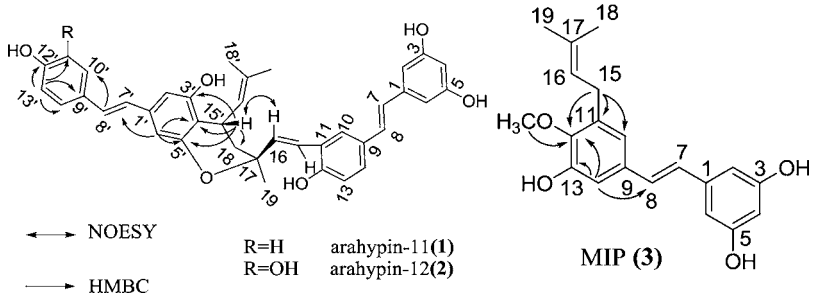

Figure 2. Selected HMBC correlations of arahypin-11 (**1**), arahypin-12 (**2**), and MIP (**3**).

a spore suspension, *R. oligosporus* culture powder (5.0 g) was stirred in 2.0 L of sterilized water overnight at room temperature. 3T3-L1 preadipocytes (5 passages) were kindly provided by Professor Benny Tan Kwon Huat from the Department of Pharmacology, National University of Singapore.

Reagents. HPLC grade solvents for preparation of mobile phases were from Tedia Co. Inc. (Fairfield, OH, USA). Analytical grade reagents used for peanut seed extraction and silica gel chromatography were from Fisher Scientific, UK, Ltd. (Loughborough, UK). Resveratrol (99%), 3-isobutyl-1-methylxanthine (IBMX), dexamethasone (DEX), thiazolyl blue tetrazolium bromide (MTT), and Oil Red O were obtained from Sigma Chemical Co. (St Louis, MO, USA). Recombinant human insulin from yeast was purchased from Roche Applied Science (Indianapolis, IN, USA), and rosiglitazone was from Cayman Chemical Co. (Ann Arbor, MI, USA). Other chemicals were of analytical grade unless otherwise specified.

Instruments. HPLC analysis was performed on a Waters HPLC system (Milford, MA, USA) with an Alliance 2695 separation module and a 2996 photodiode array (PDA) detector. The LC-MS system consisted of HPLC (Ultimate 3000) interfaced to a Bruker Amazon X mass spectrometer with an ion trap mass analyzer. HR-MS analysis was carried out on a Bruker microOTOFQ II mass spectrometer. Optical rotations were measured on a polAAr 3001 polarimeter with a sodium lamp of wavelength 589 nm and recorded as $[\alpha]_{\lambda}^{T^{\circ}C}$ (c = g/100 mL, solvent). NMR data were recorded in deuterated acetone with a Bruker Avance 500 (AV500) spectrometer (Karlsruhe, Germany). A Synergy HT microplate reader (Biotek, Winnoski, VT, USA) was used in absorbent and fluorescent assays.

Peanut Processing. Black skin peanut seeds (1.2 kg) were surface-sterilized with 70% ethanol for 3 min before imbibing distilled water overnight at room temperature. They were chopped in a food blender to obtain 3−7 mm pieces, which were then soaked in 2 L of *R. oligoporus* spore suspension at a concentration of 2.5 g/L for 5 min. After being sieved to remove small particles, the peanut pieces were blotted with paper towels and air-dried until no water spots were left on absorbent paper. An approximately 1 cm peanut pieces layer was placed on plastic trays and incubated in the dark at room temperature for 5 days. The trays were shaken by hand every day to scatter the peanut pieces, which were blotted with paper towels to remove excess moisture and prevent bacterial growth during incubation.

Isolation of New Stilbenoids. Stressed peanut pieces were extracted with 4.0 L of methanol by shaking overnight. The combined extract was filtered and concentrated to 1.2 L, which was defatted with 400 mL of *n*-hexane three times. Silica gel powder (50 g) and isopropanol (150 mL) were added to the methanol solution, and the mixture was evaporated until a free-flowing yellow powder appeared. The silica powder with peanut extract was applied to a chromatographic column (60 mm i.d.) packed with silica gel (suspended in *n*-hexane) to a height of 200 mm. The column was sequentially eluted with a gradient of *n*-hexane/acetone (4:1, 3:1, 2:1, 1:1, 1:2). Each fraction was collected as two 600 mL portions. The method of silica gel chromatography was modified from a previous method reported by Sobolev et al.[3]

HPLC analysis was carried out on a Waters 2695 system equipped with a PDA detector covering 210−400 nm range. The separation was performed on a Waters C_{18} column (5 μm, 4.6 mm × 100 mm) with 0.05% acetic acid (A) and acetonitrile (B) as the mobile phase. The

Table 1. NMR Data[a] of Arahypin-11 (1) and Arahypin-12 (2)

	arahypin-11			arahypin-12		
	δ_H (J in Hz)	δ_C	HMBC correlations	δ_H (J in Hz)	δ_C	HMBC correlations
1		141.5			141.5	
2	6.51, d (2.2)	106.3	C-3, C-4, C-6, C-7	6.50, d (2.2)	106.3	C-3, C-4, C-6, C-7
3		160.3			160.3	
4	6.25, t (2.2)	103.4	C-2, C-3, C-5, C-6	6.24, t (2.2)	103.3	C-2, C-3, C-5, C-6
5		160.3			160.3	
6	6.51, d (2.2)	106.3	C-2, C-4, C-5, C-7	6.50, d (2.2)	106.3	C-2, C-4, C-5, C-7
7	6.86, d (16.2)	127.8	C-1, C-2, C-6, C-8, C-9	6.86, d (16.4)	127.8	C-1, C-2, C-6, C-8, C-9
8	7.03, d (16.2)	129.7	C-1, C-7, C-9, C-10	6.97, d (16.4)	129.7	C-1, C-7, C-9, C-10
9		130.8			130.8	
10	7.58, d (2.0)	126.9	C-8, C-11, C-12, C-15	7.58, d (2.2)	126.9	C-8, C-11, C-12, C-15
11		125.6			125.6	
12		155.9			156.0	
13	6.83, d (8.4)	117.1	C-9, C-11, C-12, C-14	6.83, d (8.2)	117.5	C-9, C-11, C-12, C-14
14	7.28, dd (8.4, 2.0)	128.0		7.28, dd (8.2, 2.2)	128.0	
15	6.90, d (16.0)	125.4	C-10, C-12, C-16, C-17	6.89, d (16.4)	125.4	C-10, C-12, C-16, C-17
16	6.52, d (16.0)	134.7	C-11, C-15, C-18, C-19	6.52, d (16.4)	134.7	C-11, C-15, C-18, C-19
17		78.1			78.1	
18	a: 2.28 (13.7, 6.8) b: 1.75, m	41.3	C-16, C-17, C-19, C-4′, C-15′, C-16′	a: 2.27 (13.7, 6.7) b: 1.73, m	41.3	C-16, C-17, C-19, C-11′, C-15′, C-16′
19	1.53,S	30.0	C-16, C-17, C-18	1.52, S	30.0	C-16, C-17, C-18
1′		125.6			139.0	
2′	6.52, d, 1.8	107.1	C-3′, C-4′, C-6′, C-7′	6.50, d, 1.8	107.1	C-3′, C-4′, C-6′, C-7′
3′		158.1			158.1	
4′		113.0			113.0	
5′		157.0			157.0	
6′	6.64, d, 1.8	108.2	C-2′, C-4′, C-5′, C-7′	6.62, d, 1.8	108.2	C-2′, C-4′, C-5′, C-7′
7′	6.86, d, 16.4	127.2	C-1′, C-2′, C-6′, C-8′, C-9′	6.81, d, 16.4	127.3	C-1′, C-2′, C-6′, C-8′, C-9′
8′	6.98, d, 16.4	129.4	C-1′, C-7′, C-9′, C-10′	6.96, d, 16.4	129.7	C-1′, C-7′, C-9′, C-10′
9′		130.8			131.3	
10′	7.43, d (8.7)	129.4	C-12′, C-14′	7.08, d (1.8)	114.4	C-8′, C-11′, C-12′, C-14′
11′	6.84, d (8.7)	117.1	C-9′, C-12′, C-13′, C-14′		146.8	
12′		158.8			146.9	
13′	6.84, d (8.7)	117.1	C-9′, C-11′, C-12′, C-14′	6.80, d (8.1)	116.9	C-9′, C-11′, C-12′, C-14′
14′	7.43, d (8.7)	129.4	C-10′, C-12′	6.91, dd (8.1, 1.8)	120.6	
15′	3.80, m	31.0	C-18, C-3′, C-4′, C-5′, C-16′, C-17′	3.79, m	30.8	C-18, C-3′, C-4′, C-5′, C-16′, C-17′
16′	5.21, d, (9.2)	129.6		5.20, d, (9.3)	129.6	C-4′, C-19′
17′		134.3			134.2	
18′	1.82, s	18.8	C-16′, C-17′, C-19′	1.81, s	18.7	C-16′, C-17′, C-19′
19′	1.76, s	26.6	C-16′, C-17′, C-18′	1.76, s	26.6	C-16′, C-17′, C-18′

[a]Recorded in acetone-d_6.

column temperature was room temperature, and the injection volume was 5 μL. The gradient elutions were as follows: initial conditions, 50% A/50% B, held for 5 min, increased linearly to 10% A/90% B in 40 min, held isocratic for 5 min, decreased to initial conditions in 5 min, and held isocratic for 10 min. The flow rate was 0.2 mL/min. The LC conditions for LC-MS analysis were the same as those applied in the HPLC analysis. For ESI-MS, both the positive and negative ion modes were used for characterization of stilbene phytoalexins. The ion source voltage and the nebulizer pressure were maintained at 4.5 kV and 4 bar, respectively. The flow rate of dry gas was 8.0 L/min, and the temperature was set at 220 °C. Full scan mass spectra were recorded in the range of m/z 70−1200 amu. Peaks with novel molecular weights were targeted as new stilbenoids (Figure 2) in peanuts. The fraction eluted with *n*-hexane/acetone (2:1) containing compound **3** (molecular weight 326), the fraction eluted with *n*-hexane/acetone (1:1) containing compound **1** (molecular weight 588), and the fraction eluted with *n*-hexane/acetone (1:2) compound **2** (molecular weight 604) were evaporated, dissolved in methanol, and subjected to a final purification using semipreparative HPLC.

Semipreparative HPLC separation was performed on a Waters 717 system equipped with a PDA detector using a YMC-Pack ODS-AM C_{18} (10 × 250 mm, 5 μm) column maintained at room temperature, and the injection volume was 100 μL. The mobile phases and gradient programs were identical to the conditions in the HPLC analysis above except the flow rate was set at 3 mL/min. The fractions corresponding to the peaks of the new compounds were repeatedly collected under the multiwavelength UV monitoring. The structures of the collected compounds were elucidated by spectroscopic analysis including HRESIMS, 1D NMR (^{1}H, ^{13}C, DEPT), and 2D NMR (^{1}H−^{1}H COSY, HMQC, HMBC, NOESY). 1D and 2D NMR were recorded using standard pulse programs from Bruker pulse sequences library. Mixing time for NOESY was 500 ms. In HMQC experiment, the J_{CH} value is 170 Hz. For HMBC experiments, the long-range coupling constant is 10 Hz.

***1** (Arahypin-11):* 3.0 mg (yield 2.5 μg/g, fresh wt); yellow solid; $[\alpha]_D^{23}$ 0 (c 0.4, $CHCl_3$); UV λ_{max} 221, 312 nm; ^{1}H NMR and ^{13}C NMR data are listed in Table 1; HRESIMS, m/z 587.2447 $[M - H]^-$, calcd for $C_{38}H_{35}O_6$, 587. 2439.

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2 (Arahypin-12): 5.0 mg (yield 4.2 μg/g, fresh wt); yellow brown solid; $[\alpha]_D^{23}$ 0 (*c* 0.4, $CHCl_3$); UV λ_{max} 225, 320 nm; ^1H NMR and ^{13}C NMR data are listed in Table 1; HRESIMS, *m/z* 603.2399 $[M - H]^-$, calcd for $C_{38}H_{35}O_7$, 603.2388.

3 (3,5,3′-Trihydroxy-4′-methoxy-5′-isopentenylstilbene, MIP): 5.5 mg (yield 4.6 μg/g, fresh wt); pale yellow solid; UV λ_{max} 223, 309 nm; ^1H NMR and ^{13}C NMR data are listed in Table 2; HRESIMS, *m/z* 325.1452 $[M - H]^-$, calcd for $C_{20}H_{21}O_4$, 325.1445.

Table 2. NMR Data[a] of MIP (3)

	δ_H (*J* in Hz)	δ_C	HMBC correlations
1		140.7	
2	6.56, d (2.1)	106.1	C-3, C-4, C-6, C-7
3		159.8	
4	6.30, t (2.1)	103.1	C-2, C-3, C-5, C-6
5		159.8	
6	6.56, d (2.1)	106.1	C-2, C-3, C-4, C-7
7	6.90, d (16.7)	128.8	C-1, C-2, C-6, C-8, C-9
8	6.97, d (16.7)	129.3	C-1, C-7, C-9, C-10, C-14
9		134.6	
10	6.88, d (2.1)	120.3	C-8, C-9, C-11, C-12, C-14, C-15
11		136.3	
12		146.8	
13		151.2	
14	6.99, d (2.1)	112.9	C-8, C-10, C-12, C-13
15	3.36, d (7.3)	29.4	C-10, C-11, C-12, C-16, C-17
16	5.32, t (7.3)	124.3	C-18, C-19
17		132.7	
18	1.77, bs	18.1	C-16, C-17, C-19
19	1.75, bs	26.0	C-16, C-17, C-18
OMe	3.80, s	61.0	C-12

[a]Recorded in acetone-d_6.

Cell Culture, Adipocyte Differentiation, and Oil Red O Staining. 3T3-L1 preadipocytes were maintained in Dubecco's modified Eagle's medium (DMEM) (Gibco BRL, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco BRL) and antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin, Gibco BRL) at 37 °C under a humidified 5% CO_2 atmosphere. In adipogenesis study, 3T3-L1 preadipocytes were seeded in a 96-well plate at a density of 1× 10 ^{4}/well and maintained for 2 additional days until confluence (designated day 0). For adipocyte differentiation, the cells were incubated in a differentiation medium (10% FBS/DMEM containing 0.5 mM IBMX, 10 μg/mL insulin, and 0.25 μM DEX) for 2 days. The medium was then changed to 10% FBS/DMEM containing 10 μg/mL insulin. After another 2 days, the medium was replaced with 10% FBS/DMEM and the cells continued to differentiate until day 6. Test compounds were administered during the differentiation process, and on day 6 the cells were subjected to Oil Red O staining to quantify intracellular lipid content as described previously.[12,13] Briefly, the cells were fixed for 1 h at room temperature with 10% formalin in PBS, washed three times with PBS, and then stained for 1 h with filtered Oil Red O (0.5% in 60% isopropanol/40% PBS). After three washings with distilled water, the stained lipid droplets were dissolved in isopropanol and quantified by measuring the absorbance at 510 nm.

Cell Viability Assay. The cells were treated with the tested stilbene compounds at indicated concentrations in the differentiation medium for 48 h. Then the cells were incubated with MTT solution (0.5 mg/mL) for 3 h at 37 °C, and the supernatant was replaced by DMSO to dissolve the violet precipitate formazan. The absorbance was measured at 490 nm on a microplate reader.

RESULTS AND DISCUSSION

Profiles of Stilbene Phytoalexins in Stressed Peanut Seeds. The peanuts were cut into pieces to enlarge the infected surface area where the peanut stilbenoids were found to be most concentrated.[4] Our previous results had shown that peanut seeds stressed by *R. oligoporus* would produce a significantly higher level of stilbene derivatives compared with the unstressed controls.[7] The three main peaks of black peanut methanol extract were assigned as SB-1 (*m/z* 345.1, 360 nm), arachidin-1 (*m/z* 313.1, 340 nm), and arachidin-3 (*m/z* 297.1, 336 nm) according to their characteristic UV spectra and molecular weights through HPLC and LC-MS analysis. SB-1 (33 mg, yield 27.5 μg/g, fresh wt), arachidin-1 (30 mg, yield 25 μg/g, fresh wt), and arachidin-3 (8.5 mg, yield 7.1 μg/g, fresh wt) were isolated from different eluting fractions using semipreparative HPLC, and their structures were further confirmed according to 1D NMR spectral data reported previously.[2,4] The three new stilbenoids, arahypin-11, arahypin-12, and MIP, were identified because their UV spectra and molecular weights were different from those of known stilbenoids from stressed peanut seeds.[3−7] Arahypin-11 ($C_{38}H_{36}O_6$) and arahypin-8 are isomers with similar UV spectra but exhibit significantly different chromatographic behaviors under the same gradient elution conditions.

Structure Elucidation of the New Stilbenes. The NMR proton spectrum of compound **1** (arahypin-11) is similar to that of arahypin-8,[7] which has three methyl groups. The HMBC correlations from H-18′ (δ_H 1.82) and H-19′ (δ_H 1.76) to C-16′ (δ_C 129.6) and C-17′ (δ_C 134.3) indicate the presence of two prenyl-derived methyl groups. However, the two protons at δ_H 7.43 and the two protons at δ_H 6.84 suggest the existence of a 1,4-disubstituted benzene ring. Therefore, **1** is formed by two different monomers. The entire structure of **1** is established by assigning signals from ^1H, ^{13}C, COSY, HMQC, and HMBC experiments. The NMR data are summarized in Table 1.

The HMBC correlations from H-11′ and -13′ (δ_H 6.84) to C-9′ (δ_C 130.8), C-12′ (δ_C 158.8), and C-14′ (δ_C 129.4) combining HMBC correlations from H-8′ (δ_H 6.98) to C-10′ (δ_C 129.4) and from H-6′ (δ_H 6.64) to C-4′ (δ_C 113.0), C-5′ (δ_C 157.0), and C-7′ (δ_C 127.2) revealed the skeleton of one unreported monomer. The connection of the two monomers was established through HMBC from H-15′ (δ_H 3.80) to C-18 (δ_C 41.3), C-3′ (δ_C 158.1), C-4′, and C-5′. In addition, the relative stereochemistry of arahypin-11 was measured by NOESY experiment. To our surprise, there are no correlations between H-15′ and H-19 (δ_H 1.53), which was found in arahypin-8. Instead, there are correlations between H-15′ and H-16 (δ_H 6.52) as highlighted by circles shown in Figure 3, which proved that H-15′ and H-19 are on different sides of the six-membered ring. The relative stereochemistry of compound **1** was established as shown in Figure 2.

The proton spectrum of compound **2** (arahypin-12) is quite similar to the spectrum of compound **1** except for the disappearance of the four proton signals, which represent the 1,4-disubstituted benzene ring. In addition, the ^{13}C spectra showed two carbons at δ_C 146.8 and δ_C 146.9, indicating two adjacent phenol carbons. Therefore, we supposed that C-11′ was oxidized, and this hypothesis was confirmed by 2D NMR experiments. The entire structure of compound **2** is established by assigning signals from ^1H, ^{13}C, COSY, HMQC, and HMBC experiments. The NMR data are summarized in Table 1.

The structure of compound **3** (MIP) was established by analysis of its 1D and 2D NMR data summarized in Table 2. The HMBC correlations from H-15 (δ_H 3.36) to C-10 (δ_C 120.3), C-11 (δ_C 136.4), C-12 (δ_C 146.8), C-16 (δ_C 124.3), and

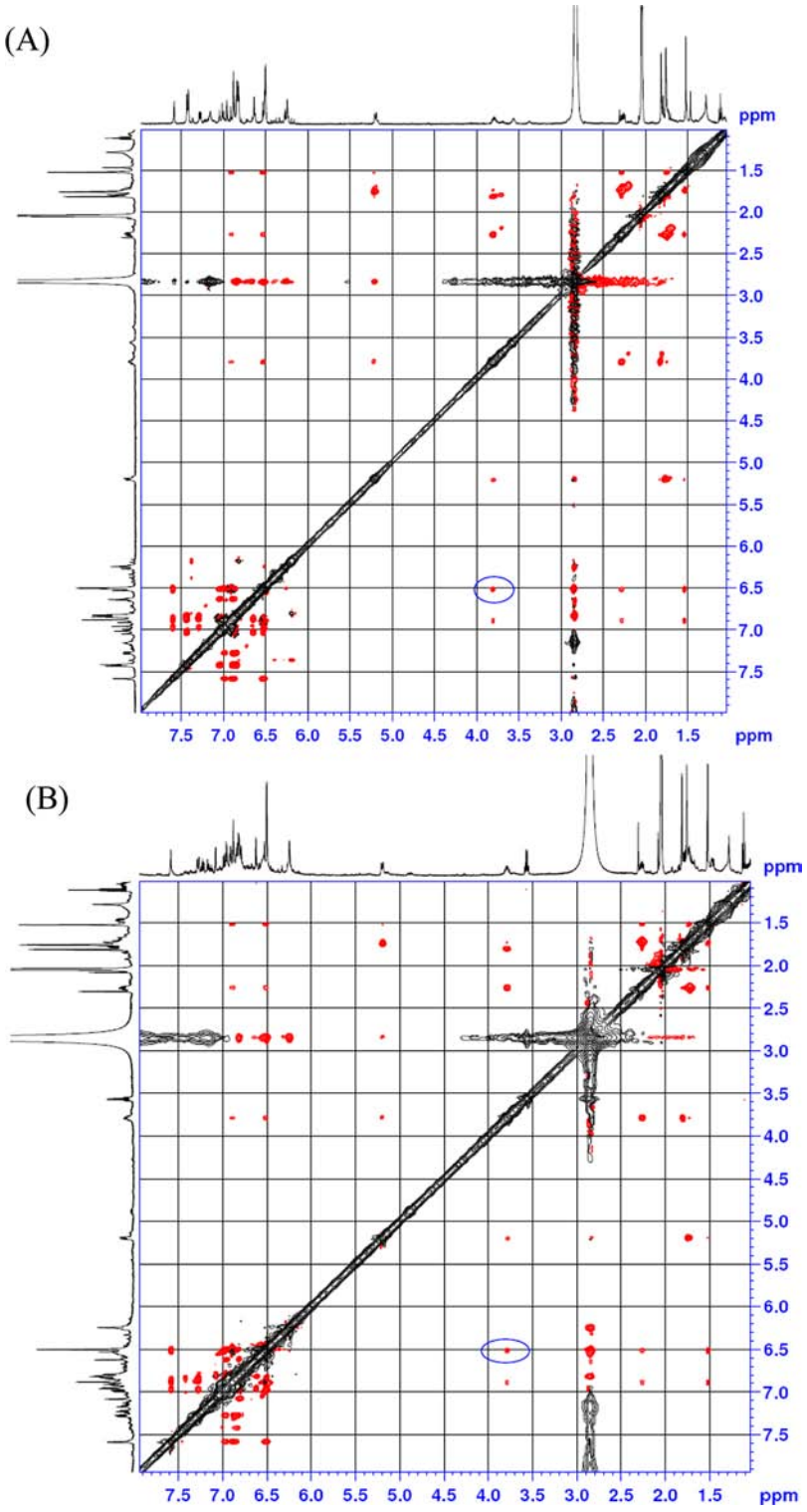

Figure 3. NOESY spectra of (A) arahypin-11 (**1**) and (B) arahypin-12 (**2**).

C-17(δ_C 132.7) suggest the presence of a methylene group in the isopentenyl moiety connected with a benzene ring. The methoxy group (δ_H 3.80, δ_C 61.0) showed correlation with C-12, indicating it is a substituent on the benzene ring as well. The HMBC experiments also revealed correlations from H-14 (δ_H 6.99) to C-8 (δ_C 129.3), C-12, and C-13 (δ_C 151.2), which are indicative of the presence of an olefinic double bond in the stilbene skeleton. By combination with other data in Table 2, the structure of compound **3** was established as shown in Figure 2.

The construction pattern of arahypin-11 (**1**) and arahypin-12 (**2**) was currently specific for peanut seeds challenged by *R. oligoporus* because stilbene dimers connected through prenyl groups were reported only in our previous study.[7] The skeleton of arahypin-11 (**1**) and arahypin-12 (**2**) cannot be categorized into any of 29 known construction patterns of stilbene oligomers summarized by Shen et al.,[14] indicating unknown

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biological potential of the molecules to be explored. The dihydro derivative of MIP (**3**) was first isolated from *G. glabra*,[15] the content of which (yield 0.35%, fresh wt) was much higher than that of MIP (**3**) in stressed peanuts. Given the fact that the concentrations of the prenylated stilbenoids were low in fungal-stressed peanut seeds, chemical synthesis study will be needed for efficient production of these compounds, and some researchers have made achievements in this direction.[8] It is also noticeable that the stereochemistry of arahypin-11 (**1**) and arahypin-12 (**2**) was different from that of arahypin-8, and all three compounds have chiral carbons but show no optical rotation activity. However, this does not exclude the possibility that these stilbene dimers formed through an enzymatic reaction. In a recent study by Wan et al.,[16] the authors showed that enzymes could also catalyze the reactions to produce stilbene dimers as racemic mixtures in a nonstereoselective way.

Arachidin-1 Inhibits 3T3-L1 Adipocyte Differentiation. The effect of the six isolated compounds plus resveratrol on adipogenesis was evaluated in 3T3-L1 cells. Quantitative analysis of intracellular lipids by Oil Red O staining (Figure 4) showed that arachidin-1 displayed a dose-dependent

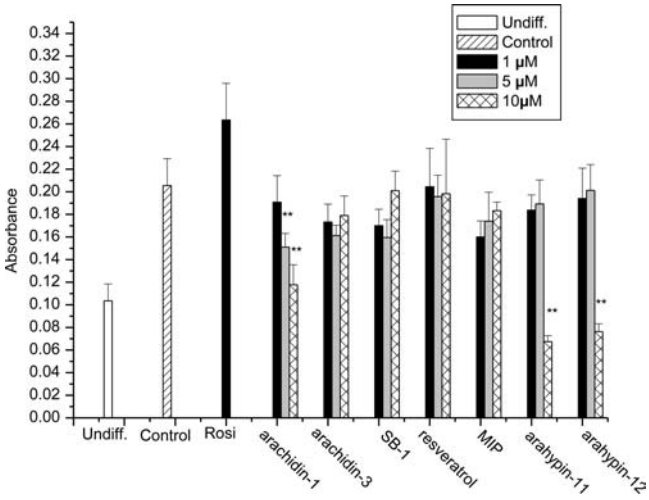

Figure 4. Inhibitory effect of peanut stilbenes on adipogenesis in 3T3-L1 cells. Undifferentiated preadipocytes (Undiff.), adipocytes (Control), and adipocytes differentiated with stilbenes were subjected to Oil Red O staining. Rosiglitazone (Rosi) was taken as a positive control for promoting adipogenesis. Error bars represent the standard deviation, $n = 4$. **,$P < 0.01$ when compared with control group.

inhibition of adipogenesis in 3T3-L1 cells. The lipid accumulation level of 3T3-L1 adipocytes differentiated with 5 and 10 μM arachidin-1 was reduced to 73 and 57%, respectively, of that of control adipocytes ($P < 0.01$). Arachidin-1 was a prenylated analogue of piceatannol, and the latter was reported recently to be a potent inhibitor for adipogenesis in 3T3-L1 cells.[12] Compared with resveratrol and arachidin-3, arachidin-1 possesses an extra hydroxyl group at the 3′-position, which may enhance its antiadipogenic activity. More studies on the molecular action mechanism of arachidin-1 in 3T3-L1 cells are needed to elucidate the relationship between the structure and biological effect of the compound. The MTT assay result (Figure 5) showed that arachidin-1 inhibited adipogenesis without cytotoxic effect on the viability of the differentiating preadipocytes. Because arahypin-11 (**1**) and arahypin-12 (**2**) displayed a dose-dependent cytotoxicity in MTT assay, further investigation would be focused on their

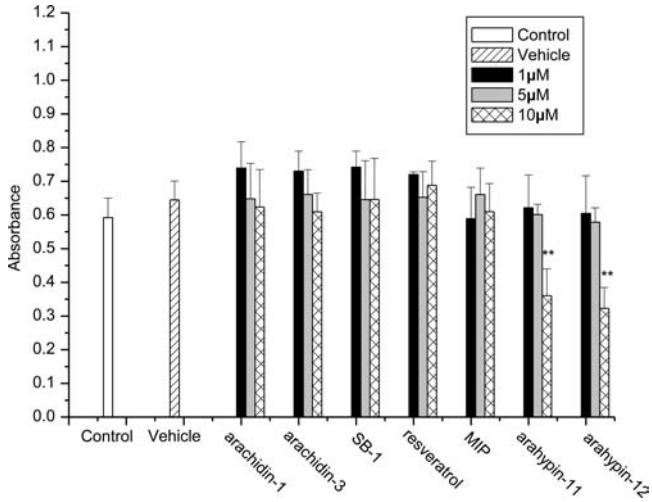

Figure 5. Viability of 3T3-L1 preadipocytes treated with peanut stilbenes in the differentiation medium for 48 h as assessed by MTT assay. Error bars represent the standard deviation, $n = 4$. **, $P < 0.01$ when compared with the control group treated with the differentiation medium alone.

anticancer activities in different cell lines. Interestingly, in our previous study[7] the two dimers, arahypin-8 and arahypin-9, did not show cytotoxic effects on 3T3-L1 preadipocytes at the same concentration range. Although arahypin-8, arahypin-9, arahypin-11 (**1**), and arahypin-12 (**2**) share the same construction pattern, the difference in their monomeric units and relative stereoconfigurations may explain why these compounds exhibited different bioactivities in 3T3-L1 cells.

In summary, the present study revealed the potential of fungal-stressed black skin peanut seeds as a source of new monomeric and dimeric stilbenoids. The structural and biological characterizations of new and known peanut stilbenoids were also performed. The results showed that the number and positions of hydroxyl groups and stereoconfigurations tend to be crucial factors in determining antiadipogenic and cytotoxic activities of individual peanut stilbenoids sharing similar structural patterns. Further investigations on improving the production of bioactive prenylated stilbenoids from peanut seeds and applying these natural phytochemicals in animal studies for chemopreventive purposes are important for exploiting peanut stilbenoids as nutraceutical ingredients.

■ ASSOCIATED CONTENT

Supporting Information

LC-MS chromatogram, UV spectra, HR-MS, 1D and 2D NMR spectra (^{1}H, ^{13}C, DEPT, ^{1}H−^{1}H COSY, HMQC, and HMBC) of arahypin-11 (**1**), arahypin-12 (**2**), and MIP (**3**). This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(D.H.) E-mail: chmhdj@nus.edu.sg. Phone: (65)-6516-8821. Fax: (65)-6775-7895. (J.W.) E-mail: chmwujie@nus.edu.sg. Phone: (65)-6516-4406.

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Notes

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