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Synthesis and Characterization of New Stilbenoid Derivatives as Potential Vasodilators†

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The development of antihypertensive drug has become a major concern in pharmacological and medical fields as hypertension is always related to many chronic diseases such as stroke and other coronary diseases. Stilbenoids, such as resveratrol, have been reported for their potency as vasodilators that are able to work directly on the muscles in blood vessel walls by dilating and preventing the tightening of blood vessels. In this study, a series of new stilbenoid derivatives with different positions and numbers of the ethoxy group have been synthesized via Wittig reaction using commercially available dihydroxybenzoic acids and hydroxybenzaldehydes; the *trans* isomers were then isolated and purified using column chromatography technique. The chemical structures of the synthesized stilbenoid derivatives were characterized by FT-IR, ^{1}H-NMR and ^{13}C-NMR spectroscopy. *In vitro* aortic ring assays were conducted to evaluate the vasodilatory potential of the synthesized stilbenoid derivatives on endothelium-intact aortic rings isolated from male Sprague Dawley (SD) rats. It was found that all the newly synthesized stilbenoid derivatives possessed vasodilatory properties. Compound **12a** with 2, 3, and 4′ ethoxy substitutions showed the highest R_{max} value of 25.99 ± 4.39% compared to the other stilbenoid derivatives.

Key words: Stilbenoids; *In vitro* aortic ring assays; Wittig reaction; resveratrol; vasodilator; antihypertensive

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Stilbenoids are a group of naturally occurring phenolic compounds that share a common stilbene backbone structure, C6-C2-C6, but differ in the type and position of substituents in the aromatic rings [1]. The presence of the ethylene bridge joining the two aromatic rings contributes to the formation of *cis* isomer (Z), and the relatively more stable *trans* isomer (E) [1, 2]. Within the past decade, stilbenoids had been gaining much interest on their biological as well as pharmacological effects including anticancer, anti-inflammatory, anti-ageing, anti-obesity, anti-cardiovascular diseases, anti-neurodegeneration, antioxidative, antibacterial, and antifungal properties [1, 3, 4].

Resveratrol (*trans*-3,4′,5-trihydroxystilbene) is one of the most widely studied stilbenoids, naturally present in certain fruits and plants, including grapes, peanuts, and mulberries in response to stress, injury, ultraviolet irradiation, and fungal infection, which is well known for its cardioprotective effects [3]. This originated from an epidemiological finding that linked the low incidences of cardiovascular diseases of the French population, despite their diet high in saturated fats, with their daily consumption of moderate quantities of red wine [5, 6]. Many studies had shown resveratrol, the most important dietary source in red wine, as the most important factor contributing to the "French Paradox" [5, 6].

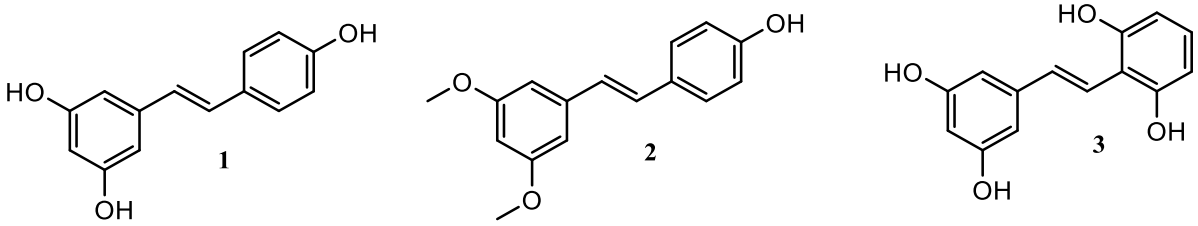

Figure 1. The structures of (**1**) resveratrol (*trans*-3,4′,5-trihydroxystilbene), (**2**) pterostilbene (*trans*-3,5-dimethoxy-4′-hydroxystilbene), and (**3**) gnetol (*trans*-3,5,2′,6′-tetrahydroxystilbene)

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There are a few biological mechanism pathway studies of selected stilbenoids on rats cardiac muscle and *in vitro* isolated rat aortic rings. Resveratrol increased endothelial nitric oxide synthase (eNOS) expression and nitric oxide (NO) content, as well as partially reversed the endothelial dysfunction found in spontaneously hypertensive rats (SHRs) [8,9]. Besides, resveratrol can effectively suppress Angiotensin II-induced hypertrophy in rat vascular smooth muscle cells [10]. There are other potential therapeutic methoxylated and hydroxylated stilbenoids such as pterostilbene (*trans*-3,5-dimethoxy-4′-hydroxystilbene) and gnetol (*trans*-3,5, 2′, 6′-tetrahydroxystilbene) (**Figure 1**), which inhibit ET-1-induced hypertrophy in isolated neonatal rat cardiomyocytes via activation of AMPK [1].

Nawaz *et al.* studied the chemical structures of resveratrol derivatives and their pharmacological activities and proved the hydroxylated resveratrol derivatives (stilbenoids) exhibited favorable therapeutic potentials [3]. Although resveratrol has been reported of its possibilities in vasodilatory activities, however, its utilization and development in products are limited due to the poor chemical stability, poor solubility, and low bioavailability[11]. However, pterostilbene (*trans*-3,5-dimethoxy-4′-hydroxystil-bene), a dimethylated analogue of resveratrol used in Ayurvedic medicine to treat coronary heart disease, was interestingly reported to have ~75% higher oral bioavailability and ~90 mins longer half-life than resveratrol [12].

Although many stilbenoids have been synthesized, not much information about their vasodilatory effects can be found. For example, Muriasa *et al.* synthesized a series of stilbenoids but studied only on their antioxidant properties [11]. Furthermore, until today there is no systematic study on how the position and number of either hydroxyl or ethoxy group present in aromatic rings of stilbenoids can actually affect the vasodilatory activity of blood vessels. Danielle compared the effects of resveratrol with other stilbenoids (pterostilbene and gnetol) on the structural and mechanical properties of mesenteric arteries but did not emphasize on the effect of the position and number of hydroxyl groups present in the stilbenoids [13]. Hence, it is interesting to investigate the vasodilatory effect of stilbenoids with different hydroxyl and ethoxy group positions modified from resveratrol structure. In the present study, the vasodilatory effects of the newly synthesized stilbenoid derivatives were evaluated using endothelium-intact isolated rat aortic ring assay.

EXPERIMENTAL SECTION

1. Chemistry

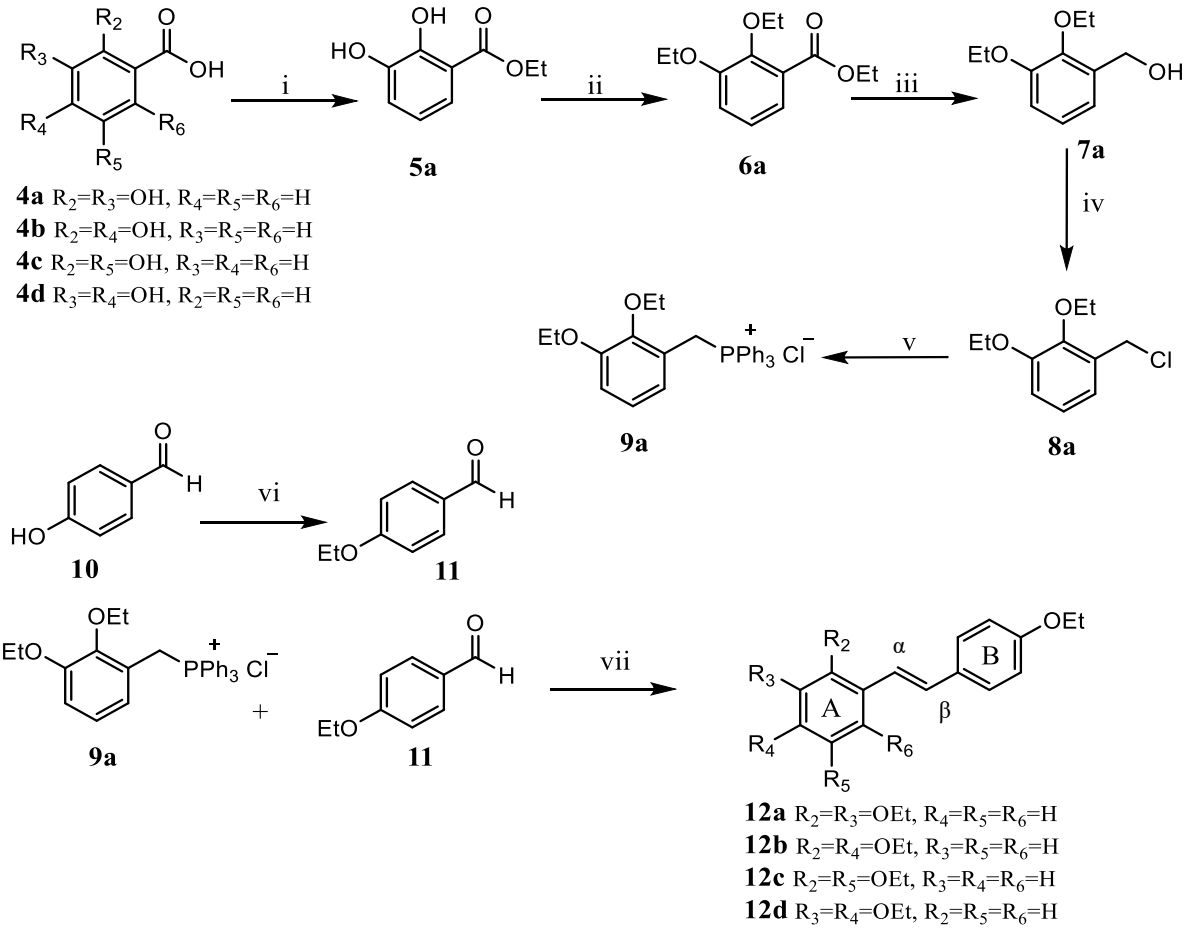
All chemicals and solvents were commercially available and used without further purification. Fourier transform infrared (FT-IR) spectra were recorded using Perkin Elmer 2000-FTIR spectrophotometer in the range of 4000 to 600 cm^{-1}. Nuclear magnetic resonance (FT-NMR) spectra were recorded using Bruker-Avance 500 MHz ultrashield spectrometer equipped with ultrashield magnets. Deuterated chloroform ($CDCl_3$) was used as the solvent and TMS as the internal standard. Thin-layer chromatography (TLC) was performed using aluminium plates coated with silica, and spots were visualized under UV light to confirm the completion of the reaction. Chromatographic separation of mixtures was performed in open glass columns packed with silica gel 60 G. The melting points of the products were observed from the Gallenkamp apparatus without any calibration.

1.1. General procedure for the synthesis of compounds **5a-5d**

Compound **5a** was synthesized from 2,3-dihydroxycarboxylic acid (**4a**) via Fisher esterification. 2,3-Dihydroxybenzoic acid (3.50 g, 22.7 mmol) was first dissolved in ethanol in a 100 mL round bottom flask. A catalytic amount of concentrated sulfuric acid was added and the reaction mixture was refluxed at 70-75°C. After 24 hours, the resultant mixture was cooled to room temperature and excess solvent was evaporated off. The crude product was dissolved in diethyl ether and transferred into a 150 mL separating funnel. The organic layer was then extracted with two aliquots of 0.25 M sodium hydrogen carbonate, followed by distilled water. The organic layer was collected in a 250 mL beaker and excess solvent was evaporated in a fume hood to yield the desired compound, **5a** without further purification. **5b, 5c**, and **5d** were synthesized according to the described method, wherein **4a** was replaced by: 2,4-dihydroxybenzoic acid, **4b**; 2,5-dihydroxybenzoic acid, **4c**; and 3,4-dihydroxybenzoic acid, **4d**, respectively.

1.2. General procedure for the synthesis of compounds **6a-6d**

Compound **6a** was synthesized from **5a** and bromoethane via Williamson etherification. A mixture of **5a** (2.50 g, 13.7 mmol) and acetone (50 mL) was stirred at 50°C in the presence of potassium carbonate anhydrous and a catalytic amount of potassium iodide. Bromoethane (1.1 equiv.) was then added into the mixture and refluxed at 75-80°C for 12 hours. The resultant mixture was filtered and the excess solvent was evaporated in a fume hood to yield the desired compound, **6a** without further purification. **6b, 6c**, and **6d** were synthesized according to the described method, wherein **5a** was replaced by **5b, 5c**, and **5d**, respectively.



Scheme 1. The synthesis of stilbenoid derivative analogue **12a**. Reagents and conditions: i) EtOH, H_2SO_4, reflux; ii) bromoethane, K_2CO_3, KI, acetone, reflux; iii) $NaBH_4$, THF-MeOH, reflux; iv) $SOCl_2$, pyridine, r.t.; v) triphenylphosphine, THF, 120°C; vi) bromoethane, K_2CO_3, KI, acetone, reflux; vii) NaOH, DCM, reflux; The same method was used in the synthesis **12b**, **12c**, and **12d**.

1.3. General procedure for the synthesis of compounds **7a-7d**

Compound **7a** was synthesized from **6a** via reduction using $NaBH_4$. A mixture of **6a** (2.00 g, 8.3 mmol) and granular $NaBH_4$ (7.0 equiv.) in THF (25 mL) was refluxed at 100°C. After an hour, methanol (50 mL) was added dropwise into the mixture over 30 minutes. The mixture was cooled down to room temperature after 4 hours of reflux. The mixture was neutralized by step-wise addition of 12% HCl. The reaction mixture was filtered, then the filtrate was dissolved in ethyl acetate and extracted with distilled water. Excess solvent was evaporated in a fume hood to yield the desired compound, **7a** without further purification. **7b, 7c**, and **7d** were synthesized according to the described method, wherein **6a** was replaced by **6b, 6c**, and **6d**, respectively.

1.4. General procedure for the synthesis of compounds **8a-8d**

Compound **8a** was synthesized from **7a** via reaction with $SOCl_2$. In a round bottom flask, **7a** (1.50 g, 7.6 mmol) was dissolved in THF (10 mL), and a few drops of pyridine was added into the mixture. The flask was then immersed in an ice bath. The mixture was stirred for 15 minutes before the addition of $SOCl_2$ (1.25 equiv). The mixture was continuously stirred for 8 hours at room temperature. The resultant mixture was poured into cold distilled water and extracted with diethyl ether. The organic layer was extracted with another portion of distilled water and excess solvent was evaporated in a fume hood to yield the desired compound, **8a** without further purification. **8b, 8c**, and **8d** were synthesised according to the described method, wherein **7a** was replaced by **7b, 7c**, and **7d**, respectively.

1.5. General procedure for the synthesis of compounds **9a-9d**

Compound **9a** was synthesized from **8a** via reaction with triphenylphosphine. In a round bottom flask, **8a** (1.00 g, 4.7 mmol) was dissolved in THF (5 mL). Triphenylphosphine (1.0 equiv) in THF (5 mL) was then added into the mixture. The mixture was refluxed at 100°C for 12 hours until some white solid was observed. The white solid phosphonium salt was filtered and rinsed with THF to yield the desired

compound, **9a** without further purification. **9b, 9c**, and **9d** were synthesized according to the described method, wherein **8a** was replaced by **8b, 8c**, and **8d**, respectively.

1.6. Synthesis of 4-ethoxybenzaldehyde, compound **11**

Compound **11** was synthesized from 4-hydroxybenzaldehyde (**10**) and bromoethane via Williamson etherification. A mixture of **10** (1.50 g, 12.3 mmol) and acetone (50 mL) was stirred at 50°C in the presence of potassium carbonate anhydrous and a catalytic amount of potassium iodide. Bromoethane (1.1 equiv.) was then added into the mixture and refluxed at 75-80°C for 12 hours. The resultant mixture was filtered and excess solvent was evaporated in a fume hood. The obtained solid was recrystallized from ethanol to yield the desired compound, **11**.

1.7. General procedure for the synthesis of compounds **12a-12d**

Compound **12a** was synthesized from **9a** and **11** via Wittig reaction. In a round bottom flask, **9a** (2.50 g, 5.6 mmol) and **11** (1.0 equiv) were dissolved in DCM (50 mL), and sodium hydroxide (3.5 equiv.) dissolved in distilled water (10 mL) was then added into the mixture. The mixture was refluxed at 65-70°C for 6 hours. The resultant mixture was neutralized by step-wise addition of 12% HCl. Then it was filtered. The filtrate was dissolved in ethyl acetate and extracted with distilled water. Excess solvent was evaporated in a fume hood. The crude product was purified by silica gel column chromatography with *n*-hexane–EtOAc (95:5) as the eluent to give the *trans* isomer of compound **12a. 12b, 12c**, and **12d** were synthesized according to the described method, wherein **9a** was replaced by **9b, 9c**, and **9d**, respectively.

(E)-1,2-diethoxy-3-(4-ethoxystyryl)benzene (12a)
Mp: 70-72°C, off-white crystal, yield: 38%. **IR (cm^{-1})**: 3049 (C_{sp2}-H stretching), 2983, 2929 and 2879 (C_{sp3}-H stretching), 1600 and 1575 (aromatic C=C stretching), 1513 (alkene C=C stretching); **^{1}H-NMR (500 MHz, CDCl$_3$)** δ, ppm: 7.49 (d, *J*=8.5 Hz, 2H), 7.38 (d, J_{trans}=16.5 Hz, 1H), 7.24 (dd, *J*=8.0 Hz, 1.0 Hz, 1H), 7.09 (d, J_{trans}=16.5 Hz, 1H), 7.04 (t, *J*=7.0 Hz, 1H), 6.92 (d, *J*=8.5 Hz, 2H), 6.82 (dd, *J*=8.0 Hz, 1.0 Hz, 1H), 4.12-4.06 (m, 6H), 1.50-1.43 (m, 9H); **^{13}C-NMR (125 MHz, CDCl$_3$)** δ, ppm: 158.63, 152.49, 146.11, 132.21, 130.48, 129.02, 127.79, 123,79, 121.22, 117.53, 114.67, 112.19, 69.16, 64.20, 63.50, 15.79, 14.99, 14.87.

(E)-2,4-diethoxy-1-(4-ethoxystyryl)benzene (12b)
Mp: 94-96°C, yellow crystal, yield: 36%. **IR (cm^{-1})**: 3032 (C_{sp2}-H stretching), 2978, 2929 and 2879 (C_{sp3}-H stretching), 1600 and 1571 (aromatic C=C stretching), 1505 (alkene C=C stretching); **^{1}H-NMR (500 MHz, CDCl$_3$)** δ, ppm: 7.49 (d, *J*=8.5 Hz, 1H), 7.45 (d, *J*=8.5 Hz, 2H), 7.28 (d, J_{trans}=16.5 Hz, 1H), 7.00 (d, J_{trans}=16.5 Hz, 1H), 6.90 (d, *J*=8.5 Hz, 2H), 6.51 (dd, *J*=8.0 Hz, 2.5 Hz, 1H), 6.48 (d, *J*=2.0 Hz, 1H), 4.11-4.05 (m, 6H), 1.51-1.43 (m, 9H); **^{13}C-NMR (125 MHz, CDCl$_3$)** δ, ppm: 159.45, 158.16, 157.24, 131.17, 127.42, 126.97, 121.45, 119.86, 114.59, 105.56, 99.95, 63.95, 63.55, 63.47, 14.88.

(E)-1,4-diethoxy-2-(4-ethoxystyryl)benzene (12c)
Mp: 82-84°C, off-white crystal, yield: 39%. **IR (cm^{-1})**: 3041 (C_{sp2}-H stretching), 2978, 2933 and 2879 (C_{sp3}-H stretching), 1604 (aromatic C=C stretching), 1505 (alkene C=C stretching); **^{1}H-NMR (500 MHz, CDCl$_3$)** δ, ppm: 7.49 (d, *J*=9.0 Hz, 2H), 7.37 (d, J_{trans}=16.5 Hz, 1H), 7.18 (d, *J*=3.0 Hz, 1H), 7.10 (d, J_{trans}=16.5 Hz, 1H), 6.92 (d, *J*=8.5 Hz, 2H), 6.85 (d, *J*=9.0 Hz, 1H), 6.78 (dd, *J*=9.0 Hz, 3.0 Hz, 1H), 4.10-4.04 (m, 6H), 1.50-1.44 (m, 9H); **^{13}C-NMR (125 MHz, CDCl$_3$)** δ, ppm: 158.58, 153.14, 150.64, 130.61, 128.75, 127.99, 127.80, 121.31, 114.64, 114.01, 113.97, 112.13, 65.02, 64.01, 63.50, 15.10, 15.04, 14.88.

(E)-1,2-diethoxy-4-(4-ethoxystyryl)benzene (12d)
Mp: 150-152°C, yellow crystal, yield: 50%. **IR (cm^{-1})**: 3054 (C_{sp2}-H stretching), 2986, 2934 and 2886 (C_{sp3}-H stretching), 1602 and 1581 (aromatic C=C stretching), 1509 (alkene C=C stretching); **^{1}H-NMR (500 MHz, CDCl$_3$)** δ, ppm: 7.44 (d, *J*=9.0 Hz, 2H), 7.08 (d, *J*=2.0 Hz, 1H), 7.02 (dd, *J*=8.5 Hz, 2.0 Hz, 1H), 6.93 (s, 2H), 6.90 (d, *J*=8.5 Hz, 2H), 6.88 (d, *J*=8.5 Hz, 1H), 4.20-4.06 (m, 6H), 1.52-1.43 (m, 9H); **^{13}C-NMR (125 MHz, CDCl$_3$)** δ, ppm: 158.42, 148.85, 148.40, 130.90, 130.28, 127.43, 126.41, 126.35, 119.61, 114.67, 113.47, 111.04, 64.61, 63.49, 14.91, 14.87.

2. Aortic Ring Assay

Phenylephrine hydrochloride (PE) and acetylcholine (Ach) were purchased from Acros Organics, Belgium. Both PE and Ach were diluted with distilled water to 0.1 mM as stock solutions. The stilbenoid derivatives were dissolved using <1% of Tween 80 into 8 mg/ml stock solutions. All the chemicals were then kept in the freezer (Pensonic, PFZ-230) at -20°C for future use.

2.1. Aortic rings preparation

The aortic ring assay was conducted (n=6 for each different derivative) using male Sprague Dawley (SD) rats weighing 200-250 g. The SD rats were acclimated in animal transit room for 12 h light-dark cycles at room temperature with water and food provided. The whole experimental study was carried out based on the Guideline in Care and Use of Laboratory Animal by Universiti Sains Malaysia (USM/Animal Ethics Approval/2016/(103)/(775)). Before the isolation of the aorta from the SD rats, Krebs-Henseleit (Krebs) solution was prepared (118.0 mM NaCl, 4.7 mM KCl, 25.0 mM $NaHCO_3$, 2.5 mM $CaCl_2$, 11.0 mM D-glucose, 1.2 mM KH_2PO_4, and 1.2 mM $MgSO_4$, pH 7.4). After completely dissolved, the Krebs' solution

was continuously aerated with carbogen (95% O_2 and 5% CO_2) in a Petri dish and the temperature was maintained at 37°C. The SD rats were executed by overdose inhalation of CO_2 and its thoracic aorta was immediately excised and placed in the Krebs' solution. The adipose tissues were carefully removed and the aorta was trimmed into 2-3 mm ring segments and suspended in organ baths containing 10 ml of Krebs' solution using two needle hooks, where one hook was fixed to L-shaped braces and another hook was connected to the force-electricity transducer (GRASS Force-Displacement Transducer FT03C Isometric Measurement). The suspended aortic rings were left to equilibrate for 45 minutes. Meanwhile, the Krebs' solution was changed at 15-minute intervals and the resting tension was adjusted to 1.0 g upon necessary. Once the tension of the suspended aortic rings stabilized, the contractile agent, PE (1 µM) and the relaxing agent, Ach (1 µM) were added into the organ baths. At least 60% of vascular response should be achieved to ensure the validity of the aortic rings. After that, the aortic rings were rinsed three times with Krebs' solution at 15-minute intervals before testing the stilbenoid derivatives.

2.2. Vascular response of stilbenoid derivatives in PE-precontracted aortic rings

The endothelium-intact aortic rings were precontracted by adding PE (1 µM) into the organ baths and left for at least 30 minutes until the plateau stage. Then, 100 µl of 8 mg/ml stilbenoid derivatives (**12a-12d**) was added into the respective organ baths and left for 10 minutes. Therefore, the final concentration of each compound in its respective organ bath was 0.08 mg/ml. The vascular response was detected by the force-electricity transducer and amplified by Quad Bridge amp (AD instrument, Australia), and ultimately converted into digital signals by PowerLab 26T (AD instrument, Australia). The maximum relaxation (R_{max}) values obtained are tabulated in **Table 2** and expressed as mean ± S.E.M.

RESULTS AND DISCUSSION

1. Chemistry

Various commercially available dihydroxybenzoic acids and 4-hydroxybenzaldehyde were used as starting materials for synthesizing a series of new stilbenoid derivatives, employing the formation of triphenylphosphine-derived semi-stabilized ylides under the conventional Wittig reaction method, giving the quantitative yield of an E/Z mixture in the ratio of ~86:14. According to **Scheme 1**, 2,3-dihydroxybenzoic acid (**4a**) was esterified and etherified to form ethyl 2,3-diethoxybenzoate **(6a)** before reduced to form benzyl alcohol (**7a**) and followed by the reaction with thionyl chloride to form benzyl chloride (**8a**). The phosphonium salt (**9a**) was prepared by reacting benzyl chloride (**8a**) with triphenylphosphine crystal with heating in THF solvent before it was reacted with 4-ethoxybenzaldehyde (**11**) under alkaline conditions. The crude product was purified by silica gel column chromatography with *n*-hexane–EtOAc (95:5) as the eluent to give the *trans* (E) isomer of compound **12a.** Stilbenoid derivatives **12b**, **12c**, and **12d** were synthesized using the same method in which the starting materials were replaced by 2,4-dihydroxybenzoic acid (**4b**), 2,5-dihydroxybenzoic acid (**4c**), and 3,4-dihydroxybenzic acid (**4d**), respectively. The chemical structures of synthesized stilbenoid derivatives were elucidated by FT-IR, ^{1}H-NMR, and ^{13}C-NMR spectroscopy.

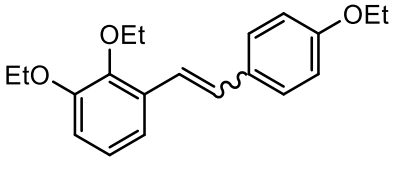
Table 1 shows the yield and E/Z ratio of the synthesized stilbenoid derivatives. The E/Z ratio was determined directly from the ^{1}H-NMR spectra of the crude products (**Figure 2**) by comparing the integrated areas of the visible peaks of particular protons that existed in both the E and Z isomers. The formula used to determine the E/Z ratio is as below:

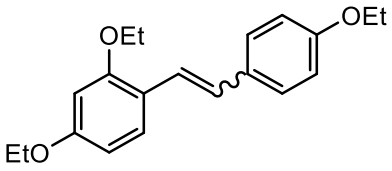
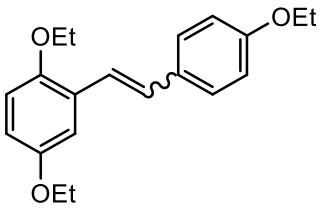
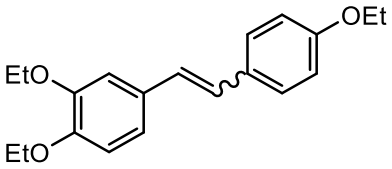
$$\%\,(E) = \frac{i\,(E)}{i\,(E) + i\,(Z)}(100) \quad \text{——— (1)}$$

$$\%\,(Z) = \frac{i\,(Z)}{i\,(E) + i\,(Z)}(100) \quad \text{——— (2)}$$

where i = integration area of the proton peak in ^{1}H-NMR spectrum

Table 1. E/Z ratio of the synthesized stilbenoid derivative analogues **12a-12d**

Compound	Yield of E/Z mixture [%]	E/Z Ratio
 **12a**	42	87:13

 **12b**	40	91:9
 **12c**	45	74:26
 **12d**	59	82:18

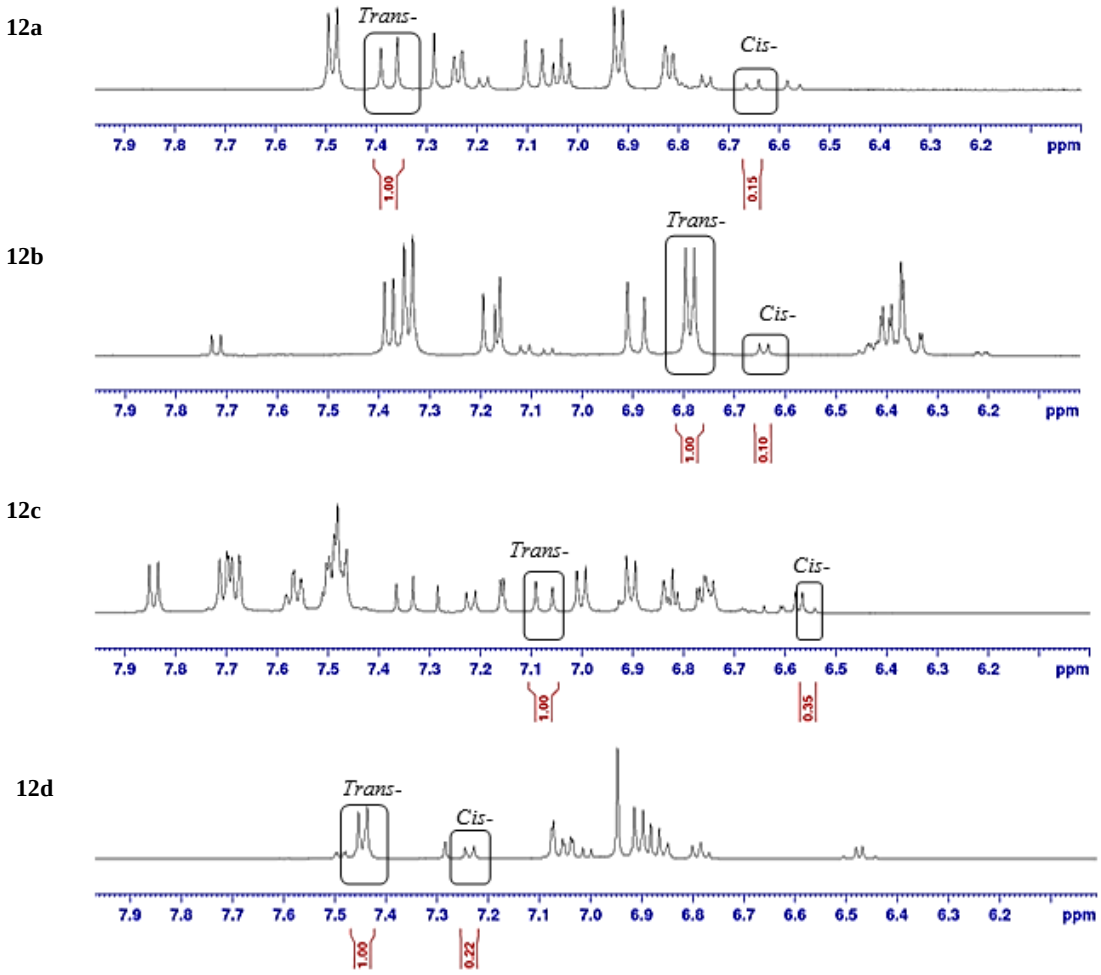


Figure 2. ^{1}H-NMR spectra data of the crude products containing the *cis* (Z) and *trans (*E) isomers of the synthesized stilbenoid derivative analogues **12a-12d**

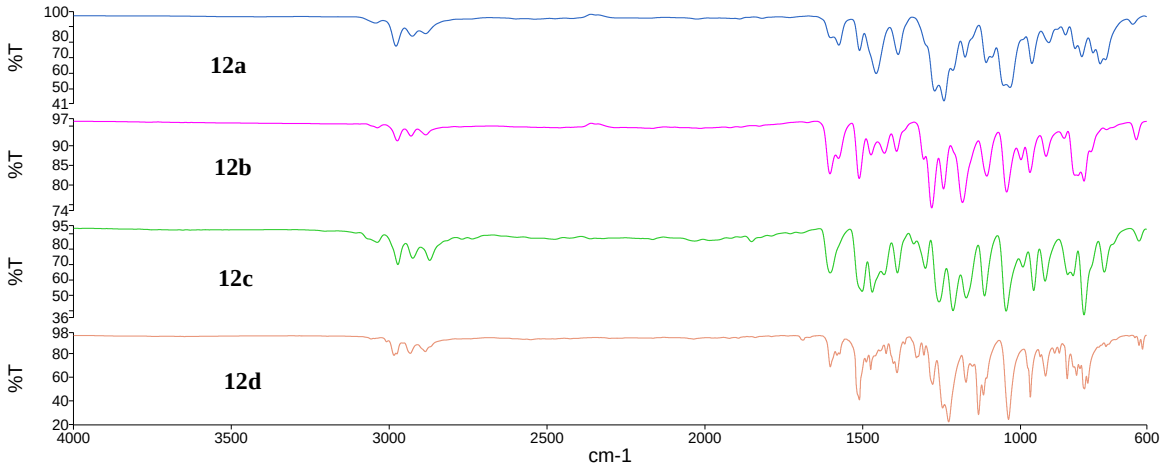


Figure 3. FTIR spectra of the *trans (*E) isomers of the synthesized stilbenoid derivative analogues **12a-12d**

The *trans* (E) stilbenoid derivatives of **12a-12d** were successfully separated from their mixtures containing both *cis* and *trans* isomers. The structures of the *trans* (E) stilbenoid derivatives of **12a-12d** were confirmed by their spectra data. The IR spectra of the synthesized stilbenoid derivative analogues **12a-12d** are compared in **Figure 2**. Aromatic C_{sp2}-H stretching was assigned at the range of 3032-3054 cm^{-1}. C_{sp3}-H stretching of alkyl group was assigned to the absorption bands 2879-2986 cm^{-1}. The aromatic C=C stretching was present in the range of 1571-1604 cm^{-1}, whereas the alkene C=C stretching was shown in the range of 1505-1513 cm^{-1}.

The ^{1}H-NMR and ^{13}C-NMR spectra data of the synthesized stilbenoid derivative analogues **12a-12d** are compared in **Figure 3** and **Figure 4**, respectively. The alkyl chain protons appeared as multiplets with a chemical shift around 1.43-1.52 ppm and 4.04-4.20 ppm in the ^{1}H-NMR spectra, while the alkyl chain carbons had the chemical shift around 14.87-15.78 ppm and 63.47-69.16 ppm in the ^{13}C-NMR spectra. The aromatic protons and carbons had the chemical shift between 6.48 ppm to 7.49 ppm and 99.95 ppm to 159.45 ppm, in the ^{1}H-NMR and ^{13}C-NMR spectra, respectively.

The two olefinic protons (H_α and H_β) of the *trans* (E) isomers of the stilbenoid derivative analogues **12a-12c** appeared as two characterizable doublets between 7.00 ppm to 7.38 ppm with the coupling constant, *J* value of 16.5 Hz. The proton peaks of the two olefinic protons of compound **12d** overlapped and appeared as a singlet at 6.93 ppm due to their almost symmetrical-like structures. It was observed that the olefinic carbon peaks of the *trans* (E) isomer of **12d** were very close to each other, having the chemical shift of 126.35 ppm and 126.41 ppm. The olefinic carbons of the *trans* (E) isomers of the other stilbenoid derivatives, **12a-12c** had the chemical shift between 121 ppm to 129 ppm.

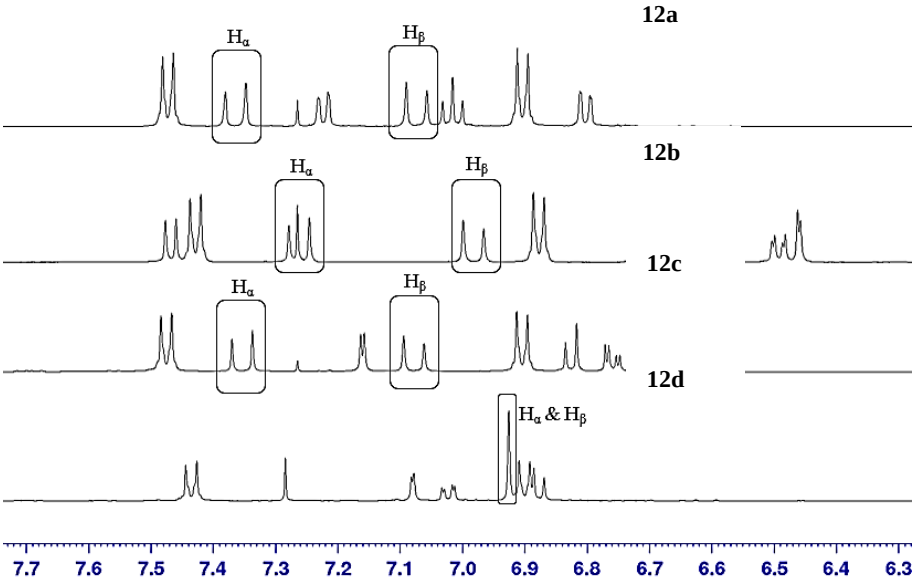


Figure 4. ^{1}H-NMR spectra data of the *trans (*E) isomers of the synthesized stilbenoid derivative analogues **12a-12d**

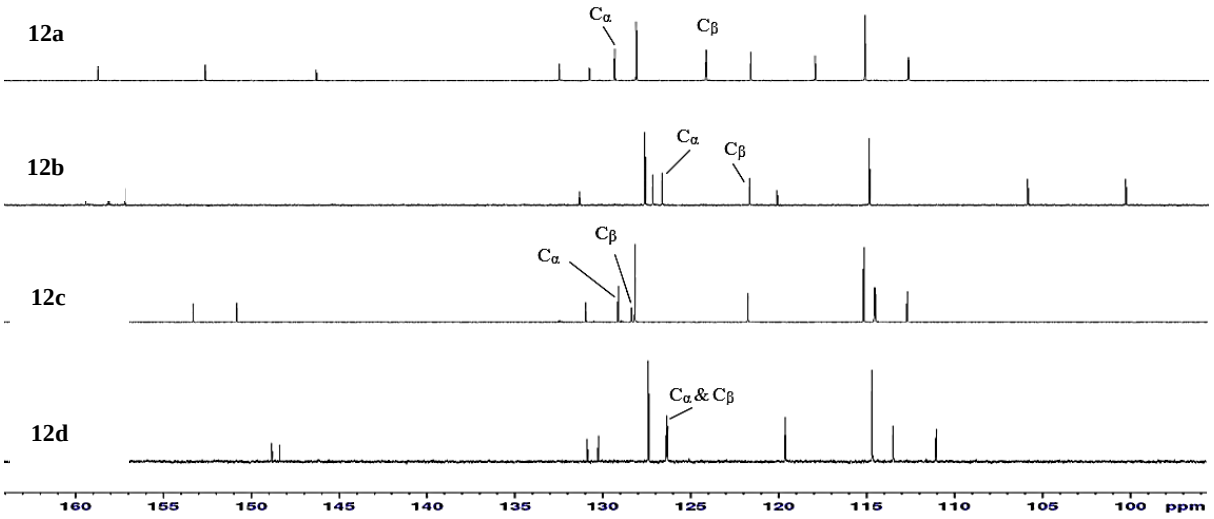


Figure 5. ^{13}C-NMR spectra data of the *trans (*E) isomers of the synthesized stilbenoid derivative analogues **12a-12d**

Table 2. The maximum relaxation (R_{max}) value of the endothelium-intact isolated aortic rings in response to the *trans (*E) isomers of the synthesized stilbenoid derivative analogues **12a-12d**

Compound	R_{max} values (%)
12a	25.99 ± 4.39
12b	22.66 ± 2.77
12c	3.51 ± 4.63
12d	20.25 ± 4.77

Notes: R_{max} = maximal relaxation; Results expressed as mean ± S.E.M. (n = 6).

2. Aortic Ring Assay

Rat aortic ring assay is one of the "golden tools" in pharmacological research fields in those related to vasculature studies due to its ease of preparation, less vulnerable to cause the intima surface damage of the endothelium as well as to minimize the orientation change of the smooth muscle cells [14,15]. In this study, a single concentration of each stilbenoid derivative was applied instead of cumulative addition, therefore the maximum relaxation (R_{max}) value was used to evaluate the potential of vasodilatory effects elicited by each compound. R_{max} value is strictly depended on the affinity, specificity, and selectivity of the test compound binding to the vasoconstriction- or vasodilation-mediated receptors located on the membrane of blood vessels.

The vascular response towards the stilbenoid derivatives **12a-12d** was primarily evaluated by using endothelium-intact isolated aortic rings. As shown in **Table 2**, all the synthesized stilbenoid derivatives **12a-12d** showed vasodilation response (positive R_{max} value) instead of vasoconstriction response (negative R_{max} value). This proved that the structures of *trans* (E) stilbenoids containing ethoxy substitutions could be potential vasodilators.

The order of vasodilatory effects of the stilbenoid derivatives declined as such: **12a** >**12b** >**12d** >**12c**. Therefore, two preliminary hypotheses on the chemical structures of the synthesized stilbenoid derivatives (**12a-12d**) towards the vasodilatory effects could be made: (i) the diethoxy substitutions that occupied ortho or meta to each other was more favorable than that of the para positions, and (ii) 2-ethoxy substitution was more favorable than 3- or 4-ethoxy substitution on the A ring.

Compound **12a** with 2, 3, and 4′ ethoxy substitutions exhibited the highest vasodilatory effect at 0.08 mg/mL final concentration in an organ bath with R_{max} value of 25.99 ± 4.39 % compared to other stilbenoid derivatives (**12b-12d**). It was also found that the R_{max} value of compound **12a** was over one quarter the maximal relaxation of resveratrol, which indicated that compound **12a** could potentially elicit over 100% of relaxation if applied in cumulative concentration manner [16]. This also indicated that compound **12a** could enhance the expression of eNOS for NO production in the endothelium and also activate the NO signalling cascade including both the sGC and cGMP signalling pathways which result in vasodilation. Therefore, **12a** could be used for future

mechanism study to discover its pathways to cause a vasodilatory effect.

CONCLUSION

A series of new stilbenoid derivative analogues (**12a-12d**) were successfully synthesized under Wittig reaction with E/Z ratio of ~83:17. The *trans* (E) isomer was found in a greater amount than the *cis* Z) isomer due to its relatively more stable structure with fewer steric interaction. Compound **12a** with 2, 3, and 4′ ethoxy substitutions exhibited the strongest vasodilatory effect in endothelium-intact isolated rat aortic rings compared to the other stilbenoid derivatives. Further mechanism study shall be carried out to reveal the mediated vasodilation-causing signalling pathways.

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