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Synthesis, Characterization and Cytotoxicity Study of Aminostilbenes on A549 Human Lung Cancer Cells

Nurain Syazwani Mohd Zaki[1], Nik Nur Syazni Nik Mohamed Kamal[2], Unang Supratman[3], Desi Harneti[3] and Mohamad Nurul Azmi Mohamad Taib[1]*

[1]Natural Products and Synthesis Organic Laboratory (NPSOLab), School of Chemical Sciences Universiti Sains Malaysia, 11800 Penang, Malaysia

[2]Advance Medical and Dental Institute, Universiti Sains Malaysia, 13200 Kepala Batas, Penang, Malaysia

[3]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran 45363 Jatinangor, Indonesia

*Corresponding author (e-mail: mnazmi@usm.my)

Resveratrol is a stilbene derivative that is a natural polyphenolic compound found in grapes and wine. It was first isolated from *Veratrum grandiflorum* and found to have various pharmacological and biological properties. In the last decade, resveratrol attracted significant attention because of its anticancer, anti-inflammatory, antibacterial, antiviral, neuroprotective, and antioxidant properties. In this study, seven aminostilbene analogues were synthesized, characterized and evaluated for their cytotoxic activities against the human lung cancer (A549) cell line using an MTT cell proliferation assay. Among these, compound **4b** exhibited good cytotoxicity towards A549 cells after 72 h of incubation, with an IC_{50} value of 20 µM. The structures of the synthesized compounds were characterized using Fourier Transform Infrared (FT-IR) and Nuclear Magnetic Resonance (NMR) spectroscopy in combination with High-Resolution Mass Spectrometry (HRMS).

Keywords: Stilbenes; aminostilbene; MTT assay; cytotoxic activity; A549 lung cancer

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Resveratrol (3,5,4'-trihydroxystilbene) was discovered in 1940 and first isolated from the roots of *Veratrum grandiflorum*, also known as white hellebore [1-3]. can be found naturally in food such as grapes, cranberries, blueberries and peanuts [4]. This compound is a phytoalexin that is involved in the plant's defence system and responds to external stress, such as bacteria and fungi [5]. In the structure of resveratrol, one aromatic ring possesses two hydroxyl (−OH) groups at C-3 and C-5, while the other aromatic ring has one −OH group at C-4' [6]. Due to the presence of an ethylene group, resveratrol has two possible stereoisomers, i.e., *cis* and *trans* (Figure 1). Common resveratrol isolated from natural products was discovered to be the *trans* isomer [7]. Over time, resveratrol's diverse biological properties have been recognized and revealed, primarily due to the abundance and variety of its molecular targets.

Among the key benefits of *trans*-resveratrol are its antioxidant, anti-inflammatory, anticancer, estrogenic, neuroprotective, cardioprotective, anti-atherosclerosis, anti-aging, anti-diabetic, anti-osteoporosis, and anti-obesity properties [8]. Yet, relatively few studies have been undertaken to determine the *cis* isomer's biological effects. Early research has demonstrated that *cis*-resveratrol has antioxidant, antibacterial, and anti-platelet aggregation properties [9-10]. However, it is known that both stereoisomeric forms of resveratrol possess anticancer properties with different mechanistic actions *in vitro* and *in vivo*. Gaspari *et al*.[11] studied the inhibitory activities of the *cis* and *trans* isomers of combretastatin A4 (CA-4) on tubulin polymerization. These activities resulted in a cytotoxic effect that frequently hindered tumour-specific vasculature and cancer cell proliferation [12]. It was discovered that *cis*-CA-4 analogues promoted the process of depolymerization, inhibiting the formation of microtubules, while *trans*-CA-4 isomers of stilbene derivatives enhanced the process of tubulin polymerization. Despite differing biological activities, the *trans* form appears to be thermodynamically more stable and active. Other known analogues of *trans*-resveratrol such as piceatannol and pterostilbene (Figure 1) are potent anticancer drugs for breast, colon, ovarian, lung and prostate cancers [13-16].

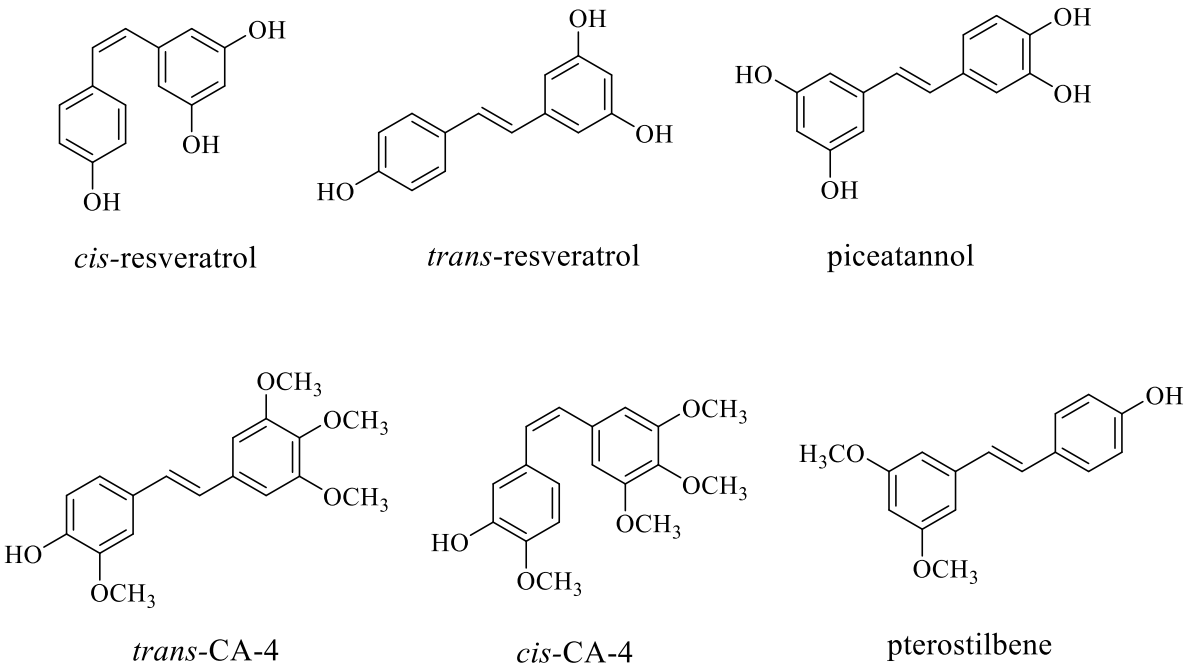


Figure 1. The chemical structures of resveratrol and its analogues.

Cancer is a major worldwide problem that has increased significantly over the years [17]. According to the American Cancer Society, 18 million people were diagnosed with cancer in 2020, while World Cancer Research International documented that 1.7 million deaths were due to lung cancer worldwide [17]. Lung cancer generally is a type of cancer that causes abnormal cell growth in lung tissues. Thus, the development of new therapeutic drugs that are less toxic and highly effective has taken centre stage in human endeavours. It was reported that substituted aniline has good potential as an active component of anticancer drugs or as an intermediate for oligomers with multifunctional capabilities [18-19]. For example, 2-aminostilbene which possesses nitro- and methoxy-groups on its aromatic ring exhibited good cytotoxicity against breast cancer cells (MDA-MB-231) and pancreatic cancer cells (PSN1) [20]. Durrant *et al.* [21] reported that trimethoxy aminostilbene was highly potent in inducing cell death in ovarian cancer cells. In the present study, we report the synthesis of aminostilbene derivatives, their characterisation by spectroscopic analyses, and the evaluation of their cytotoxicities against human lung cancer (A549) and normal healthy human lung (BEAS-2B) cells using the MTT assay method.

EXPERIMENTAL

1. General

All the chemicals and reagents used in this study were obtained from Acros Organics Co. (Belgium), Sigma-Aldrich Co. (USA) and Merck Chemical Co. (Germany). These chemicals were employed without further purification. ^{1}H (500 MHz) and ^{13}C (125 MHz) nuclear magnetic resonance (NMR) spectra were recorded with a Bruker Avance 500 MHz spectrometer (Billerica, Massachusetts, United States) using $CDCl_3$, acetone-d_6, and DMSO-d_6 as solvents. Data were analysed using the Top Spin 3.6.2 software package. Infrared FTIR spectra were recorded using a Perkin-Elmer System 2000 FTIR spectrometer (Waltham, Massachusetts, United States) in the range of 4000–400 cm^{-1}. High-resolution mass spectroscopy (HRMS) was performed using a Waters Xevo QTOF MS (Milford, Massachusetts, United States) and reported in m/z. Purification via column chromatography was conducted using silica gel 60 from Merck (Germany). Analytical thin layer chromatography (TLC) was performed using aluminium TLC sheets (silica gel F254) from Merck (Germany). The developed TLC was then visualized under a Spectroline model ENF-260C/PE (230 Volt / 50 Hz / 0.17 Amp) (Melville, United States) UV lamp at 254 and 365 nm.

2. Chemicals

The chemicals used in this study were 3,5-dimethoxy-benzaldehyde, *o*-anisaldehyde, 4-methoxybenzaldehyde, 3,4-dimethoxybenzaldehyde, 3,4,5-trimethoxybenzal-dehyde, 4-isopropylbenzaldehyde, 1-naphthaldehyde, tetrahydrofuran (THF), 2-iodoaniline, triethylamine, methyltriphenylphosphonium bromide, potassium tert-butylate, dimethylformamide (DMF), palladium (II) acetate, hydrochloric acid, ethanol, ammonium chloride, *n*-hexane, ethyl acetate, chloroform, acetone, deuterated chloroform ($CDCl_3$), deuterated acetone (acetone-d_6) and deuterated dimethylsulfoxide (DMSO-d_6). These were obtained from Acros Organics Co. (Belgium), Sigma-Aldrich Co. (USA) and Merck Chemical Co. (Germany). DMF was pre-dried over 4 Å molecular

sieves (Sigma-Aldrich, USA) under nitrogen. Cisplatin was purchased from Merck KGaA (Darmstadt, Germany).

3. Cell Culture and Reagents

Human lung cancer cells (A549) and healthy human lung cells (BEAS-2B) were obtained from the American Type Culture Collection (ATCC) (Manassas, Virginia, USA). The culture medium, i.e., Dulbecco's Modified Eagle's Medium (DMEM) and 1:1 mixture of DMEM with Ham's F-12 (DMEM/F-12), 10 % (v/v) foetal bovine serum, 100 U/mL penicillin-streptomycin (PenStrep), Trypsin-EDTA and phosphate-buffered saline (PBS) tablets were purchased from GIBCO (Grand Island, New York, USA). MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) used for the MTT assays was purchased from Merck Milli-pore (Merck KGaA, Darmstadt, Germany). Dimethylsulfoxide (DMSO) was purchased from Fischer Scientific (Hampton, New Hampshire, USA).

4. General Procedures for Synthesis

4.1. Synthesis of Stilbene Derivatives (3a-g)

N-(2-iodophenyl)acetamide (1.0 equiv.) was dissolved in 15 mL of dry dimethyl-formamide (DMF) under inert conditions. The solution was refluxed for a few minutes. Then, palladium (II) acetate (0.01 equiv.) and triethylamine (3.5 equiv.) were added to the solution, followed by styrene (1.2 equiv.). The mixture was heated with vigorous stirring at 120 °C under inert conditions for 12 h. The reaction progress was monitored by TLC. After the starting material was fully consumed, the reaction was quenched with aqueous ammonium chloride solution (10 mL), extracted three times with ethyl acetate (3 × 10 mL), and washed with plenty of distilled water (3 × 20 mL). The organic layer was collected and dried over anhydrous sodium sulphate. The solvent was removed using a rotary evaporator, and the crude product was purified *via* column chromatography (*n*-hexane/ethyl acetate, 80:20) to obtain the desired stilbene products.

(*E*)-*N*-(2-(3,5-dimethoxystyryl)phenyl)acetamide (3a): White solid; Mp: 170-173°C; Yield: 70%, IR *v* (cm^{-1}): 3265 (m, N-H), 2945 (w, C_{sp3}-H), 1644 (s, C=O), 1595 (s, C=C), 1295 (s, C-N), 1056 (s, C_{sp3}-O) 744 (s, C-H). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.75 (d, *J* = 7.6 Hz, 1H, H-6'), 7.45 (d, *J* = 7.6 Hz, 1H, H-3'), 7.23 (t, *J* = 7.6 Hz, 1H, H-5'), 7.20 (br s, 1H, NH), 7.12 (t, *J* = 7.6 Hz, 1H, H-4'), 7.04 (d, *J* = 16.0 Hz, 1H, H-8), 6.86 (d, *J* = 16.0 Hz, 1H, H-7), 6.59 (s, 2H, H-2, H-6), 6.36 (s, 1H, H-4), 3.76 (s, 6H, 2 × OCH_3, H-1", H-2"), 2.15 (s, 3H, CH_3, H-8'). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 168.4, 161.1, 139.0, 134.7, 132.7, 130.0, 128.5, 127.0, 125.5, 124.1, 124.0, 104.9, 100.1, 55.4, 24.3. HRMS (+ESI) $[M+H]^+$: 298.1424, $C_{18}H_{20}NO_3$ requires 298.1443.

(*E*)-*N*-(2-(4-isopropylstyryl)phenyl)acetamide (3b): Light brown solid; Mp: 160-163°C; Yield: 10%, IR *v* (cm^{-1}): 3286 (m, N-H), 2921 (w, C_{sp3}-H), 1656 (s, C=O), 1578 (m, C=C), 1298 (s, C-N), 761 (s, C-H). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.78 (d, *J* = 7.7 Hz, 1H, H-6'), 7.45 (d, *J* = 7.7 Hz, 1H, H-3'), 7.38 (d, *J* = 8.0 Hz, 2H, H-2, H-6), 7.23 (t, *J* = 7.7 Hz, 1H, H-4'), 7.18 (d, *J* = 8.0 Hz, 2H, H-3, H-5), 7.11 (t, *J* = 7.7 Hz, 1H, H-5'), 7.02 (d, *J* =16.1 Hz, 1H, H-8), 6.92 (d, *J* = 16.1 Hz, 1H, H-7), 2.86 (m, 1H, H-1"), 2.16 (s, 3H, CH_3, H-8'), 1.21 (s, 3H, CH_3, H-2"), 1.20 (s, 3H, CH_3, H-3"). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 168.7, 148.9, 139.0, 132.9, 129.4, 128.2, 126.9, 126.7, 126.5, 125.5, 123.9, 122.5, 117.3, 33.9, 24.4, 23.9. HRMS (+ESI) $[M+H]^+$: 280.1717, $C_{19}H_{22}NO$ requires 280.1701.

(*E*)-*N*-(2-(2-(naphthalen-1-l)vinyl)phenyl)acetamide (3c): Light brown solid; Mp: 165-167°C; Yield: 40%, IR *v* (cm^{-1}): 3279 (m, N-H), 3043 (w, C_{sp3}-H), 1653 (s, C=O), 1576 (m, C=C), 1297 (s, C-N), 741 (s, C-H). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 8.16 (d, *J* = 7.7 Hz, 1H, H-7), 7.88 (d, *J* = 7.7 Hz, 1H, H-10), 7.85 (t, *J* = 7.7 Hz, 2H, H-9, H-5), 7.77 (d, *J* = 15.8 Hz, 1H, H-12), 7.72 (d, *J* = 7.5 Hz, 1H, H-6'), 7.65 (d, *J* = 7.7 Hz, 1H, H-3), 7.53 (d, *J* = 7.5 Hz, 1H, H-3'), 7.50 (t, *J* = 7.7 Hz, 2H, H-8, H-4), 7.34 (t, *J* = 7.5 Hz, 1H, H-5'), 7.23 (t, *J* = 7.5 Hz, 1H, H-4'), 7.17 (d, *J* = 15.8 Hz, 1H, H-11), 2.19 (s, 3H, CH_3 H-8'). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 168.5, 134.7, 133.7, 131.3, 130.4, 129.9, 128.7, 128.5, 127.1, 126.6, 126.3, 126.0, 125.6, 124.1, 124.0, 123.6, 24.3. HRMS (+ESI) $[M+H]^+$: 288.1371, $C_{20}H_{18}NO$ requires 288.1388.

(*E*)-*N*-(2-(3,4,5-rimethoxystyryl)phenyl)acetamide (3d): White solid; Mp: 200-202°C; Yield: 55%, IR *v* (cm^{-1}): 3174 (m, N-H), 2929 (w, C_{sp3}-H), 1642 (s, C=O), 1575 (m, C=C), 1299 (s, C-N), 1040 (m, C_{sp3}-O), 746 (s, C-H). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.81 (d, *J* = 7.6 Hz, 1H, H-6'), 7.70 (br s,1H, NH), 7.50 (d, *J* = 7.6 Hz, 1H, H-3'), 7.30 (t, *J* = 7.6 Hz, 1H, H-4'), 7.19 (t, *J* = 7.6 Hz, 1H, H-5'), 7.03 (d, *J* = 15.9 Hz, 1H, H-8), 6.92 (d, *J* = 15.9 Hz, 1H, H-7), 6.72 (s, 2H, H-2, H-6), 3.92 (s, 6H, 2 × OCH_3, H-1", H-3"), 3.87 (s, 3H, CH_3, H-2"), 2.23 (s, 3H, CH_3, H-8'). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 169.1, 153.5, 138.5, 135.4, 132.7, 130.5, 129.3, 128.3, 127.0, 124.2, 123.4, 123.2, 104.0, 60.9, 56.2, 24.1. HRMS (+ESI) $[M+H]^+$: 328.1542, $C_{19}H_{22}NO_4$ requires 328.1549.

(*E*)-*N*-(2-(3,4-dimethoxystyryl)phenyl)acetamide (3e): Light brown solid; Mp: 149-151°C; Yield: 65%, IR *v* (cm^{-1}): 3325 (m, N-H), 2936 (w, C_{sp3}-H), 1679 (s, C=O), 1579 (m, C=C), 1290 (s, C-N), 1021 (m, C_{sp3}-O), 754 (s, C-H). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.82 (d, *J* = 7.7 Hz, 1H, H-6'), 7.51 (d, *J* = 7.7 Hz, 1H, H-3'), 7.28 (t, *J* = 7.7 Hz, 1H, H-5'), 7.18 (t, *J* = 7.7 Hz, 1H, H-4'), 7.07 (d, *J* = 8.0 Hz, 1H, H-6), 7.03 (s, 1H, H-2), 6.99 (d, *J* = 16.0 Hz, 1H, H-8), 6.93 (d, *J* = 16.0 Hz, 1H, H-7), 6.88 (d, *J* = 8.0 Hz, 1H, H-5), 3.94 (s, 3H, CH_3, H-2"), 3.91 (s, 3H, CH_3, H-1"), 2.23 (s, 3H, CH_3, H-8'). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 168.7,

149.5, 149.3, 134.7, 132.7, 130.7, 130.3, 128.3, 127.0, 125.8, 124.3, 121.8, 120.1, 111.5, 109.5, 56.2, 24.5. HRMS (+ESI) $[M+H]^+$: 298.1448, $C_{18}H_{20}NO_3$ requires 298.1443.

(***E***)-***N***-**(2-(4-methoxystyryl)phenyl)acetamide (3f):** White solid; Mp: 147-149°C; Yield: 72%, IR *v* (cm^{-1}): 3289 (m, N-H), 2927 (w, C_{sp3}-H), 1644 (s, C=O), 1512 (s, C=C), 1291 (s, C-N), 1032 (m, C_{sp3}-O), 745 (s, C-H). ^1H NMR ($CDCl_3$, 500 MHz) δ: 7.76 (d, *J* = 7.7 Hz, 1H, H-6'), 7.44 (d, *J* = 7.7 Hz, 1H, H-3'), 7.38 (d, *J* = 8.0 Hz, 2H, H-2, H-6), 7.22 (t, *J* = 7.7 Hz, 1H, H-5'), 7.11 (t, *J* = 7.7 Hz, 1H, H-4'), 6.93 (d, *J* = 16.1 Hz, 1H, H-8), 6.88 (d, *J* = 16.1 Hz, 1H, H-7), 6.85 (d, *J* = 8.0 Hz, 2H, H-3, H-5), 3.78 (s, 3H, CH_3, H-1"), 2.16 (s, 3H, CH_3, H-8'). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 168.4, 159.7, 134.5, 132.2, 130.5, 129.8, 129.3, 128.0, 127.9, 126.8, 124.0, 121.2, 114.2, 55.3, 24.3. HRMS (+ESI) $[M+H]^+$: 268.1333, $C_{17}H_{18}NO_2$ requires 268.1338.

(***E***)-***N***-**(2-(2-methoxystyryl)phenyl)acetamide (3g):** Light brown solid; Mp: 145-148°C; Yield: 20%, IR *v* (cm^{-1}): 3288 (m, N-H), 2921 (w, C_{sp3}-H), 1657 (s, C=O), 1597 (m, C=C), 1297 (s, C-N), 1021 (s, C_{sp3}-O), 738 (s, C-H). ^1H NMR (Acetone-d_6, 500 MHz) δ**:** 8.90 (br s, 1H, NH), 7.74 (d, *J* =7.7 Hz, 1H, H-6'), 7.63 (d, *J* = 7.7 Hz, 1H, H-3'), 7.62 (dd, *J* = 2.0 Hz, 1.5 Hz, 2H, H-4, H-6), 7.29-7.23 (m, 3H, H-5', H-8, H-7), 7.16 (t, *J* = 7.7 Hz, 1H, H-4'), 7.02 (d, *J* = 7.6 Hz, 1H, H-3), 6.95 (t, *J* = 7.6 Hz, 1H, H-5), 3.89 (s, 3H, OCH_3, H-1"), 2.15 (s, 3H, CH_3, H-8'). ^{13}C NMR (Acetone-d_6, 125 MHz) δ: 169.2, 158.0, 136.8, 129.9, 129.4, 128.3, 127.4, 126.7, 126.1, 125.9, 125.8, 125.2, 121.5, 119.9, 112.0, 55.9, 23.9. HRMS (+ESI) $[M+H]^+$: 268.1325, $C_{17}H_{18}NO_2$ requires 268.1338.

4.2. Synthesis of Aminostilbene Derivatives (4a-g)

The appropriate stilbene (**3a–g**) (1.0 equiv.) was stirred into 20 mL tetrahydrofuran (THF). Then, a mixture of 2 M hydrochloric acid (HCl) and ethanol (1:1; 2 mL) was added to the reaction flask, and the mixture was heated at 140 °C for 20 h with continuous stirring. Upon completion (monitored by TLC), the reaction was quenched with aqueous ammonium chloride solution (10 mL). The mixture was extracted three times with ethyl acetate (3 × 10 mL) and washed with plenty of distilled water (3 × 20 mL). The organic fractions were then collected and dried over anhydrous sodium sulphate. The solvent was removed using a rotary evaporator, and the dry crude product was purified *via* column chromatography with an *n*-hexane/ ethyl acetate (70:30) solvent system.

(***E***)**-2-(3,5-dimethoxystyryl)aniline (4a):** Dark brown sticky oil; Yield: 65%; IR *v* (cm^{-1}): 3452, 3373 (w, N-H), 2961 (w, C_{sp3}-H), 1592 (s, C=C), 1202 (m, C-N), 1062 (m, C_{sp3}-O), 751 (m, C-H). ^1H NMR ($CDCl_3$, 500 MHz) δ: 7.32 (d, *J* = 7.6 Hz, 1H, H-3'), 7.07 (d, *J* = 16.0 Hz, 1H, H-8), 7.03 (t, *J* = 7.6 Hz, 1H, H-4'), 6.84 (d, *J* = 16.0 Hz, 1H, H-7), 6.73 (t, *J* = 7.6 Hz, 1H, H-5'), 6.65 (d, *J* = 7.6 Hz, 1H, H-6'), 6.59 (s, 2H, H-2, H-6), 6.32 (s, 1H, H-4), 3.75 (s, 6H, CH_3, H-1", H-2"). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 161.0, 143.9, 139.6, 130.3, 128.8, 127.3, 124.7, 123.7, 119.2, 116.3, 104.5, 99.9, 55.4. HRMS (+ESI) $[M+H]^+$: 256.1335, $C_{16}H_{18}NO_2$ requires 256.1338.

(***E***)**-2-(4-isopropylstyryl)aniline (4b):** Dark brown sticky oil; Yield: 52%; IR *v* (cm^{-1}): 3433, 3359 (w, N-H), 2956 (m, C_{sp3}-H), 1573 (m, C=C), 1256 (m, C-N), 745 (m, C-H). ^1H NMR ($CDCl_3$, 500 MHz) δ: 7.37 (d, *J* = 8.1 Hz, 2H, H-3, H-5), 7.32 (d, *J* = 7.6 Hz, 1H, H-3'), 7.15 (d, *J* = 8.1 Hz, 2H, H-2, H-6), 7.04 (d, *J* = 16.0 Hz, 1H, H-8), 7.02 (t, *J* = 7.6 Hz, 1H, H-4'), 6.89 (d, *J* = 16.0 Hz, 1H, H-7), 6.73 (t, *J* = 7.6 Hz, 1H, H-5'), 6.64 (d, *J* = 7.6 Hz, 1H, H-6'), 2.84 (m, 1H, H-1"), 1.19 (s, 3H, CH_3, H-2"), 1.18 (s, 3H, CH_3, H-3"). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 148.5, 143.8, 135.2, 130.3, 128.4, 127.2, 126.7, 126.4, 124.1, 123.3, 119.1, 116.2, 33.9, 23.9. HRMS (+ESI) $[M+H]^+$: 238.1584, $C_{17}H_{20}N$ requires 238.1596.

(***E***)**-2-(2-(naphthalen-1-yl)vinyl)aniline (4c):** Dark brown sticky oil; Yield: 49%; IR *v* (cm^{-1}): 3440, 3369 (w, N-H), 1575 (m, C=C), 1258 (m, C-N), 750 (s, C-H). ^1H NMR ($CDCl_3$, 500 MHz) δ: 8.12 (d, *J* = 8.0 Hz, 1H, H-7), 7.80 (d, *J* = 8.0 Hz, 1H, H-10), 7.73 (d, *J* = 8.0 Hz, 1H, H-5), 7.69 (d, *J* = 15.7 Hz, 1H, H-12), 7.67 (d, *J* = 7.6 Hz, 1H, H-3'), 7.47-7.41 (m, 3H, H-4, H-8, H-9), 7.40 (d, *J* = 7.8 Hz, 1H, H-3), 7.13 (d, *J* = 15.7 Hz, 1H, H-11), 7.08 (t, *J* = 7.6 Hz, 1H, H-4'), 6.80 (t, *J* = 7.6 Hz, 1H, H-5'), 6.70 (d, *J* = 7.6 Hz, 1H, H-6'). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 144.0, 135.3, 133.7, 131.4, 130.0, 129.3, 128.8, 128.6, 128.0, 127.3, 126.1, 125.8, 125.7, 124.1, 123.8, 123.6, 119.2, 116.3. HRMS (+ESI) $[M+H]^+$: 246.1281, $C_{18}H_{16}N$ requires 246.1283.

(***E***)**-2-(3,4,5-trimethoxystyryl)aniline (4d):** Dark brown sticky oil; Yield: 69%; IR *v* (cm^{-1}): 3449, 3368 (w, N-H), 2937 (w, C_{sp3}-H), 1581 (m, C=C), 1240 (m, C-N), 1065 (m, C_{sp3}-O), 751 (m, C-H). ^1H NMR ($CDCl_3$, 500 MHz) δ: 7.32 (d, *J* = 7.6 Hz, 1H, H-3'), 7.04 (t, *J* = 7.6 Hz, 1H, H-4'), 7.01 (d, *J* = 16.0 Hz, 1H, H-8), 6.85 (d, *J* = 16.0 Hz, 1H, H-7), 6.76 (t, *J* = 7.6 Hz, 1H, H-5'), 6.68 (d, *J* = 7.6 Hz, 1H, H-6'), 6.66 (s, 2H, H-2, H-6), 3.84 (s, 6H, CH_3, H-1", H-3"), 3.80 (s, 3H, CH_3, H-2"). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 153.4, 142.5, 137.9, 133.2, 130.6, 128.6, 127.2, 124.5, 123.4, 120.1, 116.9, 103.5, 60.9, 56.1. HRMS (+ESI) $[M+H]^+$: 286.1435, $C_{17}H_{20}NO_3$ requires 286.1443.

(***E***)**-2-(3,4-dimethoxystyryl)aniline (4e):** Dark brown sticky oil; Yield: 75%; **IR *v* (cm^{-1}):** 3440, 3372 (w, N-H), 2957 (w, C_{sp3}-H), 1513 (s, C=C), 1264 (s, C-N), 1024 (m, C_{sp3}-O), 752 (m, C-H). ^1H NMR ($CDCl_3$, 500 MHz) δ: 7.32 (d, *J* = 7.6 Hz, 1H, H-3'), 7.03 (t, *J* = 7.6 Hz, 1H, H-4'), 6.99 (s, 1H, H-2), 6.98 (d, *J* = 8.1 Hz, 1H, H-6), 6.97 (d, *J* = 16.0 Hz, 1H, H-8), 6.86 (d, *J* = 16.0 Hz, 1H, H-7), 6.78 (d, *J* = 8.1 Hz, 1H, H-5), 6.76 (t, *J* = 7.6 Hz, 1H, H-5'), 6.69 (d, *J* = 7.6 Hz, 1H, H-

6'), 3.84 (d, J = 12.3 Hz, 6H, 2 × OCH_3, H-1'', H-2''). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 149.1, 148.9, 143.4, 130.7, 130.0, 128.4, 127.1, 124.3, 122.3, 119.7, 119.4, 116.4, 111.2, 108.7, 55.9. HRMS (+ESI) $[M+H]^+$: 256.1343, $C_{16}H_{18}NO_2$ requires 256.1338.

(***E***)**-2-(4-methoxystyryl)aniline (4f):** Dark brown sticky oil; Yield: 68%; IR v (cm^{-1}): 3440, 3371 (w, N-H), 2955 (w, C_{sp3}-H), 1574 (m, C=C), 1248 (s, C-N), 1031 (m, C_{sp3}-O), 750 (m, C-H). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.37 (d, J = 8.7 Hz, 2H, H-2, H-6), 7.31(d, J = 7.6 Hz, 1H, H-3'), 7.02 (t, J = 7.6 Hz, 1H, H-4'), 6.95 (d, J = 16.1 Hz, 1H, H-8), 6.86 (d, J = 16.1 Hz, 1H, H-7), 6.83 (d, J = 8.7 Hz, 2H, H-3, H-5), 6.73 (t, J = 7.6 Hz, 1H, H-5'), 6.64 (d, J = 7.6 Hz, 1H, H-6'), 3.76 (s, 3H, CH_3, H-1''). ^{13}C NMR ($CDCl_3$, 125 MHz) δ:159.2, 143.6, 130.4, 129.9, 128.3, 127.6, 127.0, 124.3, 122.1, 119.2, 116.2, 114.1, 55.3. HRMS (+ESI) $[M+H]^+$: 226.1239, $C_{15}H_{16}NO$ requires 226.1232.

(***E***)**-2-(2-methoxystyryl)aniline (4g):** Dark brown sticky oil; Yield: 60%; IR v (cm^{-1}): 3471, 3382 (w, N-H), 3025 (w, C_{sp3}-H), 1574 (m, C=C), 1240 (s, C-N), 1020 (m, C_{sp3}-O), 740 (s, C-H). ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 7.82 (d, J = 7.6 Hz, 1H, H-6), 7.42 (d, J = 7.7 Hz, 1H, H-3'), 7.37 (d, J = 16.1 Hz, 1H, H-8), 7.29 (t, J = 7.6 Hz, 1H, H-4), 7.26 (d, J = 16.1 Hz, 1H, H-7), 7.07 (d, J = 7.6 Hz, 1H, H-3), 7.04-7.01 (m, 2H, H-4', H-5), 6.73 (d, J = 7.7 Hz, 1H, H-5'), 6.64 (t, J = 7.7 Hz, 1H, H-6'), 3.89 (s, 3H, CH_3, H-1''). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 157.3, 143.0, 129.6, 129.4, 129.3, 128.9, 128.7, 126.7, 123.9, 121.0, 120.2, 113.1, 111.8, 111.5, 55.3. HRMS (+ESI) $[M+H]^+$: 226.1289, $C_{15}H_{16}NO$ requires 226.1232.

5. Evaluation of Cell Viability by MTT Assay

In this study, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye reduction assay was used to measure cellular metabolic activity as an indicator of the cytotoxicity of each compound (**4a-g**) against human lung cancer cell (A549) and normal cell (BEAS-2B) lines. The cells were seeded in 96-well plates at a density of 1×10^4 cells/well and incubated in 5 % CO_2 humidified at 37 °C for growth. The cells were treated with different concentrations of each compound ranging from 25 µM to 100 µM for 24, 48 and 72 h. The untreated cells receiving <0.1 % DMSO (v/v) in the culture medium were used as the negative control, while cisplatin (*cis*-diamminedichloroplatinum) was used as the positive control in this study. During the incubation period, 10 µL (5 mg/mL) of MTT reagent was added to each well, followed by 3 h incubation at 37 °C. Next, the medium was aspirated, and 100 µL of DMSO was added to solubilize the formazan crystal. The intensity of the purple colour obtained was directly proportional to the cell number, thus indicating cell viability. The reduction of the MTT solution was determined spectrophotometrically at 570 nm using a microplate reader (PowerWave XS, BioTek, USA), with 630 nm as the reference wavelength. The solution's optical density (O.D.) value directly represents relative cell numbers. The O.D. values were converted into percentages of cell viability using the following formula:

$$\text{Cell viability (\%)} = \left[\frac{\text{O.D.treatment} - \text{O.D.blank}}{\text{O.D.untreated} - \text{O.D.blank}}\right] \times 100\% \quad (1)$$

6. Selectivity Index (SI)

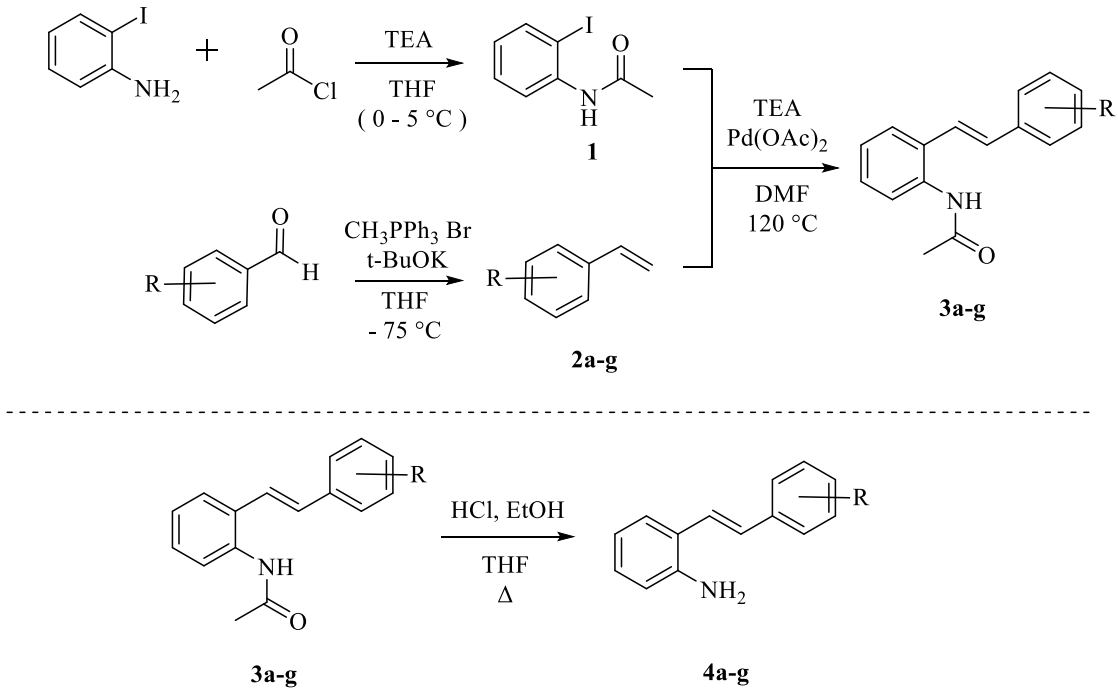
The degree of selectivity for each aminostilbene **4a-g** was expressed by its selectivity index (SI) value as suggested by Badisa *et al.* [22]. A high value (SI>2) suggests selective toxicity against A549 cancer cells, while a low SI value (<2) indicates the compound exhibits general toxicity which can cause cytotoxicity in normal cells. The SI value for each tested compound was calculated using the formula:

$$SI = IC_{50} \text{ normal cell} / IC_{50} \text{ cancer cell} \quad (2)$$

RESULTS AND DISCUSSION

1. Synthesis of Aminostilbenes 4a-g

The synthetic pathway for aminostilbene is summarized in Scheme 1. 2-iodoacetamide (**1**) was prepared from the reaction of a cooled solution of 2-iodoaniline and triethylamine with acetyl chloride at 0 - 5 °C. Different substituted benzaldehydes were reacted with methyltriphenylphosphonium bromide *via* the Wittig reaction to produce styrene derivatives **2a-g**. The synthesis of stilbenes **3a-g** was carried out using the cross-coupling reaction between styrene and acetamide **1**, known as the Heck reaction. Palladium (II) acetate was utilized as the catalyst in this reaction, resulting in 10-72 % yields of stilbenes **3a-g** [23-24]. Then, the stilbene derivatives **3a-g** underwent a deacetylation reaction in the presence of ethanol and HCl (2 M) with a 1:1 ratio and were refluxed for 20 h to afford crude aminostilbenes. Finally, the crude aminostilbenes were purified *via* column chromatography to obtain the aminostilbenes **4a-g** with moderate yields (49-75 %). The structures of all the synthesized compounds were validated using ^{1}H- and ^{13}C-NMR, FTIR, and HRMS spectroscopic data.



Where R = a: 3,5-dimethoxy (OMe)
b: 4-isopropyl
c: 2-naphthalene
d: 3,4,5-trimethoxy (OMe)
e: 3,4-dimethoxy (OMe)
f: 4-methoxy (OMe)
g: 2-methoxy (OMe)

Scheme 1. Synthetic routes for aminostilbenes **4a-g**.

Based on the FTIR analyses, all the synthesized aminostilbenes showed characteristic bands which confirmed the formation of aminostilbene and its derivatives. Two bands resonated at 3359–3471 cm^{-1}, indicating the presence of a primary amine (-NH_2) resulting from the amide hydrolysis of stilbene. A weak band at 2956–3025 cm^{-1} was assigned to the **(**C_{sp3}-H) stretch of the methyl group attached to the aromatic ring, except in the case of compound **4c** which did not have a methyl group. This was further validated by the presence of the (C=C) aromatic stretch in the range of 1513–1592 cm^{-1}, indicating the formation of an aromatic ring. In addition, a medium band at ~750 cm^{-1} due to aromatic (C-H) bending was observed in every synthesized compound. A strong and sharp band was observed in the range of 1202–1264 cm^{-1} due to the (C-N) stretching of the aromatic amine. Compounds **4a** and **4d–g** exhibited a stretching band (C_{sp3}-O) in the region of 1020–1062 cm^{-1} which indicated the presence of a methoxy group.

Compound **4e**, bearing dimethoxy groups at the C-3 and C-4 positions, was isolated with the highest yield (75 %). Herein, **4e** is selected as an example for the spectral discussion of this series of compounds. In the ^{1}H NMR spectrum of **4e,** significant feature signals were observed in the range of δ_H 3.83–7.32. Two singlets were observed at δ_H 3.83 and 3.85, corresponding to the methoxy group present in the compound. A doublet resonated at δ_H 6.86 and 6.97 with a large coupling constant, J = 16.0 Hz, which corroborated the presence of a *trans*-isomer. In the ^{13}C NMR spectrum of **4e**, a single peak was observed at δ_C 55.9, corresponding to the presence of the methoxy group. By comparing the ^{13}C NMR and DEPT-90 spectra, five quaternary carbons were identified: C-1' (δ_C 143.0), C-2' (δ_C 123.9), C-1 (δ_C 129.6), C-3 (δ_C 129.3) and C-4 (δ_C 128.7). In addition, singlet peaks showed up at δ_C 116.4 and 130.0, corresponding to the signal of an ethylene group (C-8 and C-7).

2. Cytotoxicity Analysis

The MTT assay evaluated the cytotoxic activity of compounds **4a-g** against human lung cancer cell (A549) and normal cell (BEAS-2B) lines. The cytotoxicity of each compound was evaluated based on the IC_{50} values obtained. IC_{50} is the concentration of the tested compound that is required to produce 50 % inhibition of cell growth after an exposure time of 24-72 h. In this study, cisplatin (15 µM) was used as the positive control. The efficacy of each compound

was evaluated according to its IC_{50} value as follows: excellent/potent activity (< 1 µM), good activity (1-20 µM), moderate activity (20-100 µM), low activity (100-200 µM), and inactive (> 200 µM) [25]. Among these compounds, **4b** exhibited good cytotoxicity towards A549 cells after 72 h incubation, with an IC_{50} value of 20 µM.

While other compounds displayed weak effects with high IC_{50} values ranging from 21.5–61.4 µM after 24-72 h, it is worth noting that compounds with values higher than 50 µM were considered inactive [26]. Interestingly, most compounds showed no cytotoxic activity against normal human lung (BEAS-2B) cells with IC_{50} >100 µM. The exceptions were **4b** and **4c**, both of which showed slight toxicity against BEAS-2B with IC_{50} values of 71.9 and 81.9 µM. Cisplatin, a conventional chemotherapeutic agent, was used as the positive control in this study. Its structure consists of two labile chlorine and two inert ammonia molecules while aminostilbene **4b** consists of two aromatic rings linked with an olefinic bond, bearing an amino group at the *o*-position. Both compounds had differences in their chemical structures despite a small difference in the IC_{50} values obtained. Based on the MTT assays, cisplatin displayed good cytotoxic effects against both cell lines (A549 and BEAS-2B) with IC_{50} values of 19.9 and 20.0 µM, respectively. Based on the SI values obtained in Table 1, all the final products demonstrated high selectivity in inducing cytotoxic activity against lung cancer cells (A549) compared to normal lung cells (BEAS-2B). Taken together, the derivatives tested in this study were potent in inhibiting human lung cancer A549 cells with minimal cytotoxic effects towards normal BEAS-2B cells. These preliminary findings could be used as stepping stones for the development of future anticancer drugs.

Table 1. IC_{50} values of aminostilbenes **4a-g**.

Compounds	IC_{50} values (µM) (Mean ± SD)[a]			Selective Index (IC_{50} in normal cell/IC_{50} in cancer cells)
	Time (h)	A549	BEAS-2B	
4a	24	>100	>100	-
	48	52.2 ± 1.3	>100	1.9
	72	21.5 ± 1.7	>100	4.6
4b	24	51.0 ± 1.7	>100	1.9
	48	40.1 ± 3.1	77.6 ± 3.0	1.9
	72	20.0 ± 0.7	71.9 ± 3.3	3.5
4c	24	59.3 ± 1.6	>100	1.6
	48	25.1 ± 1.2	93.5 ± 1.0	3.7
	72	23.3 ± 0.2	81.9 ± 2.9	3.5
4d	24	>100	>100	-
	48	68.5 ± 5.1	>100	1.4
	72	59.4 ± 4.4	>100	1.6
4e	24	91.5 ± 2.5	>100	1.1
	48	76.0 ± 0.4	>100	1.3
	72	61.4 ± 4.7	>100	1.6
4f	24	>100	>100	-
	48	>100	>100	-
	72	>100	>100	-
4g	24	>100	>100	-
	48	57.3 ± 2.5	>100	1.7
	72	52.2 ± 0.9	>100	1.9
Cisplatin	24	60.0 ± 5.8	77.7 ± 5.9	1.3
	48	27.7 ± 1.3	36.7 ± 1.7	1.3
	72	19.9 ± 0.4	20.0 ± 0.4	1.1

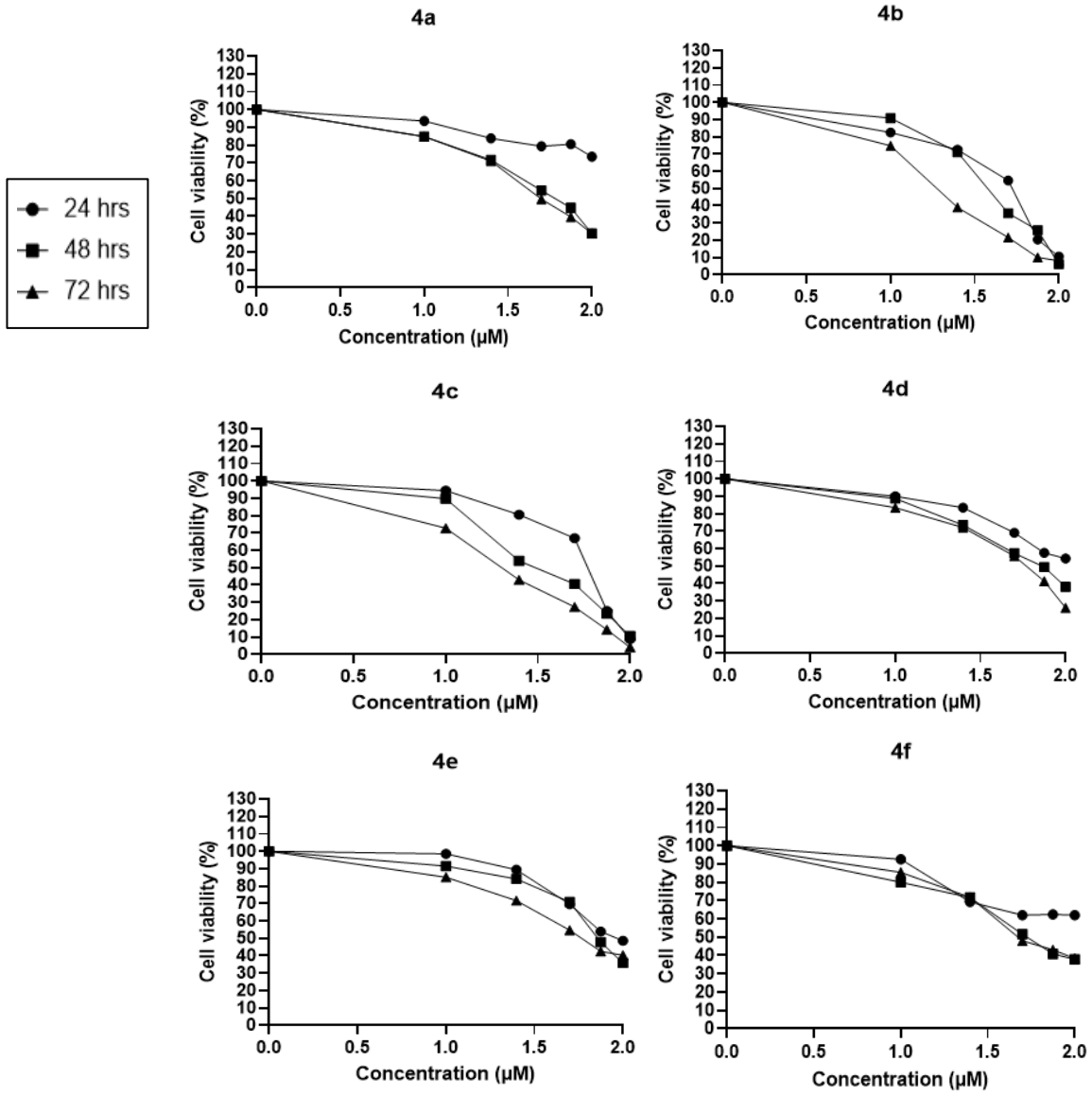
[a]The values shown are the means ± standard deviation of at least three independent experiments.

The structure-activity analysis (SAR) was focused on discovering the chemical or functional group(s) that boosts the biological activities of the parent compound. Based on the above results, we suggest that the isopropyl group located at the *para*-position in **4b** could enhance the cytotoxic activity of the parent compound toward A549 cancer cells. This was because **4b** exhibited the most potent cytotoxicity with an IC_{50} value of 20 µM compared to other aminostilbene analogues. In addition, as the toxicities of **4a**-**4g** were compared, it was revealed that the potency increased in the following order: 4-isopropyl (**4b)** > 3,5-OMe (**4a)** > Phenyl (**4c)** > 2-OMe (**4g)** > 3,4,5-OMe (**4d)** > 3,4-OMe (**4e)** > 4-OMe (**4f)**. Evidently, substitution with alkoxy and phenyl groups contributed to moderate cytotoxic activity against A549 cells, with IC_{50} values ranging from 21.5-100 µM.

3. Aminostilbene-induced Dose- and Time-dependant Cytotoxic Effects

If the cytotoxic effects vary with time, this indicates that the effects of the drugs are time-dependent. In contrast, dose-dependent implies the cytotoxic effects vary according to the dose or concentration of drugs used in the treatment.

As shown in Figure 2, both compounds **4b** and **4c** reduced the number of viable human lung cancer A549 cells in a dose-dependent manner, with a pronounced effect at higher concentrations. For instance, at 24 h of treatment, compound **4b** exhibited a reduction in cell viability of approximately 54 % at a concentration of 50 µM. At the same time, marginal cell death was observed at 100 µM. On the other hand, **4c** displayed minimal cell viability (27 %) after 48 h of treatment at 50 µM. More than 90% of cancer cells assayed died at 100 µM. The MTT assay data demonstrated that the cytotoxic effects of **4b** and **4c** on A549 cells were also time-dependent. At 50 µM, both **4b** and **4c** reduced cell viability to approximately 35 % and 30 % after 48 h, respectively. For both compounds, reduction of cell viability was once again found to be greatest after 72 h of treatment, with more than 75 % of cells dead. On the other hand, 50 µM cisplatin inhibited the viability of A549 cells to 80 % at 48 h.



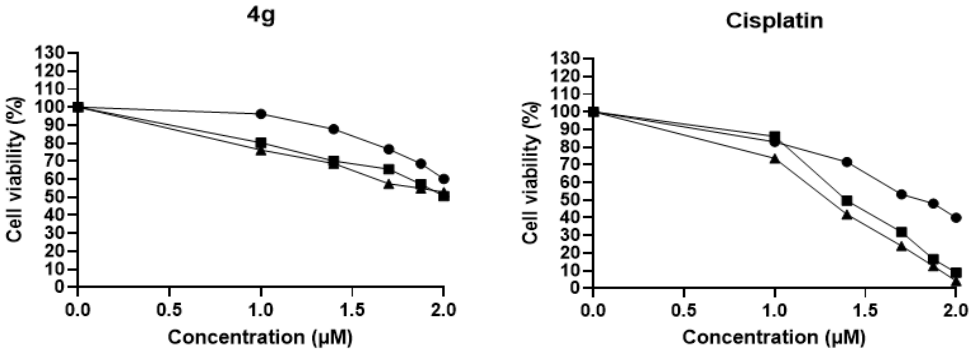


Figure 2. Dose-response curve of aminostilbenes on A549 cancer cells.

The best concentration range for a compound to exhibit dose- and time-dependent cytotoxic effects on A549 cells was reported to be between 1.5-2.0 μM in 24-48 h [27-28]. These findings highlighted the importance of further investigation into the cytotoxic effects of these compounds on cancer cells.

CONCLUSION

A total of seven aminostilbene derivatives **4a-g** were successfully synthesised *via* the deacetylation of stilbenes, with moderate yields of 49-75 %. In addition, all the synthesized aminostilbenes were tested for their cytotoxic activity against human lung cancer cell (A549) and normal cell (BEAS-2B) lines. Based on our results, compound **4b** exhibited good cytotoxicity towards A549 cells with an IC_{50} value of 20 μM at 72 h post-treatment. Further research introducing structural variation into the backbone of **4b** and modifying the structures may lead to diminished cytotoxic effects on normal healthy lung (BEAS-2B) cells. Based on SAR analysis, the presence of an isopropyl group at the *p*-position of an aromatic ring significantly affects its cytotoxic effect on A549 cells. Our findings suggest that aminostilbene derivatives have good potential in the development of anticancer drugs.

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