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## Stilbene heterocycles: Synthesis, antimicrobial, antioxidant and anticancer activities

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### Abstract

Stilbenes have long been in focus of scientific interest due to their diverse biological activities. In order to explore molecules possessing superior biological profile, a series of novel stilbene derivatives (**9-19**) containing pyridine moiety have been synthesized and evaluated for their antimicrobial, antioxidant and anticancer potential. Compounds **11**, **16**, **17**, **18** and **19** exhibited activity against *P. aeruginosa* (MIC = 50 µg/mL) on par with ampicillin. With regards to fungicidal activity against *C. albicans*, compounds **11**, **13** and **14** showed comparable activity with nystatin (MIC = 3.1 µg/mL). Further, compounds **17** and **19** possess good antioxidant properties. In addition, compound **19** exhibited strong anticancer activity with IC<sub>50</sub> values of 12.38 ± 6.83 µM and 14.52 ± 2.21 µM against two cancer cell lines PC-3 and MCF-7 respectively. Thus, compound 3-Bromo-3', 4'-dihydroxy stilbene-2-nitrogen (**19**) may be considered a lead molecule possessing diverse biological activity for further development.

**Keywords:** Stilbene heterocycles, antimicrobial, antioxidant, anticancer

### 1. Introduction

Phytoalexins are naturally occurring stilbenes present in many plant species, which protects plants from pathogen attacks and regulates many biological functions [1, 2]. In recent days hydroxylated stilbenes have attracted the attention of many scientific groups because of their remarkable biological properties and therapeutic potentials including anti-inflammatory [3], antioxidative [4], lipid-lowering [5], radical scavenging [6], neuroprotection [7], anticarcinogenic [8-12], antiviral [13] and also platelet aggregation inhibition activity [14]. In addition, numerous studies have indicated that hydroxy stilbenes exhibit nitric oxide production inhibitory, antimicrobial and anti-malarial activities [15-22]. One of the best representative compounds of this group is *trans*-3, 5, 4'-trihydroxy stilbene (resveratrol), a phytoalexin found in grapes, fruits and vegetables of several plants.

Some experimental evidence shows that, methoxy substituted stilbenes are good cancer chemo-preventive and most of methoxylated resveratrol derivatives exhibit potent cytotoxic and pro-apoptotic activity against cancer cells [23-26]. In general, the biological activity of the compounds are mainly influenced by their chemical structure and constituent groups attached to it. In view of these wide biological applications of stilbenes, we thought that it would be worthwhile to explore new class of stilbene compounds and investigate their biological profile. In our present work, we have synthesized a series of novel pyridine moiety containing stilbene compounds and evaluated their antimicrobial, antioxidant and anticancer potential.

### 2. Materials and Methods

**2.1** Solvents and organic reagents were purchased from Sigma- Aldrich, Merck (Germany) and Loba Chemie (India) and were used as such without any further purification. Reactions were monitored using thin-layer chromatography (TLC) on pre-coated silica gel plates (silica gel 60 F<sub>254</sub>, Merck). Chromatographic separation of mixtures was performed in open glass columns packed with silica gel (Merck Grade 7734, 70-230 mesh) and eluted with ethyl acetate - hexane solvent mixture. Melting points were determined on Acro Steel Pvt. Ltd., melting point apparatus (using a calibrated thermometer). <sup>1</sup>H and <sup>13</sup>C NMR spectra recorded on Bruker 400MHz NMR spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shifts expressed in δ (ppm). Mass spectra recorded using GC-MS-QP2010S (Direct probe).

#### 2.1.1 Preparation of 6-bromopyridine-2-carbaldehyde (**1**)

The key intermediate 6-bromopyridine-2-carbaldehyde (**1**) was prepared by known procedure [27] starting from 2-amino-6-methylpyridine. The reaction proceeds in three steps.

In the first step, 2-amino-6-methylpyridine (60 g, 0.55 mol) was treated with HBr (1.0L, 48% in water) and liquid bromine (80 mL) at 0-10 °C followed by NaNO<sub>2</sub> (100 g, 1.45 mol) addition in water to give 2-bromo-6-picoline (82 g, 0.47 mol). This was then treated with NBS (167 g, 0.94 mol) using CCl<sub>4</sub> (750 mL) as a solvent and column purification of crude mass over silica gel using EtOAc/Hexane =1:9 as an eluent gave 2-bromo-6-dibromomethyl-pyridine (135 g, 0.41 mol) as a white solid which was hydrolyzed with CaCO<sub>3</sub> (92 g, 0.92 mol) in water at reflux temperature. Column purification over silica gel afforded compound **1** (63 g, 0.34 mol) as a pure white solid with overall yield of about 62%.

### 2.1.2 General procedure for synthesis of benzyltriphenyl phosphonium halide salts (2-8)

A mixture of substituted benzyl chloride / bromide (1 eq.) and triphenylphosphine (1 eq.) in toluene was heated under reflux for 10-12 h. TLC was checked for the completion of reaction. After the completion of reaction, the reaction mixture was cooled slowly to room temperature and the solid thus obtained was filtered under suction, washed with cold toluene and air dried to afford compounds **2-8** as white to off- white solids.

### 2.1.3 General procedure for synthesis of Stilbene derivatives (9-15)

To a solution of appropriate phosphonium salt **2-8** (1 eq.) in CH<sub>2</sub>Cl<sub>2</sub> at 25-30 °C was added potassium t-butoxide (1.5 eq.) and the resulting solution was stirred under nitrogen for 2 h. A solution of aldehyde **1** (1 eq.) in CH<sub>2</sub>Cl<sub>2</sub> was added dropwise over 30 min, and stirring continued for 2-6 h at room temperature. After the completion of reaction, the resulting mixture was quenched into water, extracted with dichloromethane. The organic phase was washed with water, dried over anhydrous sodium sulfate and evaporated solvent under reduced pressure. The residue thus obtained was purified over silica gel column chromatography (220-400 mesh) (1:40 silica gel ratio) using EtOAc/Hexane mixture (1:9) as a mobile phase to afford pure material of **9-15**.

#### 2.1.3.1 3-Bromo-3', 5'-dimethoxystilbene-2-nitrogen (9)

Reaction of 6-bromopyridine-2-carbaldehyde (**1**) (2.0 g, 10.75 mmol), [3, 5-di (methoxy) benzyl] triphenyl phosphonium bromide (**2**) (5.3 g, 10.75 mmol) and potassium t-butoxide (1.9 g, 17 mmol) in DCM (50 mL) gave compound **9** (2.1 g, 6.54 mmol)

Compound **9** obtained as pale yellow solid; Yield 61.0%; m.p. 56-58 °C; <sup>1</sup>H NMR: (CDCl<sub>3</sub>) δ: 3.74 (s, 6H), 6.41 (dd, 1H, *J* = 2.0 Hz), 6.48 (dd, 2H, *J* = 2.0Hz), 6.63 (d, 1H, *J* = 12.4 Hz), 6.83(d, 1H, *J* = 12.4 Hz), 7.18-7.20 (dd, 1H, *J* =7.2 Hz), 7.30-7.37 (m, 2H); <sup>13</sup>C NMR δ: 55.31, 100.47, 106.61, 122.85, 126.02, 129.46, 134.59, 137.85, 138.12, 141.34, 157.32, 160.75; GC MS (*m/z*): 319.

#### 2.1.3.2 3-Bromo-3', 4', 5'-trimethoxystilbene-2-nitrogen (10)

Reaction of 6-bromopyridine-2-carbaldehyde (**1**) (2.5 g, 13.44 mmol), [(3, 4, 5-trimethoxy) benzyl] triphenyl phosphonium chloride (**3**) (6.43 g, 13.44 mmol) and potassium t-butoxide (2.3 g, 20.5 mmol) in DCM (50 mL) gave compound **10** (2.6 g, 7.48 mmol).

Compound **10** obtained as yellow solid; Yield 56.0%; m.p. 60-62 °C; <sup>1</sup>H NMR: (CDCl<sub>3</sub>) δ: 3.65 (s, 3H), 3.85 (s, 3H), 3.95 (s, 3H), 6.55 (s, 2H), 6.58 (d, 1H, *J* = 12.4 Hz), 6.86 (s, 1H),

6.94 (d, 1H, *J* = 12.4 Hz), 7.21-7.23 (dd, 1H, *J* =7.6 Hz), 7.27-7.36 (m, 2H); <sup>13</sup>C NMR δ: 56.02, 56.36, 56.40, 97.27, 113.59, 116.46, 122.54, 125.56, 127.53, 129.92, 137.68, 141.28, 142.64, 150.10, 152.07, 158.06; GC MS (*m/z*): 349.

#### 2.1.3.3 3-Bromo-stilbene-2-nitrogen (11)

Reaction of 6-bromopyridine-2-carbaldehyde (**1**) (2.5 g, 13.44 mmol), benzyl triphenyl phosphonium chloride (**4**) (5.22g, 13.44mmol) and potassium t-butoxide (2.3 g, 20.5 mmol) in DCM (50 mL) gave compound **11** (2.5 g, 9.62 mmol).

Compound **11** obtained as colorless liquid; Yield 72.0%; <sup>1</sup>H NMR: (CDCl<sub>3</sub>) δ: 6.59 (d, 1H, *J* = 12.4 Hz), 6.84 (d, 1H, *J* = 12.4 Hz), 7.07 (m, 1H), 7.25 (m, 7H); <sup>13</sup>C NMR δ: 122.56, 125.96, 127.85, 128.37, 128.73, 128.81, 129.19, 134.62, 136.26, 137.84, 141.45, 157.43; GC MS (*m/z*): 259.

#### 2.1.3.4 3-Bromo-3', 4'-(methylenedioxy) stilbene-2-nitrogen (12)

Reaction of 6-bromopyridine-2-carbaldehyde (**1**) (2.0 g, 10.75 mmol), [3, 4-(Methylenedioxy) benzyl] triphenyl phosphonium chloride (**5**) (4.6 g, 10.75 mmol) and potassium t-butoxide (1.9 g, 17 mmol) in DCM (50 mL) gave compound **12** (2.2 g, 7.3 mmol).

Compound **12** obtained as light brown viscous liquid; Yield 68.0%; <sup>1</sup>H NMR: (CDCl<sub>3</sub>) δ: 5.92 (s, 2H), 6.47 (d, 1H, *J* = 12.4 Hz), 6.69 – 6.82 (m, 4H), 7.14 (dd, 1H, *J* = 1.0 Hz, *J* = 7.6 Hz), 7.24-7.32 (m, 2H); <sup>13</sup>C NMR δ: 101.08, 108.28, 109.02, 122.65, 122.86, 125.73, 127.99, 130.09, 134.09, 137.96, 141.44, 147.38, 147.55, 157.47; GC MS (*m/z*): 303.

#### 2.1.3.5 3-Bromo-4'-methoxystilbene-2-nitrogen (13):

Reaction of 6-bromopyridine-2-carbaldehyde (**1**) (2.0 g, 10.75 mmol), [4-(methoxy) benzyl] triphenyl phosphonium bromide (**6**) (5.0g, 10.75mmol) and potassium t-butoxide (1.9 g, 17 mmol) in DCM (50 mL) gave compound **13** (2.1 g, 7.24 mmol).

Compound **13** obtained as pale yellow viscous liquid; Yield 67.0%; <sup>1</sup>H NMR: (CDCl<sub>3</sub>) δ: 3.77 (s, 3H), 6.48 (d, 1H, *J* = 12.4 Hz), 6.75 – 6.81 (m, 3H), 7.13 – 7.15 (dd, 1H, *J* = 1.2 Hz, *J* = 7.2 Hz), 7.23 – 7.30 (m, 4H); <sup>13</sup>C NMR δ: 55.20, 113.74, 122.52, 125.73, 127.58, 128.64, 130.95, 135.83, 138.59, 142.06, 157.81, 159.40; GC MS (*m/z*): 289.

#### 2.1.3.6 3-Bromo-3', 4'-dimethoxystilbene-2-nitrogen (14)

Reaction of 6-bromopyridine-2-carbaldehyde (**1**) (2.5 g, 13.44 mmol), [3, 4-(dimethoxy) benzyl] triphenyl phosphonium chloride (**7**) (6.0 g, 13.44 mmol) and potassium t-butoxide (2.3 g, 20.5 mmol) in DCM (50 mL) gave compound **14** (3.1 g, 9.7 mmol).

Compound **14** obtained as pale yellow viscous liquid; Yield 73.0%; <sup>1</sup>H NMR: (CDCl<sub>3</sub>) δ: 3.74 (s, 3H), 3.87 (s, 3H), 6.49 (d, 1H, *J* = 12.4 Hz), 6.75 – 6.79 (m, 2H), 6.86 – 6.89 (m, 1H), 6.95 – 6.97 (m,1H), 7.17 (d, 1H, *J* = 7.2 Hz), 7.26 – 7.34 (m, 2H); <sup>13</sup>C NMR δ: 55.37, 55.48, 110.43, 111.49, 121.91, 122.41, 125.38, 127.00, 128.40, 134.07, 137.46, 140.93, 148.08, 148.56, 157.33; GC MS (*m/z*): 319.

#### 2.1.3.7 3-Bromo-6'-nitrostilbene-2-nitrogen (15)

Reaction of 6-bromopyridine-2-carbaldehyde (**1**) (2.5 g, 13.44 mmol), (2-nitrobenzyl) triphenyl phosphonium chloride (**8**)

(6.0 g, 13.44 mmol) and potassium t-butoxide (2.3 g, 20.5 mmol) in DCM (50 mL) gave compound **15** (2.87 g, 9.4 mmol).

Compound **15** obtained as pale yellow solid; Yield 70.0%; m.p. 46-48 °C, <sup>1</sup>H NMR: (CDCl<sub>3</sub>) δ: 6.72 (d, 1H, *J* = 12.0 Hz), 6.84 (d, 1H, *J* = 7.2 Hz), 7.14 (d, 1H, *J* = 12.0 Hz), 7.21 – 7.28 (m, 3H), 7.43 – 7.50 (m, 2H), 8.13 (d, 1H, *J* = 7.6 Hz); <sup>13</sup>C NMR δ: 122.66, 124.62, 126.31, 128.47, 129.58, 131.53, 131.69, 133.15, 133.22, 138.08, 141.42, 147.93, 155.92; GC MS (*m/z*): 306.

### 2.1.4 General procedure for synthesis of hydroxy Stilbene derivatives (16-19)

To a solution of TEA (14.0 equiv) in chlorobenzene (6.0 equiv) under a nitrogen atmosphere at 0 -5 °C was added anhydrous AlCl<sub>3</sub> (9.0 equiv) in small portions over 30 min. The reaction mixture was then maintained at ambient temperature for 30 min under stirring. Slowly raised the temperature to 60 °C and maintained at same temperature for another 1h. Methoxy derivatives **9**, **10**, **13**, **14** (1.0 equiv) in chlorobenzene (6.0 equiv) was added subsequently in 30 min at 60–70 °C. Completion of reaction was monitored by TLC. Cooled the reaction mixture to room temperature and quenched in to ice water. The resulting mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub>; the organic layer was then washed with water dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude thus obtained was then subjected to column purification over silica gel (220-400 mesh) (mobile phase: Hexane: EtOAc: 8:2) to yield the pure hydroxy stilbene derivatives **16-19**.

#### 2.1.4.1 3-Bromo-3', 5'-dihydroxy stilbene-2-nitrogen (16)

Reaction of compound **9** (1.0 g, 3.1 mmol) with TEA (4.5 g, 44.5 mmol) and aluminum chloride (3.75 g, 28 mmol) in chlorobenzene (4.2 g, 37.5 mmol) gave compound **16** (0.33 g, 1.1 mmol) as white solid; Yield 36.0%; m.p. 188-90 °C; <sup>1</sup>H NMR: (DMSO-d<sub>6</sub>) δ: 6.08 – 6.11 (m, 3H), 6.44 (d, 1H, *J* = 12.4 Hz), 6.70 (d, 1H, *J* = 12.4 Hz), 7.18 (d, 1H, *J* = 7.6 Hz), 7.43 (d, 1H, *J* = 7.6 Hz), 7.57 (dd, 1H, *J* = 7.6 Hz), 9.18 (s, 2H); <sup>13</sup>C NMR δ: 102.41, 106.38, 122.99, 126.03, 126.24, 128.30, 134.77, 134.96, 137.43, 139.16, 139.29, 140.68, 157.04, 158.39; GC MS (*m/z*): 291.

#### 2.1.4.2 3-Bromo-3', 4', 5'-trihydroxy stilbene-2-nitrogen (17):

Reaction of compound **10** (1.0 g, 2.86 mmol) with TEA (4.1 g, 40.6 mmol) and aluminum chloride (3.5 g, 26 mmol) in chlorobenzene (3.9 g, 34.6 mmol) gave compound **17** (0.28 g, 0.91 mmol) as pale yellow solid; Yield 32.0%; m.p. 196-98 °C; <sup>1</sup>H NMR: (DMSO-d<sub>6</sub>) δ: 6.37 (s, 1H), 6.82 (d, 1H, *J* = 16.0 Hz), 6.94 (s, 1H), 7.33 (d, 1H, *J* = 7.6 Hz), 7.39 (d, 1H, *J* = 7.6 Hz), 7.62 (dd, 1H, *J* = 7.6 Hz), 7.73 (d, 1H, *J* = 16.0 Hz), 8.30 (bs, 1H), 9.20 (s, 1H), 9.28 (s, 1H); <sup>13</sup>C NMR δ: 103.56, 112.78, 113.18, 120.79, 121.26, 124.75, 129.51, 138.44, 139.77, 141.24, 147.84, 149.78, 157.84; GC MS (*m/z*): 307.

#### 2.1.4.3 3-Bromo-4'-hydroxy stilbene-2-nitrogen (18)

Reaction of compound **13** (1.0 g, 3.44 mmol) with TEA (4.9 g, 48.5 mmol) and aluminum chloride (4.1 g, 30.7 mmol) in chlorobenzene (4.6 g, 40.9 mmol) gave compound **18** (0.38 g, 1.4 mmol) as pale yellow solid; Yield 40.0%; m.p. 146-48 °C; <sup>1</sup>H NMR: (DMSO-d<sub>6</sub>) δ: 6.36 (d, 1H, *J* = 12.4 Hz), 6.65 – 6.68 (m, 2H), 7.21 – 7.26 (m, 3H), 7.43 (d, 1H, *J* = 7.6 Hz),

7.59 (dd, 1H, *J* = 7.6 Hz), 9.62 (s, 1H); <sup>13</sup>C NMR δ: 115.68, 121.76, 122.99, 125.50, 126.92, 128.92, 133.78, 140.22, 141.29, 157.14, 158.35; GC MS (*m/z*): 275.

#### 2.1.4.4 3-Bromo-3', 4'-dihydroxy stilbene-2-nitrogen (19)

Reaction of compound **14** (1.0 g, 3.1 mmol) with TEA (4.5 g, 44.5 mmol) and aluminum chloride (3.75 g, 28 mmol) in chlorobenzene (4.2 g, 37.5 mmol) gave compound **19** (0.35 g, 1.2 mmol) as yellow solid; Yield 39.0%; m.p. 156-58 °C; <sup>1</sup>H NMR: (DMSO-d<sub>6</sub>) δ: 6.32 (d, 1H, *J* = 12.4 Hz), 6.62 – 6.73 (m, 4H), 7.25 (d, 1H, *J* = 7.6 Hz), 7.42 (d, 1H, *J* = 7.6 Hz), 7.57 (dd, 1H, *J* = 7.6 Hz), 8.85 (s, 1H), 9.09 (s, 1H); <sup>13</sup>C NMR δ: 115.86, 116.46, 121.23, 123.16, 126.20, 126.39, 127.35, 135.08, 139.65, 139.76, 145.34, 146.18, 157.94; GC MS (*m/z*): 291.

## 2.2 Antimicrobial assay

Bacterial strains viz., *Bacillus subtilis* (MTCC 2616), *Staphylococcus aureus* (MTCC 6908), *Escherichia coli* (MTCC 1698), *Pseudomonas aeruginosa* (MTCC 4673), the fungal strains such as *Aspergillus niger* (MTCC 9687), *Mucor indicus* (MTCC 7135) and *Candida albicans* (MTCC 4748) were obtained from MTCC, Chandigarh and Division of Microbiology, IARI, New Delhi. The MIC (µg/mL) values of the compounds **9-15** and **16-19** were determined by disc diffusion method [28-32] using antibacterial ampicillin and antifungal drug nystatin as positive controls. Test compounds were prepared by dissolving 1 mg of each sample in 1 ml of DMSO (Dimethyl sulphoxide) and further diluted into different concentrations ranging from 3.1 – 400 µg/mL. Bacteria and fungal culture were grown in Potato Dextrose Agar (PDA, HiMedia). The plates were then incubated at 37 ± 1 °C for 24 h for bacteria and at 30 ± 1 °C for 48 h (*C. albicans*) and 72 h (*A. niger* and *M. indicus*) respectively. All the experiments were performed in triplicates.

## 2.3 Antioxidant Activity

### DPPH Radical Scavenging Activity:

The radical scavenging activity of all the compounds **9-19** towards the radical DPPH was measured as described [33, 34]. Stock solutions of compounds **9-19**, L-ascorbic acid (standard) and resveratrol (secondary reference) were prepared in absolute ethanol at a concentration of 1 mg/ mL (10 mL). These stock solutions were further diluted in ethanol to get appropriate concentrations (1–500 µg/mL). A solution of DPPH (0.13 g/ 100 mL) in ethanol was prepared. 100 µL of this DPPH solution was added to 1 mL of each different concentration (1–500 µg/mL) and the mixture was shaken vigorously. Each concentration was tested in triplicate. DPPH (100 µL) in ethanol (1 mL) was used as control. The mixtures were left for 30 min at room temperature and the absorbance (optical density) was measured at 517 nm using shimadzu UV spectrophotometer (UV-1800). The % radical scavenging activity was calculated using formula:

$$\% \text{ scavenging activity} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

The IC<sub>50</sub> (concentration causing 50% inhibition) values of each compound was determined graphically and the inhibition curve plotted for three different experiments was represented as % of mean inhibition ± standard deviation.

### ABTS Radical Scavenging Activity

The ABTS radical scavenging activity of compounds **9-19** was measured as described in the reported procedure [35]. The ABTS+• was generated by mixing equal amount of ABTS diammonium salt (7.4 mmol/L) and potassium persulfate (2.45 mmol/L in the final concentration) in water and the mixture was kept in the dark at room temperature for 15 h to allow completion of radical generation. This was further diluted with absolute ethanol (about 1:50) so that its absorbance at 734 nm was 0.70±0.02 measured on a spectrophotometer (UV-1800, shimadzu). To determine the scavenging activity, 1.0 mL of ABTS+• reagent was mixed with 0.1 mL of test samples of various concentrations (1–500 µg/mL) with control (1.0 mL of ABTS+• and 0.1 mL of absolute ethanol), and the absorbance at 734 nm was measured at 6-7 min after the initial mixing, using absolute ethanol as the blank. The percentage inhibition of the samples was calculated as:

$$\% \text{ Inhibition} = \frac{A_0 - A}{A_0} \times 100$$

Where  $A_0$  is the absorbance of the control,  $A$  is the absorbance of samples.

Each experiment was performed in triplicate. Linear regression analysis was carried out for calculating effective concentration of sample required to scavenge radical by 50% ( $IC_{50}$  value) using dose-response curve plotting between %inhibition vs concentrations.

### 2.4 Anticancer activity

*In vitro* anticancer activity was determined using MTT assay method [36]. Cell lines were maintained in Dulbecco's Modified Eagle's Medium (Sigma- Aldrich Inc., USA) supplemented with 10% fetal bovine serum (Gibco BRL., USA) in a  $CO_2$  incubator at 37 °C. PC-3 (prostate cancer), MCF - 7 (breast cancer) and HeLa (cervical cancer) cell lines were seeded into 96-well plates at the density of 10,000 cells per well. 24 h after seeding, cells were treated with different concentrations of the compounds from 100 µM serially diluted up to 3.13 µM using camptothecin (CPT) as standard and resveratrol as a secondary reference samples. The cells were later incubated for 48 h, 20 µl of MTT (5 mg/ml stock, Sigma-Aldrich Inc., USA) was added to each well and incubated for another 3 h. The purple formazan crystals formed were dissolved by adding 100 µl of DMSO to each well and absorbance was read at 570 nm in a spectrophotometer [Spectra Max 340]. The cell death was calculated as follows:

$$\text{Cell death} = 100 - [(\text{test absorbance} / \text{control absorbance}) \times 100]$$

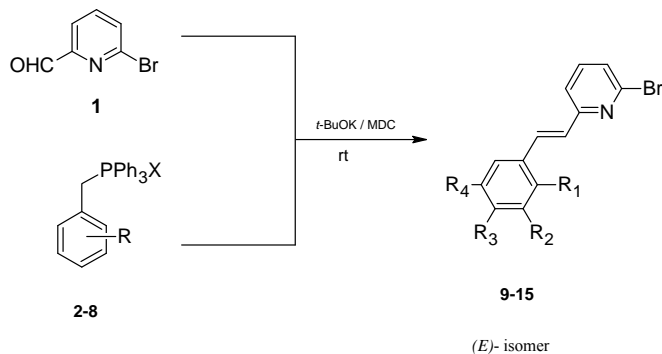
The test result with  $IC_{50}$  value was expressed as concentration at which inhibits the cell growth by 50%. All the experiments were performed in triplicate.

## 3. Results and Discussion

### 3.1 Chemistry

The overall synthetic method for the preparation of various

stilbene derivatives **9-15** containing one pyridine moiety with bromine substitution at  $\alpha$ - position in place of benzene nucleus is shown in scheme 1. The purpose of our study was to evaluate the biological profile and their therapeutic usage. According to our method, the starting material 6-bromopyridine-2-carbaldehyde (**1**) was prepared in three steps from 6-amino-2-picoline by reported procedure [27]. The Wittig reaction of compound **1** with appropriate benzyltriphenyl phosphonium salts **2-8** (Table 1) [37-40] in presence of potassium t-butoxide in dichloromethane at room temperature afforded crude stilbene derivatives. Further, column purification of these crude mixtures over  $SiO_2$  yielded pure (*E*)-stilbene derivatives **9-15** and very low to negligible amount of (*Z*)-isomers.



- 9:**  $R_1, R_3 = H; R_2, R_4 = OMe$   
**10:**  $R_1 = H; R_2, R_3, R_4 = OMe$   
**11:**  $R_1, R_2, R_3, R_4 = H$   
**12:**  $R_1, R_2 = H; R_3, R_4 = \text{Methylenedioxy}$   
**13:**  $R_1, R_2, R_4 = H; R_3 = OMe$   
**14:**  $R_1, R_2 = H; R_3, R_4 = OMe$   
**15:**  $R_2, R_3, R_4 = H; R_1 = NO_2$

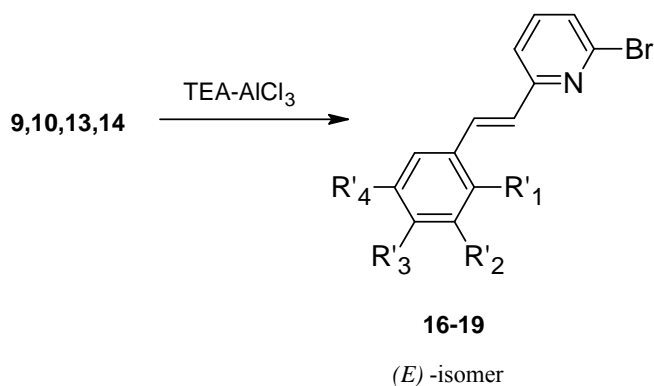
**Scheme 1:** Synthesis of compounds 9-15

**Table 1:** Substituents for Compounds (2-8)

Compound	X	R	Yield# (%)
<b>2</b>	Br	3, 5-(OMe) <sub>2</sub>	90
<b>3</b>	Cl	3, 4, 5-(OMe) <sub>3</sub>	67
<b>4</b>	Cl	H	95
<b>5</b>	Cl	3, 4-Methylene dioxy	72
<b>6</b>	Br	4-OMe	88
<b>7</b>	Cl	3, 4-(OMe) <sub>2</sub>	78
<b>8</b>	Cl	2-NO <sub>2</sub>	75

#isolated yield.

Further, methoxy derivatives **9, 10, 13** and **14** were demethylated using  $AlCl_3$ -TEA complex at 60-70 °C and after column purification over silica gel afforded hydroxy derivatives **16-19** with considerable yields. The chemical structures of all the target compounds were confirmed by  $^1H$ ,  $^{13}C$  NMR and GC-MS spectral analysis.



**16:** R<sub>1</sub>, R<sub>3</sub> = H; R<sub>2</sub>, R<sub>4</sub> = OH

**17:** R<sub>1</sub> = H; R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub> = OH

**18:** R<sub>1</sub>, R<sub>2</sub>, R<sub>4</sub> = H; R<sub>3</sub> = OH

**19:** R<sub>1</sub>, R<sub>2</sub> = H; R<sub>3</sub>, R<sub>4</sub> = OH

**Scheme 2:** Synthesis of compounds 16-19.

### 3.2 Biological profile

Compounds **9-19** were subjected for their antibacterial and antifungal activities by disc diffusion method against both Gram +ve & Gram -ve bacteria and fungi in comparison with reference samples ampicillin and nystatin respectively. The antioxidant activity by radical scavenging assay was studied by DPPH (1, 1-diphenyl-2-picrylhydrazyl) and ABTS [(2, 2'-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid)] methods and the results were reported as IC<sub>50</sub> value. The anticancer activity was studied using three human cancer cell lines PC-3 (prostate cancer), MCF 7 (breast cancer) and HeLa (cervical cancer) by MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] assay.

#### 3.2.1 Antibacterial activity

The *in vitro* antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli* is summarized in Table 2. Compounds **11**, **16**, **17**, **18** and **19** showed activity mainly against *P. aeruginosa* matching with that of ampicillin (MIC = 50 µg/mL) but inactive against Gram +ve bacteria which is quite unique.

**Table 2:** Antibacterial Activity of Compounds 9-19 Expressed as MIC (µg/mL)

Compound	Gram +ve		Gram -ve	
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>P. aeruginosa</i>	<i>E. coli</i>
<b>9</b>	—	—	—	—
<b>10</b>	—	—	—	—
<b>11</b>	—	—	50	—
<b>12</b>	—	—	—	—
<b>13</b>	—	—	—	—
<b>14</b>	—	—	—	—
<b>15</b>	—	—	—	—
<b>16</b>	—	—	50	—
<b>17</b>	100	100	50	200
<b>18</b>	—	100	50	50
<b>19</b>	200	—	50	100
Ampicillin	3.1	3.1	50	3.1

(—) means no activity up to 400 µg/mL

#### 3.2.2 Antifungal activity

The *in vitro* antifungal activity against *Aspergillus niger*, *Mucor indicus* and *Candida albicans* using nystatin as a positive control was evaluated and the values are indicated in Table 3. The MIC (µg/mL) values of compounds showed that, compounds **14**, **15** and **19** exhibited better inhibitory activity against *Mucor indicus* (MIC = 25 µg/mL) while compounds **11**, **13** and **14** showed activity similar to nystatin against *Candida albicans*.

**Table 3:** Antifungal Activity of Compounds 9-19 Expressed as MIC (µg/mL)

Compound	<i>M. indicus</i>	<i>A. niger</i>	<i>C. albicans</i>
<b>9</b>	200	50	6.2
<b>10</b>	50	200	6.2
<b>11</b>	100	—	3.1
<b>12</b>	100	100	25
<b>13</b>	200	—	3.1
<b>14</b>	25	200	3.1
<b>15</b>	25	200	50
<b>16</b>	—	—	6.2
<b>17</b>	—	—	6.2
<b>18</b>	100	—	25
<b>19</b>	25	—	6.2
Nystatin	25	25	3.1

(—) means no activity up to 400 µg/mL

#### 3.2.3 Antioxidant activity

Antioxidant activity of all the compounds was performed by DPPH method (Table 4) taking L-ascorbic acid and resveratrol as standards. As it can be seen from the table, compounds **9-15** displayed poor antioxidant activity whereas phenolic compounds **16** and **18** exhibited moderate antioxidant activity. However, compounds **17** and **19** showed very significant radical scavenging activity comparable to that of L-ascorbic acid.

**Table 4:** Dpph Radical Scavenging Activity of Compounds 9-19

Compound	IC <sub>50</sub> ± SD* (µg/mL)	Compound	IC <sub>50</sub> ± SD* (µg/mL)
<b>9</b>	5243.05 ± 63.12	<b>16</b>	227.02 ± 9.04
<b>10</b>	5194.17 ± 38.25	<b>17</b>	13.54 ± 1.08
<b>11</b>	5775.28 ± 12.47	<b>18</b>	481.12 ± 5.52
<b>12</b>	5651.76 ± 40.21	<b>19</b>	9.77 ± 1.18
<b>13</b>	5578.13 ± 26.28	Resveratrol	54.38 ± 1.84
<b>14</b>	5348 ± 7.84	L-Ascorbic acid	5.48 ± 0.14
<b>15</b>	5381.74 ± 43.72		

\* Standard deviation obtained from three separate experiments in triplicate.

Similarly, the results from ABTS radical scavenging assay (Table 5) indicated that all the compounds except phenolic derivatives **16-19** were inactive when compared to standard BHT (2, 6-ditertiary butyl-p-cresol) and resveratrol. Compounds **16** and **18** showed moderate activity while compounds **17** and **19** exhibited very significant activity and would constitute good antioxidant molecules.

**Table 5:** DPPH Radical Scavenging Activity of Compounds 9-19

Compound	IC <sub>50</sub> ± SD* (µg/mL)	Compound	IC <sub>50</sub> ± SD* (µg/mL)
<b>9</b>	12399.25 ± 43.12	<b>16</b>	2.42 ± 0.03
<b>10</b>	7208.90 ± 58.25	<b>17</b>	1.97 ± 0.02
<b>11</b>	4230.62 ± 23.97	<b>18</b>	4.84 ± 0.02
<b>12</b>	3559.77 ± 11.31	<b>19</b>	1.14 ± 0.02
<b>13</b>	4172.73 ± 4.77	Resveratrol	1.18 ± 0.04
<b>14</b>	4165.21 ± 3.68	BHT	1.71 ± 0.09
<b>15</b>	4163.57 ± 3.72		

\* Standard deviation obtained from three separate experiments in triplicate.

**Table 6:** Anticancer Activity of Compounds 9-19.

Compound	IC <sub>50</sub> ± SD* of Stilbene compounds (µM)		
	PC-3	MCF-7	HeLa
<b>9</b>	—	64.06 ± 16.6	107.62±11.23
<b>10</b>	—	—	—
<b>11</b>	—	—	—
<b>12</b>	—	97.57 ± 10.23	—
<b>13</b>	—	51.08 ± 9.72	—
<b>14</b>	—	—	—
<b>15</b>	—	—	—
<b>16</b>	—	—	—
<b>17</b>	133.97 ± 15.24	35.57 ± 15.87	82.5±12.95
<b>18</b>	—	—	—
<b>19</b>	12.38 ± 6.83	14.52 ± 2.21	81.82±12.72
Resveratrol	—	—	—
CPT	7.40 ± 6.32	12.14 ± 7.6	15.47±6.05

(—) means not effective up to 150 µM

\* Standard deviation obtained from three separate experiments in triplicate.

In summary, we have synthesized a series of (*E*)-stilbene derivatives with alpha bromo-pyridine moiety which were evaluated for their antimicrobial, antioxidant and anticancer potentials. Compounds **11** and **16-19** though did not show any activity against Gram+ve organisms but showed good inhibitory activity against *P. aeruginosa* comparable to ampicillin. As far as antifungal activity is concerned, compounds **14**, **15** and **19** showed very significant activity against *M. indicus*. On the other hand compounds **17** and **19** showed antioxidant activity similar to that of L-ascorbic acid. Similarly, the anticancer screening results indicated that the compound **19** was more potent against PC-3 and MCF 7 cancer cell lines. Of all the compounds, compound **19** (3-Bromo-3', 4'-dihydroxy stilbene-2-nitrogen) is found to show a wide range of biological profile which will definitely form a lead molecule for further study.

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#### 3.2.4 Anticancer activity

The anticancer activity of compounds **9-19** was evaluated using three human cancer cell lines PC-3 (prostatic cancer), MCF 7 (breast cancer) and HeLa (cervical cancer) by MTT assay with camptothecin (CPT) taken as a positive control and resveratrol as a reference sample (Table 6). The result from the table indicated that, compounds (**17**) and (**19**) exhibited good anticancer activity against all the 3 cell lines but **19** showed activity very similar to camptothecin against PC-3 and MCF cell lines.

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