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Medicinal Chemistry

Synthesis and Anticancer Activity of Some New Stilbene Derivatives

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Original Research Article

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Abstract: The present study was aimed to synthesize derivatives of cis stilbene and evaluate for their anti-cancer activities. Several cis stilbenes with substitutions on the olefinic carbon were synthesized and characterized by IR, NMR, and MASS spectra. Some compounds from each series were evaluated for their cytotoxicity against MCF-7 and HeLa cell lines. Among all the compounds, compound 4c with methyl ester group on olefinic carbon exhibited potent activity against MCF-7 and HeLa cell lines with IC_{50} values 22.24µM and 27.43µM respectively.
Keywords: stilbene, olefine, cytotoxicity.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and a large number of drugs have been isolated from natural sources or derived from natural product molecules, especially in cancer therapy. Stilbenes have been recognized as privileged molecules in view of their wide range of biological activities. Stilbene (E-stilbene, 1 or Z-stilbene, 2) (fig 1), itself does not occur in nature, but hydroxylated stilbenes have been found in many medicinal plants. One such example is the trans-3, 5, 4'-trihydroxystilbene (resveratrol), a phytoalexin present in grapes [1-7] and plays a role in the prevention of coronary artery disease associated with red wine consumption [8-11].

Stilbene derivatives are produced by several plants in response to pathogen attacks, and found to regulate many biological functions.

They have been shown to possess antifungul [12], antibacterial [12], cytotoxic [13], antiinflammatory [14] and anticonvulsant [15] activities. Some of them, such as resveratrol, reported to exhibit potent antioxidant activity [16], modulate the synthesis of lipids, inhibit ribonucleotide reductase, and DNA polymerase, increase the activity of Map-kinase, an enzyme potentially related to neuro degenerative diseases such as Alzheimer's and Parkinson's [17] and few stilbene derivatives found to inhibit platelet aggregation [18].

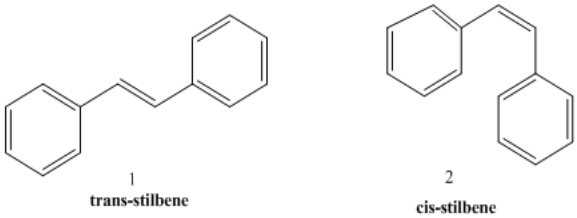
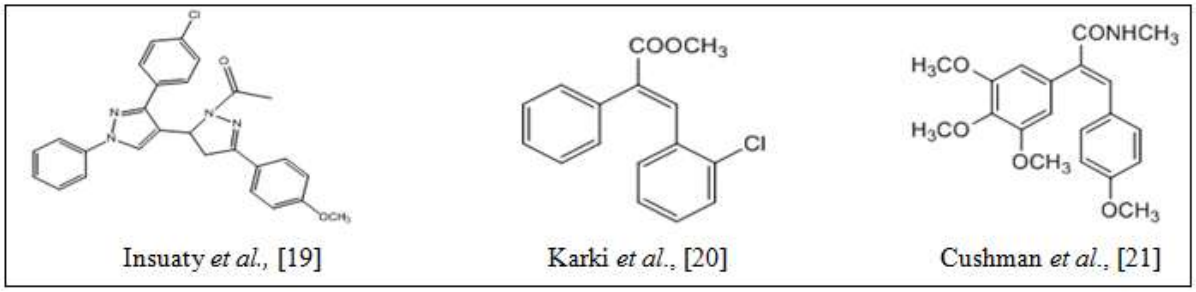


Fig-1: Structures of Stilbenes

Literature survey reveals that diaryl pyrazoles [19] and stilbenes with methoxy carbonyl group [20] were found to possess significant anticancer activity. Hence in the present study, it is proposed to synthesize stilbenes possessing 1,4-diaryl pyrazole, methoxy carbonyl group on unsaturated system of stilbenes, and also their corresponding amide derivatives. It is also proposed to synthesize derivatives with various heteryl groups replacing the pyrazole moiety. Selected synthesized compounds were evaluated for their anticancer activity.



Insuaty *et al.*, [19] Karki *et al.*, [20] Cushman *et al.*, [21]

Fig-2: Biologically active substituted stilbene and pyrazole derivatives

MATERIALS AND METHODS

All chemicals and solvents were purchased from commercial sources (Sigma Aldrich, Hymedia and sd fine) used without further purification. All compounds were characterized by spectroscopic data and compared with the data available in the literature. The NMR spectra were recorded in DMSO-d_6 or $CDCl_3$. ^{1}H NMR spectra were obtained on a Bruker Advance 3400 (1H: 400 MHz). The chemical shifts were expressed in values parts per million (ppm scale) and the J values were reported in Hertz (Hz). The peak patterns were indicated as follows: s, singlet; d, doublet; t, triplet; m, multiplet. The reactions were monitored by Thin Layer Chromatography (TLC) using silica gel 60 F254 plates (Merck). The melting points were determined on a Stuart SMP3 melting point apparatus. Elemental analyses were performed on Elementar vario MICRO CHNS Analyser.

3-(4,5-Dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT), DMEM (Dulbecco's modified Eagles medium), penicillin, streptomycin, trypsin, EDTA and Phosphate Buffered Saline (PBS) were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Fetal Bovine Serum (FBS) was bought from Gibco. 25 cm^2 and 75 cm^2 flask and 96 well plated purchased from Eppendorf India. All other reagents were of analytical grade. MCF-7 (Breast adenocarcinoma cancer cell line) and HeLa (Human Cervical Carcinoma Cell line) were purchased from NCCS, Pune.

EXPERIMENTAL

General Procedure for the Preparation of Compounds 3a-e

A mixture of phenylacetic acid 2a-b (2 mmol), formyl hetero cyclic precursors 1a-d (2 mmol), and triethylamine (0.5 ml) in acetic anhydride (5 ml) was heated at reflux for 12 h, poured into hot saturated sodium carbonate solution (50 ml), and left overnight. The mixture was extracted with ether (2 X 50 ml), and the ether extracts were discarded. The aqueous solution was acidified with dilute HCl, and the precipitated product was filtered and dried recrystallization from EtOAc-hexane gave pure products, 3a-e [21]. The precursors 1c and 1d were inturn prepared from the corresponding acetophenones and phenylhydrazine by adopting the procedure reported in literature [22]

General Procedure for the Preparation of Compounds 4a-d

Conc H_2SO_4 (0.5ml) was added to a stirred solution of carboxylic acid 3 (172mg, 0.5 mmol) in absolute methanol (20 ml), and the mixture was heated under reflux for 6 h. About 90% of the excess methanol was removed by evaporation, and the residue was poured into ice water (300 ml). The product was extracted with ether (2 x 40 ml), and the combined extracts were washed with 2% aqueous NaOH solution (2 X 50 ml) followed by water (200 ml). Evaporation of ether from the dried (Na_2SO_4) solution gave the desired products, 4a-d [21].

General Procedure for the Preparation of Compounds 5c-d

A mixture of carboxylic acid **3** (172 mg, 0.5 mmol) and thionyl chloride (1 mL) in benzene (10mL) was refluxed for 6 h. The excess thionyl chloride and benzene were removed at reduced pressure, and the resulting residue was kept under vacuum for 30 min. It was subsequently mixed with aqueous methylamine solution (40%, 5 mL) and kept at room temperature for 2 h. The precipitated product was filtered, washed sequentially with 2% NaOH solution and water, and dried. The product was purified by recrystallization from EtOAc-hexane [21].

General Procedure for the Preparation of Compounds $5c_1$-$5d_1$

A mixture of compound **3** (0.5mm) and thionyl chloride (1 mL) in benzene (10mL) was refluxed for 6 h. The excess thionyl chloride and benzene were removed at reduced pressure, and the resulting residue was kept under vacuum for 30 min. Then it was treated with a solution of ethylamine (0.5 mL) in THF (5 mL)**.** The mixture was stirred for 3 h. Solvent were removed at reduced pressure, and the residue was poured onto ice (200g). The product was extracted with ether (2 X 20 mL), washed with water, and dried (Na_2SO_4). Evaporation of ether gave crude product which was purified by column chromatography on silica gel using ether as the eluent [21].

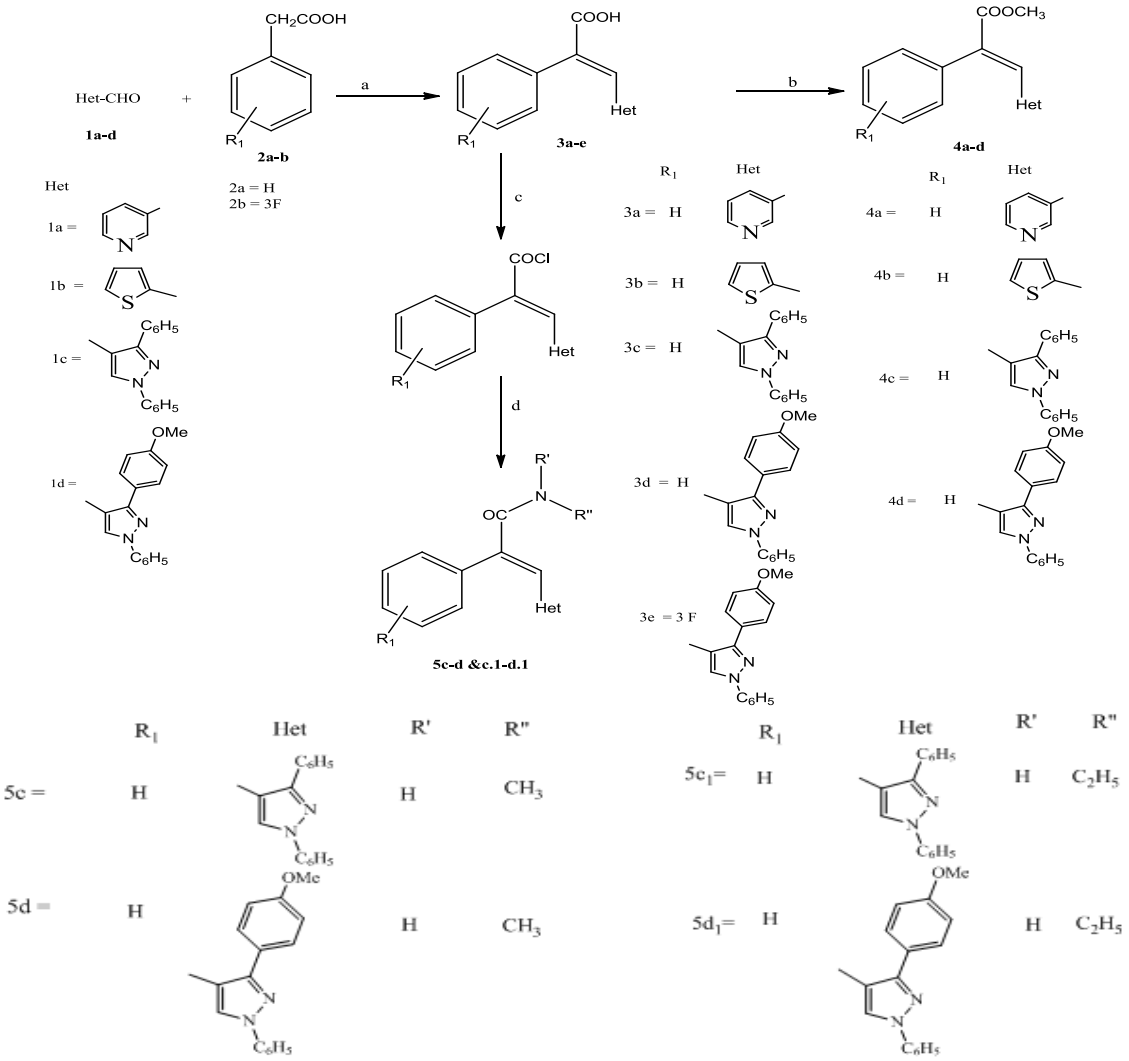
Biological assays

Cell culture

The cell lines (MCF-7 and HeLa) were maintained in culture with MEM supplemented with 10 % Fetal bovine serum (FBS) and the antibiotics penicillin/streptomycin ($0.5mL^{-1}$), in atmosphere of 5% CO_2 and 95% air at 37°C. Stock solutions of synthesized stilbene derivatives (3a, 3c & 3d, 4a-c, 5c-d & $5c_1$-$5d_1$) were made in DMSO and kept in aliquots at -20 °C. For MTT assay, each test compound was weighed separately and dissolved in DMSO, made up the final concentration with media to 1 mg/ ml and the cells were treated with series of concentrations from 10 to 100 µm of test compounds (3a, 3c & 3d, 4a-c, 5c-d & $5c_1$-$5d_1$).

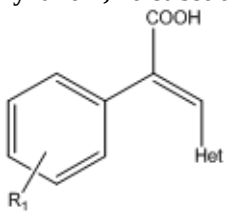
MTT assay

Inhibition of cell proliferation by cis stilbene derivatives were determined using the methyl thiazolyltetrazolium (MTT) Cell viability assay with three independent experiments with six concentrations of compounds in triplicates. MCF-7 and HeLa cells were trypsinized and performed the tryphan blue assay to know viable cells in cell suspension. Cells were counted by haemocytometer and seeded at density of 5.0 X 10^3 cells / well in 100 µl media in 96 well plate culture medium and incubated overnight at 37 °C. After incubation, the old media was taken off and added with fresh media 100 µl with different concentrations of test compound in respective wells in 96 plates. After 48 hrs, the drug solution discarded and the fresh media with MTT solution (0.5 mg/ml) was added to each well and plates were incubated at 37 °C for 3 hrs. At the end of incubation time, precipitates are formed as a result of the reduction of the MTT salt to chromophore formazan crystals by the cells with metabolically active mitochondria. The optical density of solubilized crystals in DMSO was measured at 570 nm on a microplate reader. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50 % (IC_{50}values) is generated from the dose-response curves for each cell line [23].



Scheme 1: Synthesis of new Stilbene derivatives **3a-e**, **4a-d**, **5c-d and $5c_1$-$5d_1$;** reagents and conditions: **a.** triethyl amine, acetic anhydride **b.** H_2SO_4, MeOH **c.** $SOCl_2$, benzene **d.** amines, 2h, rt.

Table-1: Physical data of heteryl and 1,4 disubstituted pyrazolyl stilbenes (3a-e)



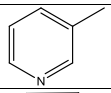
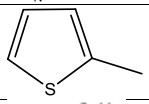
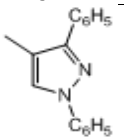
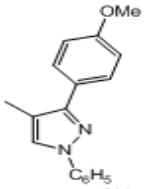
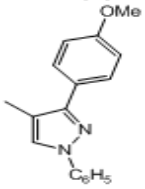
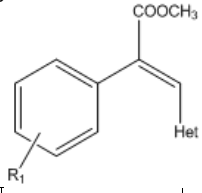
CODE	Het	R_1	Mol Formula	Mol Weight	M.P. (^{0}C)	% yield
3a		H	$C_{14}H_{11}NO_2$	225	139-141	60%
3b		H	$C_{13}H_{10}O_2S$	230	153-155	55%
3c		-H	$C_{24}H_{18}N_2O_2$	366	184-186	61%
3d		-H	$C_{25}H_{20}N_2O_3$	396	160-162	57%
3e		-3F	$C_{25}H_{19}FN_2O_3$	414	166-168	54%

Table-2: Physical data of heteryl and 1,4 disubstituted pyrazolyl stilbenes (4a-d)



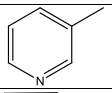
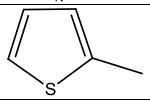
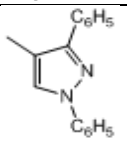
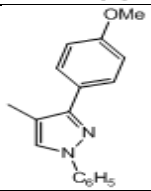
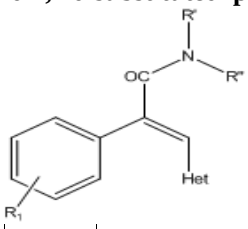
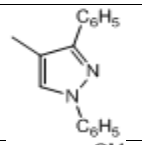
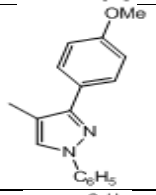
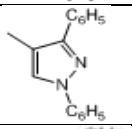
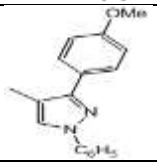
CODE	Het	R_1	Mol Formula	Mol Weight	M.P. (^{0}C)	% yield
4a		-H	$C_{15}H_{13}NO_2$	239	103-105	55%
4b		-H	$C_{14}H_{12}O_2S$	244	118-120	62%
4c		-H	$C_{25}H_{20}N_2O_2$	380	114-1116	69%
4d		-H	$C_{26}H_{22}N_2O_3$	410	110-112	51%

Table-3: Physical data of heteryl and 1,4 disubstituted pyrazolyl stilbenes (5c-d & $5c_1$-$5d_1$)



CODE	Het	R_1	R^I	R^{II}	Mol Formula	Mol Weight	M.P. (^{0}C)	% yield
5c		-H	-H	$-CH_3$	$C_{25}H_{21}N_3O$	379	108-109	70%
5d		-H	-H	$-CH_3$	$C_{26}H_{23}N_3O_2$	409	101-103	64%
$5c_1$		-H	-H	$-C_2H_5$	$C_{26}H_{23}N_3O$	393	90-93	56%
$5d_1$		-H	-H	$-C_2H_5$	$C_{27}H_{25}N_3O_2$	423	98-100	52%

Characterization data

(E)-2-phenyl-3-(pyridin-3-yl) acrylic acid (3a)

R_f = 0.43 [hexane: ethylacetate, 8:2] IR (cm^{-1}): 3350 OH, 1560 (olefinic C=C), 1678 –C=O; ^{1}H NMR at δ ppm: 7.45-7.42 (m, 2H, aromatic), 7.64-7.59 (m, 3H, aromatic), 7.80 (s, 1H, olefinic CH), 8.00-8.07 (m, 4H, aromatic), 9.63 (s, 1H, -OH); Elemental analysis: calculated for $C_{14}H_{11}NO_2$ (225); calculated; C, 74.65; H, 4.92; N, 6.22 found C, 74.62; H, 4.94; N, 6.18; ESI MS (m/z): 226 [M+1].

(E)-2-phenyl-3-(thiophen-2-yl) acrylic acid (3b)

R_f = 0.49 [hexane: ethylacetate, 8:2] IR (cm^{-1}): 3338 (OH), 1568 (olefinic C=C), 1676 -C=O ; ^{1}H NMR at δ ppm: 6.97-6.94 (t, *J* = 7.2Hz, 1H, aromatic), 7.05-7.03 (d, *J* = 8.4Hz, 1H, aromatic), 7.14-7.11 (m, 1H, aromatic), 7.43-7.42 (d, *J* = 2.8Hz, 1H, aromatic), 7.52-7.47 (m, 3H, aromatic), 7.77 (s, 1H, olefinic CH), 7.85-7.84 (d, *J* = 7.6Hz, 1H, aromatic), 9.64 (s, 1H, OH); Elemental analysis: calculated for $C_{13}H_{10}O_2S$ (230); calculated; C, 67.80; H, 4.38 found C, 67.83; H, 4.36 ESI MS (m/z): 231 [M+1].

(E)-3-(1,4-diphenyl-1H-pyrazol-3-yl)-2-phenylacrylic acid (3c)

R_f = 0.63[hexane: ethylacetate, 7:3] IR (cm^{-1})**:** 3290 (OH), 1683(C=O), 1547 (olefinic C=C). ^{1}H NMR ($CDCl_3$) δ (ppm): 7.66-7.64 (d, *J* = 6.8Hz, 6H, aromatic), 7.74 (s, 1H, olefinic CH), 7.83-7.79 (m, 6H, aromatic), 7.90-7.87 (t, *J* = 7.4Hz, 3H, aromatic), 8.55 (s, 1H, C_3-pyrazole), 9.71 (s, 1H, -OH). Elemental analysis: calculated for $C_{24}H_{18}N_2O_2$ (366); calculated; C, 78.67; H, 4.95; N, 7.65; found: C, 78.65; H, 4.93; N, 7.67; ESI MS (m/z): 367 [M+1].

3.6.4 (E)-3-(1-(4-methoxyphenyl)-4-phenyl-1H-pyrazol-3-yl)-2-phenylacrylic acid (3d)

R_f = 0.58 [hexane: ethylacetate, 7:3] IR (cm^{-1}): 3300 (OH), 1685 (C=O), 1558 (olefinic C=C). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.86 (s, 3H, $-OCH_3$), 7.04-7.02 (d, *J* = *8.4*Hz, 2H, aromatic), 7.40-7.34 (m, 2H, aromatic), 7.52-7.43 (m, 4H, aromatic), 7.67-7.65 (d, *J* = 8.4, 2H, aromatic), 7.80-7.78 (d, *J* =*9.2* Hz, 4H, aromatic), 7.85 (s,1H, olefinic CH), 8.40 (s, 1H, C_3-pyrazole), 9.72 (s, 1H, -OH). Elemental analysis: calculated for $C_{25}H_{20}N_2O_3$ (396); calculated; C, 75.74; H, 5.08; N, 7.07; found: C, 75.76; H, 5.10; N, 7.05; ESI MS (m/z): 397 [M+1].

(E)-2-(3-fluorophenyl)-3-(1-(4-methoxyphenyl)-4-phenyl-1H-pyrazol-3-yl) acrylic acid (3e)

R_f = 0.55 [hexane: ethylacetate, 8:2] IR (cm^{-1})**:** 3350 (OH), 1679 (C=O), 1560 (olefinic C=C). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.60 (s, 3H, $-OCH_3$), 7.11-7.09 (d, *J* = *4.8*Hz, 1H, aromatic), 7.33-7.29 (m, 3H, aromatic), 7.64-7.38 (m, 7H, aromatic), 7.72-7.66 (m, 2H, aromatic), 7.84 (s,1H, olefinic CH), 8.54 (s, 1H, C_3-pyrazole), 9.74 (s, 1H, -OH). Elemental analysis:

calculated for $C_{25}H_{19}FN_2O_3$ (414); calculated C, 72.45; H, 4.62; N, 6.76; found: C, 72.43; H, 4.64; N, 6.76 ESI MS (m/z): 416 [M+2].

(E)-methyl 2-phenyl-3-(pyridine-3-yl) acrylate (4a)

R_f = 0.59 [hexane: ethylacetate, 8:2] IR (cm^{-1}): 1675 (C=O), 1570 (olefinic C=C); ^{1}H NMR at δ ppm: 3.64 (s, 3H, -OCH_3), 7.05-6.84 (m, 3H, aromatic), 7.34-7.36 (t, *J* = 3.5Hz, 1H, aromatic), 7.48-7.46 (d, *J* = 8.8Hz, 1H, aromatic), 7.64-7.62 (d, *J* = 4.6Hz, 1H, aromatic), 7.76-7.74 (d, *J* = 8.8Hz, 1H, aromatic), 7.84 (s, 1H, olefinic CH), 8.02-8.01 (d, *J* = 2.56Hz, 1H, aromatic), 8.34 (s, 1H, C_3-pyrazole); Elemental analysis: calculated for $C_{15}H_{13}NO_2$ (239); calculated; C, 75.30; H, 5.48; N, 5.85 found C, 75.32; H, 5.46; N, 5.87; ESI MS (m/z): 240[M+1].

(E)-methyl 2-phenyl-3-(thiophen-2-yl) acrylate (4b)

R_f = 0.48 [hexane: ethylacetate, 8:2] IR (cm^{-1}): 1681 (C=O), 1551 (olefinic C=C): ^{1}H NMR at δ ppm: 3.68 (s, 3H, -OCH_3), 7.45-7.40 (t, *J* = 7.5Hz, 2H aromatic), 7.73-7.56 (m, 3H, aromatic), 7.80 (s, 1H, olefinic CH), 7.84-7.90 (t, *J* = 5.2Hz, 1H, aromatic), 8.02-8.06 (d, *J* = 7.94Hz, 2H, aromatic); Elemental analysis: calculated for $C_{14}H_{12}O_2S$ (244); calculated; C, 68.83; H, 4.95; found C, 68.81; H, 4.92; ESI MS (m/z): 245 [M+1].

(E)-methyl 3-(1,4-diphenyl-1H-pyrazol-3-yl)-2-phenylacrylate (4c)

R_f = 0.65 [hexane: ethylacetate, 8:2] IR (cm^{-1}): 1678 (C=O); 1562 (olefinic C=C). ^{1}H NMR at δ ppm: 3.60 (s, 3H, -OCH_3), 7.40-7.34 (m, 4H, aromatic), 7.57-7.47 (m, 6H, aromatic), 7.72-7.70 (m, 2H, aromatic), 7.85-7.82 (m, 3H, aromatic), 7.93 (s, 1H, olefinic CH), 8.43 (s, 1H, C_3-pyrazole); Elemental analysis: calculated for $C_{25}H_{20}N_2O_2$ (380); calculated; C, 78.93; H, 5.30; N, 7.36 found: C, 78.91; H, 5.28; N, 7.38; ESI MS (m/z): 381 [M+1].

(E)-methyl 3-(1-(4-methoxyphenyl)-4-phenyl-1H-pyrazol-3-yl)-2-phenylacrylate (4d)

R_f = 0.55 [hexane: ethylacetate, 7:3] IR (cm^{-1}): (1685 – C=O), 1566 (olefinic C=C).; ^{1}H NMR at δ ppm: 3.76 (s, 3H, -OCH_3), 3.87 (s, 3H, -OCH_3), 7.03-7.02 (d, *J* = 2Hz, 2H, aromatic), 7.37-7.31 (m, 4H, aromatic), 7.52-7.49 (m, 5H, aromatic), 7.64-7.62 (d, *J* = 6.8Hz, 1H, aromatic), 7.72 (s, 1H, olefinic CH), 7.80-7.78 (d, *J* = 7.2Hz, 2H, aromatic), 8.52 (s, 1H, C_3-pyrazole); Elemental analysis: calculated for $C_{26}H_{22}N_2O_3$ (410); calculated; C, 76.08; H, 5.40; N, 6.82; found: C, 76.10; H, 5.38; N, 6.80; ESI MS (m/z): 411 [M+1].

(E)-3-(1,4-diphenyl-1H-pyrazol-3-yl)-N-methyl-2-phenylacrylamide (5c)

R_f = 0.71 [hexane: ethylacetate, 8:2] IR (cm^{-1}): 3259 – NH, 1684 (C=O), 1571 (olefinic C=C): ^{1}H NMR at δ ppm: 2.62-261 (d, *J* = 5.2Hz, 3H, -CH_3), 5.60 (bs, 1H, -NH), 7.41-7.38 (m, 3H, aromatic), 7.53-7.46 (m, 6H, aromatic), 7.70 (s, H, olefinic CH), 7.83-7.79 (m, 6H, aromatic), 8.55 (s, 1H, C_3-pyrazole); Elemental analysis: calculated for $C_{25}H_{21}N_3O$ (379); calculated; C, 79.13; H, 5.58; N, 11.07 found C, 79.11; H, 5.60; N, 11.05; ESI MS (m/z): 380 [M+1].

(E)-3-(1-(4-methoxyphenyl-4-phenyl-1H-pyrazol-3-yl)-N-methyl-2-phenylacrylamide (5d)

R_f = 0.60 [hexane: ethylacetate, 8:2] IR (cm^{-1}): 3260 – NH, 1685 (C=O), 1568 (olefinic C=C). ^{1}H NMR at δ ppm:. 2.35-2.34 (d, *J* = 5.4Hz, 3H, -CH_3,), 3.87 (s, 3H, -OCH_3), 5.7 (bs, 1H, NH), 7.05-7.02 (m, 2H, aromatic), 7.40-7.33 (m, 5H, aromatic), 7.52-7.44 (m, 3H, aromatic), 7.62-7.60 (d, *J* = 8.4 Hz, 2H, aromatic), 7.72 (s, 1H, olefinic CH), 7.80-7-781 (d, *J* = 8.8 Hz, 2H, aromatic), 8.54 (s, 1H, C_3-pyrazole); Elemental analysis: calculated for $C_{26}H_{23}N_3O_2$ (409); calculated; C, 79.13; H, 5.58; N, 11.07 found C, 79.11; H, 5.60; N, 11.05; ESI MS (m/z): 410 [M+1].

(E)-3-(1,4-diphenyl-1H-pyrazol-3-yl)-N-ethyl-2-phenylacrylamide ($5c_1$)

R_f = 0.64 [hexane: ethylacetate, 7:3] IR (cm^{-1}): 3280 – NH, 1682 (C=O), 1559 (olefinic C=C). ^{1}H NMR at δ ppm: 1.24-1.20 (t, *J* = 7.4Hz, 3H, -CH_3), 3.47-3.40 (q, 2H, -CH_2), 5.49 (bt, 1H, -NH), 7.43-7.39 (m, 3H, aromatic), 7.57-7.45 (m, 7H, aromatic), 7.83-7.79 (m, 5H, aromatic), 7.92 (s, 1H, olefinic CH), 8.52 (s, 1H, C_3-pyrazole); Elemental analysis: calculated for $C_{26}H_{23}N_3O$ (393); calculated; C, 79.36; H, 5.89; N, 10.68 found C, 79.34; H, 5.91; N, 10.70; ESI MS (m/z): 394[M+1].

(E)-N-ethyl-3-(1-(4-methoxyphenyl-4-phenyl-1H-pyrazol-3-yl)-N-methyl-2-phenylacrylamide ($5d_1$)

R_f = 0.68 [hexane: ethylacetate, 7:3] IR (cm^{-1}): 3280 – NH, 1685 (C=O), 3079 –CH aromatic, 2959 –CH aliphatic, 1674 (C=O), 1564. (olefinic C=C). ^{1}H NMR at δ ppm:1.10-1.06 (t, *J* = 7.1 Hz, 3H, -CH_3), 3.37-3.30 (q, 2H, -CH_2), 3.82 (s, 3H, -OCH_3), 5.44 (bt, IH, -NH), 7.03-6.98 (m, 3H), 7.39-7.32 (m, 4H), 7.58-7.43 (m, 4H), 7.65-7.61 (m, 1H, aromatic), 7.77 (s, 1H, olefenic CH), 7.80-7.78 (d, *J* = 8.4Hz, 2H, aromatic), 8.52 (s, 1H, C_3- pyrazole); Elemental analysis: calculated for $C_{27}H_{25}N_3O_2$ (423); calculated; C, 76.57; H, 5.95; N, 9.92 found C, 76.58; H, 5.92; N, 9.90; ESI MS (m/z): 424 [M+1].

RESULTS AND DISCUSSIONS

Chemistry

All the stilbene derivatives were synthesized by base-catalyzed condensation of phenylacetic acids 2a-b with hetero aryl aldehydes 1a-d in the presence of triethylamine gave the carboxylic acids 3a-e. Esterification of compounds 3 with methanol using a catalytic amount of H_2SO_4 resulted in the corresponding esters 4a-d. Reaction of thionyl chloride with all the carboxylic acids except 3a, 3b and 3e in refluxing benzene gave the corresponding acid chlorides, which on subsequent reaction with appropriate amines, gave

compounds 5c-d & $5c_1$-$5d_1$. Physical data of the compounds is presented in tables 1, 2 and 3.

The IR spectra of stilbene derivatives 3a-e, 4a-d, 5c-d & $5c_1$-$5d_1$ showed carbonyl absorption in the range of 1674-1685 cm^{-1} and an olefinic C=C band in the range 1540-1575 cm^{-1}. OH absorption band of carboxylic acid in 3a-e was observed between 3290-3350 cm^{-1} and NH absorption band in compounds 5c-d & $5c_1$-$5d_1$ was seen in the rage of 3244-3289 cm^{-1}. In the ^1H NMR spectra of 3a-e, OH peak appeared as singlet in the range of δ 9.6-9.8, amide NH proton in 5c-d & $5c_1$-$5d_1$ appeared as broad singlet in the range of δ 5-6. Olefine moiety of stilbenes was confirmed by the presence of a singlet in the range of δ 7.70-7.90, indicating formation of stilbenes. The parent ion peak appeared on the positive mode in the mass spectrum of all the compounds further confirms the structure of stilbenes.

Cytotoxicity activity

The cytotoxicity studies of cis stilbene derivatives (3a, 3c & 3d, 4a-c, 5c-d & $5c_1$-$5d_1$) with substitution on the olefin bridge connecting the two phenyl rings were evaluated on two human cancer cell lines namely MCF-7, HeLa using MTT assay *in vitro* [23]. Semi log plot of concentration of compounds versus % inhibition of cell lines corroborated the determination of IC_{50} values of compounds using Microsoft Excel figure 3. The cytotoxicity of all the tested compounds were compared against Cisplatin as standard compound which showed cytotoxic activity with IC_{50} value of 4.15 µM against MCF-7 and 6.05 µM against HeLa cell lines and the results of anticancer activity of all tested compounds are summarized in tables 4, 5 and 6.

A COOH group was introduced on position of one of the olefinic linkage and this resulted in the formation of compounds 3a-e. Compound 3c with 1,3-diphenyl pyrazolyl group was found to be more potent against Hela cell lines with IC_{50} value of 41.39 µM, however, when the COOH group of compound 3c was converted to methyl ester 4c, the potency increased by approximately two times with IC_{50} of 27.43 µM against HeLa cell lines. However, in case of MCF-7, the ester 4c was found to be more than three times as potent as the corresponding acid 3c. However when the COOH group of compound 3c and 3d was converted to corresponding N-methyl amides 5c and 5d, potency against MCF-7 cell lines increased and where as the potency against HeLa cell lines decreased. The conversion of the acids, 3c-d to the corresponding ethyl amides $5c_1$-$5d_1$ resulted in little or no change in the potency except that the ethyl derivative $5d_1$ exhibited greater potency (IC_{50} = 31.56 µM) than the corresponding acid 3d (IC_{50} = 57.48 µM).

All the ten compounds tested, except compound 4c showed more potency against Hela cell lines when compared to MCF-7 cell lines. Among all the tested compounds, the compound 4c with methyl ester was found to be the most potent against MCF-7 and HeLa cell lines with IC_{50} values of 22.24µM and 27.43µM respectively.

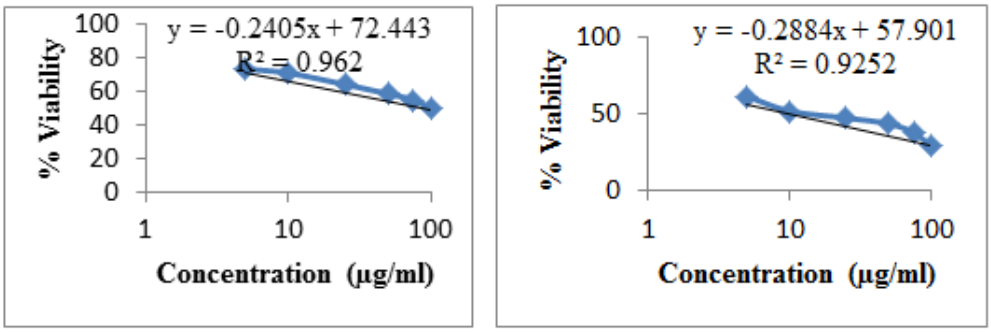
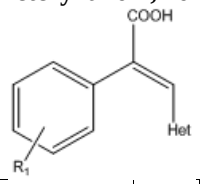


Fig-3: Dose response relationship of 4c in the number of MCF-7 and HeLa vialble cells. The IC_{50} concentration of tested compounds also depicted

Table-4: Anticancer activity of synthesized heteryl and 1,4 disubstituted pyrazolyl stilbenes (IC_{50} in µM)



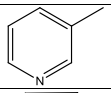
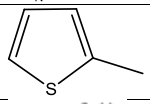
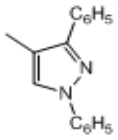
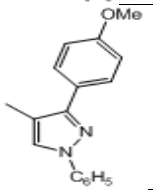
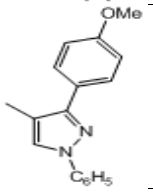
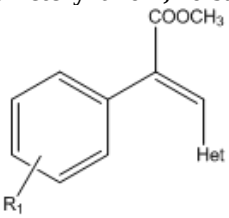
Compound	Het	R1	MCF-7	HeLa
3a		H	64.22	61.40
3b		H	-	-
3c		-H	72.99	41.39
3d		-H	58.38	57.48
3e		-3F	-	-
Cisplatin			4.15	6.05

Table-5: Anticancer activity of synthesized heteryl and 1,4 disubstituted pyrazolyl stilbenes (IC_{50} in µM)



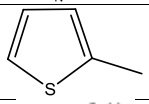
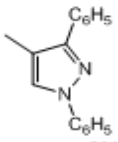
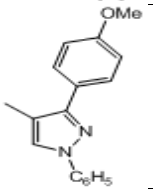
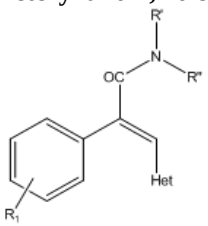
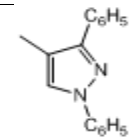
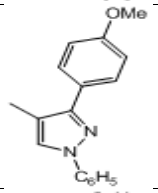
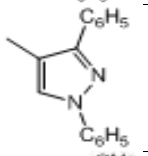
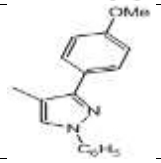
Compound	Het	R1	MCF-7	HeLa
4a		-H	72.99	41.39
4b		-H	-	-
4c		-H	22.24	27.43
4d		-H	82.58	40.28
Cisplatin			4.15	6.05

Table-6: Anticancer activity of synthesized heteryl and 1,4 disubstituted pyrazolyl stilbenes (IC_{50} in µM)



Compound	Het	R_1	R^I	R^{II}	MCF-7	HeLa
5c		-H	-H	$-CH_3$	55.77	47.71
5d		-H	-H	$-CH_3$	54.01	53.81
$5c_1$		-H	-H	$-C_2H_5$	74.62	38.91
$5d_1$		-H	-H	$-C_2H_5$	60.04	31.56
Cisplatin					4.15	6.05

CONCLUSION

The synthesis of heteroaryl stilbenes and their evaluation resulted in identification of a compound **4c** (methyl ester derivative), which showed the highest potency among all the derivatives tested. It could serve as a lead to carry out further studies in order to improve the pharmacokinetic profile.

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