

PERMUTATION AND NATURAL ACTIVITY OF SOME INNOVATIVE STILBENES SIMILARITIES AS ANTIPROLIFERATIVE AGENTS

Pankaj Kumar Jangra,

Research Scholar, Glocal School of Pharmacy,
Glocal University Mirzapur Pole Saharanpur (U.P)

Prof. (Dr.) S.K. Sharma,

Research Supervisor, Glocal School of Pharmacy,
Glocal University Mirzapur Pole Saharanpur (U.P)

Abstract:

Most active compounds of the antiangiogenic screening were further subjected for anticancer activity. Compound 5a showed maximum anticancer activity with % growth inhibition of 63.43 and 56.24 against HCT-116 and MCF-7 cell lines respectively. Further derivatization of these molecules will be helpful in the hopes of obtaining more selective and potent anticancer medicines. As a result, it is necessary to develop new therapeutic strategies for the improvement of presently available medications. There has been a lot of interest in using natural products or their derivatives to produce more effective chemotherapeutic drugs in recent decades. Natural chemicals having a stilbene backbone have shown to have promise anticancer activity in recent years, targeting a wide range of intracellular pathways. Malignant tumours are one of the world's most serious hazards to human health, and the clinical prognosis is still insufficient. In view of that some new stilbene analogues were synthesized and screened them for antiangiogenic activity. Majority of the synthesised compounds exhibited considerable antiangiogenic activity. Antiangiogenic action was discovered to be affected by the size of the bridge substituent (on the Ethylene Bridge).

Keywords: *Stilbenes, Combretastatins, Antiangiogenic, Anticancer.*

Introduction

Although significant improvements in cellular and molecular biology have improved cancer chemotherapeutic therapy, more research into new anticancer drugs is still needed to overcome resistance and toxicity problems. Because natural products are a substantial source of lead compounds with varied mechanisms

of cytostatic action [1], there has been a lot of interest in using natural products or their derivatives to produce more effective chemotherapeutic drugs in recent decades [2]. One of the most widely explored topics today is cancer prevention. Different techniques are required since the pathophysiology of cancer is complicated in so many ways. Natural chemicals having a stilbene backbone have shown to have promise anticancer activity in recent years, targeting a wide range of intracellular pathways [3a-b]. Stilbene is a flexible scaffold with two aromatic rings connected by an ethylene bridge. Some plants create stilbenes as a defence mechanism in reply to pathogen spell and other stresses. Stilbenes are prevalent in natural stuffs [4] and have a extensive array of biological roles [5]. They are classified as Z-type or E-type depending on the arrangement of their middle double bond, which can endure Z/E isomerization, resulting in a change in overall structure and a reduction in biological activity. In reality, photoisomerization is a typical obstacle in optimization research, and much quantum chemistry calculation research has concentrated on the mechanisms behind it [6], rather than optimising these molecules to increase their stability and retain their biological action [7-9]. Stilbene-containing analogues have long piqued the interest of chemists and pharmacologists due to their key biological effects, which include antioxidant, hypolipidemic, anticancer, anti-inflammatory, and antiviral capabilities [14-18]. Among these, resveratrol is one of the most investigated, with anti-proliferative, antioxidant [10a-c], anti-inflammatory, and anticancer properties [11-13] widely described. New stilbene derivatives are created, manufactured, and evaluated on different cellular targets in order to increase cancer chemopreventive and/or therapeutic activity, as well as bioavailability in comparison to the parent medication. The medicinal chemistry community has paid close attention to these potentially novel chemotherapeutic drugs. Many analogues of resveratrol have one or both aromatic rings that have been replaced differently. There are medications in clinical use that have a stilbene center, and numerous cross derivatives have been explored on various biological targets [18a-b]. Combretastatin A-4, a stilbene derivative, has emerged as a promising candidate for powerful in vitro and in vivo anticancer bioactivities throughout the process of discovering chemicals active in the prevention and treatment of cancer [19].

The ethylene bridge of CA-4 is substituted with various groups in some of the produced analogues to test the influence of such change on activity. Apart from the 3,4,5-trimethoxy group, additional groups such as 3,5-dimethoxy, 3,5-dinitro, 3-dimethylamino, and 2,3-dichloro are also tried as substituents on CA-4's rings A and B. CA-4 and other combretastatins have significant anticancer/antivascular activity, which prompted us to manufacture several novel CA-4 analogues and test them for antiangiogenic and anticancer activity in order to uncover new anticancer drugs with potent activity..

Materials and Method**Chemistry**

In the presence of triethylamine, carboxylic acids (3a-d) were produced by base catalysed condensation of o-nitrophenyl acetic acid (2) with corresponding aryl Aldehydes (1a-d). Carboxylic acids (3a-d) were esterified with methanol using a catalytic quantity of H₂SO₄ to produce matching ester derivatives (4a-d). In refluxing benzene, thionyl chloride was reacted with carboxylic acids (3a-d and 8) to provide the corresponding acid chlorides (5a-d), which were then reacted with suitable amine to give the amide derivatives (6a-n).

Recrystallization/column chromatography was used to purify all of the chemicals. TLC was used to assess the purity of the compounds. Infrared, ¹H NMR, and mass spectroscopy were used to confirm the structure of the produced compounds. (Table 1 lists the physical characteristics of the produced compounds, whereas figure 1 shows the synthesis procedure).

Silica gel was used for column chromatography (Qualigens, particle size 60-120 mm). All melting points were calculated using a DECIBEL digital melting point instrument and are displayed as-is. To validate the quality of commercial reagents utilised, compounds generated, and to observe the reactions, silica gel G'sthin layer chromatography (TLC) plates were employed. Table 1 lists the solvent systems used to perform the TLC to check the purity of the compounds produced. Iodine vapours and UV light were used to find the spots. FT-IR spectrophotometers 8400S (SHIMADZU) and SPECTRUM RX1 (PERKIN ELMER) were used to collect IR spectra (KBr Pellets). ¹H NMR spectras were recorded on 300 MHz dpx300 and av300 spectrometers using TMS as internal standard in DMSO. Mass spectras were recorded on an API 3000 LC/MS/MS Q3 (SHIMADZU) spectrometer.

General procedure of preparation of compounds**Procedure of preparation of compounds 3a-d**

A combination of benzaldehyde (1a/1b/1c/1d), p-nitrophenyl acetic acid (2 mmol), and triethylamine (0.5 ml) in acetic anhydride (5 ml) was heated at reflux for 12 hours, then transferred into a warm saturated sodium carbonate mixture (50 ml) and left for 12 hours. The ether extracts (2x50 ml) were used to extract the combination, and the ether extracts were cast-off. The acidified aqueous solution was filtered and dried, and the precipitated product was acidified with dilute HCl. Column chromatography was used to purify the product.

Procedure of preparation of compounds 4a-d

A 0.5 mmol stirred solution of carboxylic acid 3a/3b/3c/3d in absolute methanol (20 ml) was added to the combination, which was then heated in reflux for 6 hours. Evaporation removed around 90% of the surplus methanol, and the rest was placed into frozen water (300 ml). The product was excavated using ether (2x40 ml), and the collective extracts were washed with a 2 percent aqueous NaOH solution (2x50 ml) before being rinsed with water (200 ml). The required product was obtained by evaporating ether.

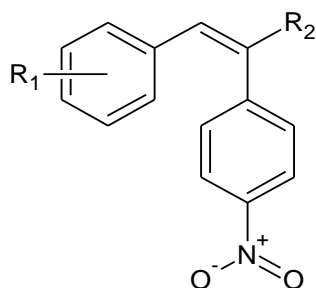
Procedure of preparation of compounds 5a-d

Refluxed for 6 hours was a combination of carboxylic acids 3a, 3b, 3c, 3d (0.5 mmol) and thionyl chloride (1 ml) in benzene (10 ml). Additional benzene and thionyl chloride were separated at decreased pressure, and the remainder was vacuum dried for 30 minutes to get the required product. Recrystallization of EtOAc-hexane purified the product.

Procedure of preparation of compounds 6a-n

A solution of acid chlorides (prepared from 3a/3b/3c/3d in 0.5 mmol scale, as stated above) in THF (5 ml) was poured to a solution of suitable amine (0.5 mmol) in THF (5 ml) (10 ml). For 3 hours, the mixture was mixed. The residue was put onto ice after the solvents were removed at decreased pressure (200 g). The ether (2x20 ml) was used to extract the product, which was then washed and dried. The unfinished product was obtained by evaporating ether. Recrystallization of EtOAc-hexane purified the product.

Table 1 Physical parameters of synthesized compounds



Compound No.	R ₁ and R ₂	M.P.°C	R _f value ^a	Recrystallization solvent	Molecular Formula

3a	R ₁ =3,5-dimethoxy R ₂ = carboxyl	100-102	0.812	EtOAc- Hexane (1:1)	C ₁₇ H ₁₅ NO ₆
4a	R ₁ =3,5-dimethoxy R ₂ = methoxycarbonyl	129-131	0.736	EtOAc- Hexane (1:1)	C ₁₈ H ₁₇ NO ₆
5a	R ₁ =3,5-dimethoxy R ₂ = chlorocarbonyl	117-120	0.803	EtOAc- Hexane (1:1)	C ₁₇ H ₁₄ ClNO ₅
6a	R ₁ =3,5-dimethoxy R ₂ = carbamothioylcarbamoyl	126-130	0.734	EtOAc- Hexane (1:1)	C ₁₈ H ₁₇ N ₃ O ₅ S
6b	R ₁ =3,5-dimethoxy R ₂ =4- fluorophenylcarbamoyl	109-112	0.676	EtOAc- Hexane (1:1)	C ₂₃ H ₁₉ FN ₂ O ₅
6c	R ₁ =3,5-dimethoxy R ₂ = ethylcarbamoyl	119-122	0.609	EtOAc- Hexane (1:1)	C ₁₉ H ₂₀ N ₂ O ₅
6d	R ₁ =3,5-dimethoxy R ₂ =2- methylphenylcarbamoyl	104-106	0.718	EtOAc- Hexane (1:1)	C ₂₄ H ₂₂ N ₂ O ₅
6e	R ₁ =3,5-dimethoxy R ₂ =2- chlorophenylcarbamoyl	81-83	0.689	EtOAc- Hexane (1:1)	C ₂₀ H ₁₉ ClN ₂ O ₅

3b	R ₁ =3-dimethylamino R ₂ = carboxyl	217-219	0.812	EtOAc- Hexane (1:1)	C ₁₇ H ₁₆ N ₂ O ₄
4b	R ₁ =3-dimethylamino R ₂ = methoxycarbonyl	212-214	0.891	EtOAc- Hexane (1:1)	C ₁₈ H ₁₈ N ₂ O ₄
5b	R ₁ =3-dimethylamino R ₂ = chlorocarbonyl	161-164	0.834	EtOAc- Hexane (1:1)	C ₁₇ H ₁₅ ClN ₂ O ₃
6f	R ₁ =3-dimethylamino R ₂ = ethylcarbamoyl	169-171	0.634	EtOAc- Hexane (1:1)	C ₁₉ H ₂₁ N ₃ O ₃
3c	R ₁ =3,5-dinitro R ₂ = carboxyl	87-89	0.311	EtOAc- Hexane (1:1)	C ₁₅ H ₉ N ₃ O ₈
4c	R ₁ =3,5-dinitro R ₂ = methoxycarbonyl	>250	0.782	EtOAc- Hexane (1:1)	C ₁₆ H ₁₁ N ₃ O ₈
5c	R ₁ =3,5-dinitro R ₂ = chlorocarbonyl	95-97	0.822	EtOAc- Hexane (1:1)	C ₁₅ H ₈ ClN ₃ O ₇
6g	R ₁ =3,5-dinitro R ₂ = ethylcarbamoyl	65-67	0.798		C ₁₇ H ₁₄ N ₄ O ₇

				EtOAc- Hexane (1:1)	
3d	R ₁ =2,3-dichloro R ₂ = carboxyl	139-141	0.570	EtOAc- Hexane (1:1)	C ₁₅ H ₉ Cl ₂ NO ₄
4d	R ₁ =2,3-dichloro R ₂ = methoxycarbonyl	>250	0.911	EtOAc- Hexane (1:1)	C ₁₆ H ₁₁ Cl ₂ NO ₄
5d	R ₁ =2,3-dichloro R ₂ = chlorocarbonyl	165-168	0.767	EtOAc- Hexane (1:1)	C ₁₅ H ₈ Cl ₃ NO ₃
6h	R ₁ =2,3-dichloro R ₂ = carbamothioylcarbamo yl	128-130	0.702	EtOAc- Hexane (1:1)	C ₁₆ H ₁₁ Cl ₂ N ₃ O 3S
6i	R ₁ =2,3-dichloro R ₂ = pyridine-4- ylcarbamo yl	161-163	0.558	EtOAc- Hexane (1:1)	C ₂₀ H ₁₃ Cl ₂ N ₃ O 3
6j	R ₁ =2,3-dichloro R ₂ = naphthalene-1- ylcarbamo yl	105-107	0.722	EtOAc- Hexane (1:1)	C ₂₅ H ₁₆ Cl ₂ N ₂ O 3
6k	R ₁ =2,3-dichloro	138-140	0.435	EtOAc- Hexane (1:1)	

	R ₂ = piperidin-1-ylcarbonyl				C ₂₀ H ₁₈ Cl ₂ N ₂ O 3
6l	R ₁ =2,3-dichloro R ₂ = piperazin-1-ylcarbonyl	171-74	0.60	EtOAc- Hexane (1:1)	C ₁₉ H ₁₇ Cl ₂ N ₃ O 3
6m	R ₁ =2,3-dichloro R ₂ =2-methoxyphenylcarbonyl	101-103	0.542	EtOAc- Hexane (1:1)	C ₂₂ H ₁₆ Cl ₂ N ₂ O 4
6n	R ₁ =2,3-dichloro R ₂ = aminoantipyrinylcarbonyl	124-126	0.763	EtOAc- Hexane (1:1)	C ₂₆ H ₂₀ Cl ₂ N ₄ O 4

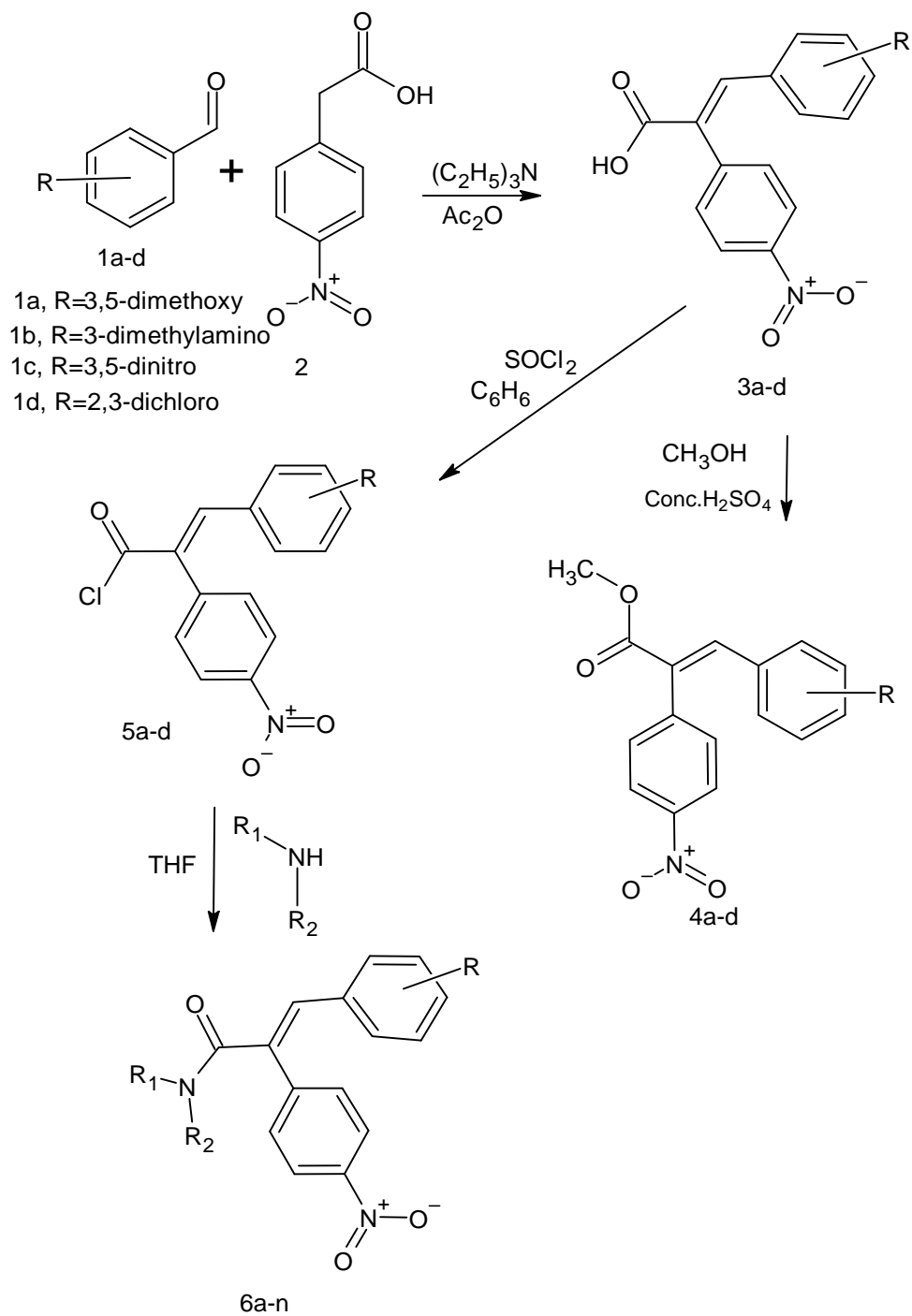


Figure 1 Scheme of synthesis of compounds

Antiangiogenesis study by chorioallantoic membrane (CAM) assay

CAM assays are often used as the primary approach to evaluate the effect of antigenic compounds. The basis of this experiment is the formation of a placental membrane, in which at a certain stage of fetal development the formation of new blood vessels in a fertilized egg takes place. The effect of test-impregnated agarose beads on the vascular membrane of open eggs on angiogenesis is evaluated. Fertilized eggs were obtained for testing from Kelchira incubators in Ghaziabad.

Procedure

Twelve eggs were used in each experiment to test one drug at a certain dose. The eggs were fertilised under ideal circumstances of 37°C and 80% relative humidity. The shells of eggs were cleaned with 70% EtOH to avoid infection. After 72 hours, a syringe was used to retrieve 8-10 ml of albumin from the lower side of the egg, and the hole was bandaged. After the upper half of the shell was removed, the eggs were covered in a plastic sheet and incubated for another 72 hours. When the diameter of the CAM was between 1.8 and 2.6 cm, the pellets containing the test chemicals were placed on it. In a 2.5 percent agarose solution, test compounds were dissolved or suspended. Following gel formation, a micropipette for viscous solutions was used to extract the bulk of agarose gel appropriate to the dosage of the test chemical to be administered to the CAM. As a result, the agarose pellets are not consistent in size. Because the half-cone-shaped agarose pellets sink somewhat into the CAM, they are stuck. The antiangiogenic impact was assessed after 24 hours, either with a stereomicroscope and observation of the avascular zone around the pellet, or with naked eye observation of the avascular zone surrounding the pellet (if clear). Antiangiogenic activity is graded on a scale of 0 to 2, with 0 indicating no impact, 1 suggesting a moderate effect, and 2 indicating a significant effect (capillary free zone is at least twice as large as the pellet). Membrane irritation and embryotoxicity can also be assessed. B-1, 4-galactan sulphate (LuPS S5), with an average molecular weight of 20000, was used as a positive control, and an agarose pellet was used as a blank [20].

Anticancer activity

Compounds showing potent antiangiogenic activity were further subjected for anticancer activity against HCT-116 (colon cancer) and MCF-7 (breast cancer) cell lines by using modified MTT assay method.

Procedure of the *in vitro* cancer activity

Human tumour cell lines from the cancer screening panel were grown in RPMI-1640 medium with 5% foetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells were injected onto 96 well

microtiter plates in 100 L at plating densities ranging from 5,000 to 40,000 cells/well, depending on the doubling period of certain cell lines. Before introducing experimental medications, the microtiter plates were incubated for 24 hours at 37°C, 5% CO₂, 95% air, and 100% relative humidity after cell inoculation. After 24 hours, two plates of each cell line were fixed in situ with trichloroacetic acid (TCA) to represent a measurement of the cell population at the moment of drug delivery (T_z). Experimental medications were solubilized in 400 times the needed final maximum test concentration in dimethyl sulfoxide (DMSO) and kept refrigerated before to use. At the moment of medication administration, an aliquot of frozen concentrate was thawed and diluted to twice the specified final maximum test concentration with complete medium containing 50 g/ml gentamicin. Additional four, 10-fold, or 12-log serial dilutions were made to provide a total of five drug concentrations plus control. By adding aliquots of 100 l of these varied drug dilutions to appropriate microtiter wells already holding 100 l of medium, the required final drug concentrations were attained.

The plates were then incubated for another 48 hours at 37°C, 5% CO₂, 95 percent air, and 100 percent relative humidity after the drug was administered. The test was completed by adding cold TCA to the adhering cells. The cells were fixed in place by gently adding 50 l of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded, and the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 l) in 0.4 percent (w/v) acetic acid was added to each well, and plates were incubated for 10 minutes at room temperature. After staining, the plates were washed five times with 1 percent acetic vinegar and air dried to eliminate any unbound colour. After solubilizing the bound dye with 10 mMTrizma base, the absorbance was measured using an automated plate reader at 515 nm. The procedure was the same for suspension cells, with the difference that the test was finished by gently pouring 50 l of 80 percent TCA into the wells to settle the cells in the bottom (final concentration, 16 percent TCA). The percentage growth was calculated using the seven absorbance measurements of time zero (T_z), control growth (C), and test growth in the presence of drug at the five concentration levels for each of the drug concentration levels (T_i). The percentage of growth inhibition was calculated using the following formula:

$$[(T_i - T_z)/(C - T_z)] \times 100 \text{ for concentrations for which } T_i \geq T_z$$

$$[(T_i - T_z)/T_z] \times 100 \text{ for concentrations for which } T_i < T_z.$$

For each experimental drug, three dosage response parameters were computed. The drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation was calculated as $(T_i - T_z)/(C - T_z) \times 100 = 50$, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. T_i

= Tz was used to calculate the medication concentration that resulted in total growth inhibition (TGI). $[(Ti-Tz)/Tz] \times 100 = -50$ was used to compute the drug concentration that resulted in a 50% reduction in measured protein at the conclusion of the drug treatment compared to the beginning (LC50), suggesting a net loss of cells following treatment. If the level of activity was attained, values were computed for each of these three parameters; however, if the effect was not reached or surpassed, the value was stated as higher or less than the maximum or lowest concentration tested [21].

Results and Discussion

In the presence of triethylamine, base catalysed condensation of p-nitrophenyl acetic acid with matching aryl Aldehydes, followed by esterification, or reaction with thionyl chloride followed by reaction with suitable amine yielded Analogues of (Z)-1-phenyl-2-(4-nitrophenyl) ethene.

Infrared, ¹H NMR, and mass spectroscopy were used to confirm the structure of the produced compounds. All of the synthesised compounds' spectral data (¹H NMR, IR, and Mass) were found to be in perfect agreement with the hypothesised structures.

Data of spectral studies

3a:(2E)-2-(4-nitrophenyl)-3-(3,5-dimethoxyphenyl)acrylic acid: FTIR (KBr) cm^{-1} 3062 and 857 (C-H), 3028 and 750 (Ar-H), 2935 and 1454 (CH₃), 1705 (C=O), 1660 (C=C), 1599 and 1420 (COO⁻), 1519 and 1340 (C-NO₂), 1239 and 1005 (C-O); ¹H NMR (DMSO) δ 9.99 (s,1H), 8.28 (d,2H), 7.89 (s,1H), 7.31 (d,2H), 6.49 (s,2H), 3.80 (s,6H); MS (TISI) 329.3 (M⁺).

4a: Methyl (2E)-2-(4-nitrophenyl)-3-(3,5-dimethoxyphenyl)acrylate: FTIR (KBr) cm^{-1} 3070 and 855 (C-H), 3032 and 752 (Ar-H), 2940 and 1455 (CH₃), 1730 (C=O), 1663 (C=C), 1605 (C=C of Ar), 1520 and 1340 (C-NO₂), 1240, 1130 and 1002 (C-O); ¹H NMR (DMSO) δ 8.29 (d,2H), 7.75 (s, 1H), 7.35 (d,2H), 6.63 (s, 2H), 3.90 (s, 3H), 3.74 (s, 6H); MS (TISI) 343.3 (M⁺).

5a:(2E)-2-(4-nitrophenyl)-3-(3,5-dimethoxyphenyl)acryloyl chloride: FTIR (KBr) cm^{-1} 3071 and 859 (C-H), 3035 and 738 (Ar-H), 2939 and 1460 (CH₃), 1756 (C=O), 1656 (C=C), 1605 (C=C of Ar), 1514 and 1340 (C-NO₂), 1250 and 1010 (C-O), 702 (C-Cl); ¹H NMR (DMSO) δ 8.24 (d,2H), 7.78 (s, 1H), 7.36 (d, 2H), 6.53 (s, 2H), 3.79 (s, 6H); MS (TISI) 347.3 (M⁺).

6a:(2E)-N-ethyl-2-(4-nitrophenyl)-3-(3,5-dimethoxyphenyl)acrylamide: FTIR (KBr) cm^{-1} 3435 and 1562 (N-H), 3070 and 850 (C-H), 3030 and 748 (Ar-H), 2939 and 1380 (CH_3), 1689 (C=O), 1651 (C=C), 1602 (C=C of Ar), 1520 and 1341 (C- NO_2), 1456 (CH_2), 1253 and 1001 (C-O); ^1H NMR (DMSO) δ 8.99 (s, 1H), 8.28 (d, 2H), 7.78 (s, 1H), 7.32 (d, 2H), 6.48 (s, 2H), 3.81 (s, 6H), 3.37 (q, 2H), 1.12 (t, 3H); MS (TISI) 356.2 (M^+).

6b:(2E)-2-(4-nitrophenyl)-3-(3,5-dimethoxyphenyl)-N-(2-chlorophenyl)acrylamide: FTIR (KBr) cm^{-1} 3434 (N-H), 3078 and 860 (C-H), 3031 and 752 (Ar-H), 2926 (CH_3), 1681 (C=O), 1632 (C=C), 1602 (C=C of Ar), 1520 and 1347 (C- NO_2), 1240 and 1009 (C-O), 709 (C-Cl); ^1H NMR (DMSO) δ 9.63 (s, 1H), 8.26 (d, 2H), 8.05 (d, 1H), 7.77 (s, 1H), 7.34-7.62 (m, 4H), 7.16 (t, 1H), 6.45 (s, 2H), 3.82 (s, 6H); MS (TISI) 438.2 (M^+).

6c:(2E)-2-(4-nitrophenyl)-3-(3,5-dimethoxyphenyl)-N-(4-fluorophenyl)acrylamide: FTIR (KBr) cm^{-1} 3428 (N-H), 3077 and 853 (C-H), 3035 and 752 (Ar-H), 2935 and 1468 (CH_3), 1690 (C=O), 1658 (C=C), 1600 (C=C of Ar), 1513 and 1340 (C- NO_2), 1230 and 1005 (C-O), 1122 (C-F); ^1H NMR (DMSO) δ 10.06 (s, 1H), 8.28 (d, 2H), 7.88 (s, 1H), 7.34 (d, 2H), 7.61 (d, 2H), 7.97 (d, 2H), 6.48 (s, 2H), 3.80 (s, 6H); MS (TISI) 422.2 (M^+).

6d:(2E)-2-(4-nitrophenyl)-3-(3,5-dimethoxyphenyl)-N-(2-methylphenyl)acrylamide: FTIR (KBr) cm^{-1} 3433 (N-H), 3066 and 850 (C-H), 3038 and 751 (Ar-H), 2935 and 1462 (CH_3), 1684 (C=O), 1662 (C=C), 1601 (C=C of Ar), 1520 and 1346 (C- NO_2), 1243 and 1005 (C-O); ^1H NMR (DMSO) δ 9.49 (s, 1H), 8.30 (d, 2H), 7.78 (s, 1H), 7.29-7.59 (m, 5H), 7.17 (t, 1H), 6.45 (s, 2H), 3.77 (s, 6H), 2.35 (s, 3H); MS (TISI) 418.2 (M^+).

6e:(2E)-N-(aminocarbonthioyl)-2-(4-nitrophenyl)-3-(3,5-dimethoxyphenyl)acrylamide: FTIR (KBr) cm^{-1} 3507, 3377 and 1599 (NH_2), 3425 and 1553 (N-H), 3058 and 860 (C-H), 3026 and 752 (Ar-H), 2935 and 1455 (CH_3), 1688 (C=O), 1650 (C=C), 1520 and 1340 (C- NO_2), 1250 and 1000 (C-O), 1125 (C=S); ^1H NMR (DMSO) δ 9.56 (s, 2H), 8.50 (s, 1H), 8.21 (d, 2H), 7.79 (s, 1H), 7.30 (d, 2H), 6.45 (s, 2H), 3.80 (s, 6H); MS (TISI) 387.2 (M^+).

3b:(2E)-3-(3,5-dinitrophenyl)-2-(4-nitrophenyl)acrylic acid: FTIR (KBr) cm^{-1} 3100 and 839 (C-H), 3034 and 744 (Ar-H), 1722 (C=O), 1660 (C=C), 1590 (COO^-), 1520 and 1340 (C- NO_2); ^1H NMR (DMSO) δ 10.00 (s, 1H), 8.41 (s, 1H), 8.30 (d, 2H), 8.22 (d, 1H), 8.05 (d, 1H), 7.49-7.77 (m, 4H); MS (TISI) 359.2 (M^+).

4b: Methyl (2E)-3-(3,5-dinitrophenyl)-2-(4-nitrophenyl)acrylate: FTIR (KBr) cm^{-1} 3050 and 879 (C-H), 3030 and 750 (Ar-H), 2926 and 1469 (CH_3), 1715 (C=O), 1670 (C=C), 1520 and 1339 (C- NO_2), 1248 and 1110 (C-O); ^1H NMR (DMSO) δ 8.37 (s, 1H), 8.23 (d, 2H), 8.18 (d, 1H), 8.05 (d, 1H), 7.79 (m, 2H), 7.50 (d, 2H), 3.97 (s, 3H); MS (TISI) 373.1 (M^+).

5b:(2E)-3-(3,5-dinitrophenyl)-2-(4-nitrophenyl)acryloyl chloride: FTIR (KBr) cm^{-1} 3050 and 857 (C-H), 3027 and 749 (Ar-H), 1759 (C=O), 1690 (C=C), 1609 (C=C of Ar), 1520 and 1344 (C- NO_2), 710 (C-Cl); ^1H NMR (DMSO) δ 8.58 (s, 1H), 8.30 (d, 2H), 8.20 (d, 1H), 8.10 (d, 1H), 7.79 (m, 2H), 7.51 (d, 2H); MS (TISI) 377.1 (M^+).

6f:(2E)-N-ethyl-3-(3,5-dinitrophenyl)-2-(4-nitrophenyl)acrylamide: FTIR (KBr) cm^{-1} 3430 and 1566 (N-H), 3048 and 856 (C-H), 3031 and 750 (Ar-H), 2930 (CH_3), 1674 (C=O), 1521 and 1340 (C- NO_2), 1466 (CH_2); ^1H NMR (DMSO) δ 9.01 (s, 1H), 8.35 (s, 1H), 8.28 (d, 2H), 8.24 (d, 1H), 8.13 (d, 1H), 7.77 (m, 2H), 7.51 (d, 2H), 3.34 (q, 2H), 1.16 (t, 3H); MS (TISI) 386.2 (M^+).

3c:(2E)-3-[3-(dimethylamino)phenyl]-2-(4-nitrophenyl)acrylic acid: FTIR (KBr) cm^{-1} 3066 and 856 (C-H), 3024 and 757 (Ar-H), 2880 and 1440 (CH_3), 1716 (C=O), 1666 (C=C), 1599 and 1410 (COO^-), 1519 and 1350 (C- NO_2), 1196 (NR_3); ^1H NMR (DMSO) δ 10.00 (s, 1H), 8.22 (d, 2H), 7.86 (d, 2H), 7.77 (s, 1H), 7.55 (d, 2H), 6.69 (d, 2H), 2.98 (s, 6H); MS (TISI) 312.2 (M^+).

4c: Methyl (2E)-3-[3-(dimethylamino)phenyl]-2-(4-nitrophenyl)acrylate: FTIR (KBr) cm^{-1} 3047 and 855 (C-H), 3019 and 750 (Ar-H), 2879 (CH_3), 1729 (C=O), 1659 (C=C), 1599 (C=C of Ar), 1527 and 1345 (C- NO_2), 1250 and 1113 (C-O), 1189 (NR_3); ^1H NMR (DMSO) δ 8.30 (d, 2H), 7.91 (d, 2H), 7.82 (s, 1H), 7.53 (d, 2H), 6.69 (d, 2H), 3.90 (s, 3H), 3.09 (s, 6H); MS (TISI) 326.2 (M^+).

5c:(2E)-3-[3-(dimethylamino)phenyl]-2-(4-nitrophenyl)acryloyl chloride: FTIR (KBr) cm^{-1} 3048 and 857 (C-H), 3026 and 749 (Ar-H), 2880 (CH_3), 1788 (C=O), 1690 (C=C), 1605 (C=C of Ar), 1519 and 1339 (C- NO_2), 1191 (NR_3), 720 (C-Cl); ^1H NMR (DMSO) δ 8.41 (s, 1H), 8.25 (d, 2H), 7.87 (d, 2H), 7.51 (d, 2H), 6.70 (d, 2H), 2.92 (s, 6H); MS (TISI) 330.1 (M^+).

6g:(2E)-3-[3-(dimethylamino)phenyl]-N-ethyl-2-(4-nitrophenyl)acrylamide: FTIR (KBr) cm^{-1} 3425 and 1559 (N-H), 3019 and 857 (C-H), 2890 (CH_3), 1680 (C=O), 1599 (C=C of Ar), 1520 and 1348 (C- NO_2), 1468 (CH_2),

1198 (NR₃), 750 (Ar-H); ¹H NMR (DMSO) δ 8.79 (s, 1H), 8.25 (d, 2H), 7.84 (d, 2H), 7.77 (s, 1H), 7.50 (d, 2H), 6.69 (d, 2H), 3.29 (q, 2H), 3.05 (s, 6H), 1.10 (t, 3H); MS (TISI) 339.0 (M⁺).

3d:(2E)-3-(2,3-dichlorophenyl)-2-(4-nitrophenyl)acrylic acid: FTIR (KBr) cm⁻¹ 3026 and 857 (C-H), 1720 (C=O), 1631 (C=C), 1600 and 1410 (COO⁻), 1519 and 1340 (C-NO₂), 750 (Ar-H), 690 (C-Cl); ¹H NMR (DMSO) δ 10.01 (s, 1H), 8.27 (d, 2H), 7.90 (d, 2H), 7.69 (s, 1H), 7.35-7.59 (m, 4H); MS (TISI) 337.5 (M⁺).

4d: Methyl (2E)-3-(2,3-dichlorophenyl)-2-(4-nitrophenyl)acrylate: FTIR (KBr) cm⁻¹ 3038 and 858 (C-H), 3020 and 749 (Ar-H), 2920 (CH₃), 1729 (C=O), 1642 (C=C), 1605 (C=C of Ar), 1518 and 1340 (C-NO₂), 1248 and 1101 (C-O), 680 (C-Cl); ¹H NMR (DMSO) δ 8.27 (d, 2H), 7.77 (d, 2H), 7.70 (s, 1H), 7.34-7.59 (m, 4H), 3.70 (s, 3H); MS (TISI) 351.5 (M⁺).

5d:(2E)-3-(2,3-dichlorophenyl)-2-(4-nitrophenyl)acryloyl chloride: FTIR (KBr) cm⁻¹ 3050 and 857 (C-H), 3023 and 749 (Ar-H), 1786 (C=O), 1642 (C=C), 1605 (C=C of Ar), 1520 and 1340 (C-NO₂), 693 (C-Cl); ¹H NMR (DMSO) δ 8.29 (d, 2H), 7.90 (s, 1H), 7.71 (d, 2H), 7.27-7.59 (m, 4H); MS (TISI) 355.6 (M⁺).

6h:(2E)-N-(aminocarbonothioyl)-3-(2,3-dichlorophenyl)-2-(4-nitrophenyl)acrylamide: FTIR (KBr) cm⁻¹ 3475, 3350 and 1601 (NH₂), 3437 (N-H), 3055 and 849 (C-H), 3030 and 749 (Ar-H), 1693 (C=O), 1657 (C=C), 1518 and 1346 (C-NO₂), 1116 (C=S), 700 (C-Cl); ¹H NMR (DMSO) δ 9.50 (s, 2H), 8.35 (s, 1H), 8.29 (d, 2H), 7.90 (s, 1H), 7.40-7.79 (m, 6H); MS (TISI) 395.6 (M⁺).

6i:(2E)-3-(2,3-dichlorophenyl)-2-(4-nitrophenyl)-N-pyridin-4-ylacrylamide: FTIR (KBr) cm⁻¹ 3444 (N-H), 3039 and 857 (C-H), 3025 and 750 (Ar-H), 1690 (C=O), 1642 (C=N-C), 1599 (C=C of Ar), 1520 and 1340 (C-NO₂), 700 (C-Cl); ¹H NMR (DMSO) δ 9.80 (s, 1H), 8.48 (d, 2H), 8.27 (d, 2H), 7.80 (d, 2H), 7.69 (s, 1H), 7.24-7.59 (m, 6H); MS (TISI) 413.5 (M⁺).

6j:(2E)-3-(2,3-dichlorophenyl)-N-1-naphthyl-2-(4-nitrophenyl)acrylamide: FTIR (KBr) cm⁻¹ 3429 (N-H), 3054 and 858 (C-H), 3020 and 761 (Ar-H), 1686 (C=O), 1650 (C=C), 1601 (C=C of Ar), 1519 and 1345 (C-NO₂), 680 (C-Cl); ¹H NMR (DMSO) δ 10.51 (s, 1H), 8.28 (d, 2H), 6.88-7.83 (m, 14H); MS (TISI) 462.7 (M⁺).

6k: 1-[(2E)-3-(2,3-dichlorophenyl)-2-(4-nitrophenyl)prop-2-enoyl]piperidine: FTIR (KBr) cm^{-1} 3010 and 858 (C-H), 2949 and 1456 (CH_2), 1676 (C=O), 1635 (C=C), 1601 (C=C of Ar), 1520 and 1340 (C- NO_2), 1200 (NR_3), 749 (Ar-H), 689 (C-Cl); ^1H NMR (DMSO) δ 8.29 (d, 2H), 7.80 (s, 1H), 7.39-7.81 (m, 6H), 3.59 (t, 4H), 1.56 (m, 6H); MS (TISI) 404.6 (M^+).

6l: 1-[(2E)-3-(2,3-dichlorophenyl)-2-(4-nitrophenyl)prop-2-enoyl]piperazine: FTIR (KBr) cm^{-1} 3439 (N-H), 3039 and 855 (C-H), 3019 and 750 (Ar-H), 2949 and 1455 (CH_2), 1667 (C=O), 1600 (C=C of Ar), 1519 and 1339 (C- NO_2), 1199 (NR_3), 689 (C-Cl); ^1H NMR (DMSO) δ 8.27 (d, 2H), 7.81 (d, 2H), 7.70 (s, 1H), 7.37-7.59 (m, 4H), 3.01 (t, 4H), 2.79 (t, 4H), 1.82 (s, 1H); MS (TISI) 405.6 (M^+).

6m:(2E)-3-(2,3-dichlorophenyl)-2-(4-nitrophenyl)-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)acrylamide: FTIR (KBr) cm^{-1} 3419 (N-H), 3040 and 857 (C-H), 3020, 749 and 705 (Ar-H), 2916 and 1471 (CH_3), 1759, 1711 and 1677 (C=O), 1599 (C=C of Ar), 1520 and 1340 (C- NO_2), 1190 (NR_3), 679 (C-Cl); ^1H NMR (DMSO) δ 9.64 (s, 1H), 8.29 (d, 2H), 7.81 (d, 2H), 7.77 (s, 1H), 7.27-7.63 (m, 8H), 6.92 (m, 1H), 2.89 (s, 3H), 2.07 (s, 3H); MS (TISI) 522.6 (M^+).

6n:(2E)-3-(2,3-dichlorophenyl)-N-(2-methoxyphenyl)-2-(4-nitrophenyl)acrylamide: FTIR (KBr) cm^{-1} 3435 (N-H), 3039 and 860 (C-H), 3025 and 749 (Ar-H), 2936 and 1466 (CH_3), 1696 (C=O), 1599 (C=C of Ar), 1520 and 1339 (C- NO_2), 1240 and 1010 (C-O), 680 (C-Cl); ^1H NMR (DMSO) δ 9.77 (s, 1H), 8.25 (d, 2H), 7.34-7.90 (m, 8H), 7.00-7.10 (m, 3H), 3.90 (s, 3H); MS (TISI) 442.7 (M^+).

Antiangiogenic activity

Table 2 shows the effects of the test substances on angiogenesis. Because most compounds demonstrated a hazardous impact at greater doses, all of the compounds were evaluated at a level of 0.01 mg/pellet, i.e. less than 40 nmol/pellet. Compounds 3a, 3d, 5a, 5b, 5c, and 5d demonstrated an antiangiogenic score of higher than 1 in (Z)-1-phenyl-2-(4-nitrophenyl) ethene analogues. Compound 5a was determined to be the most powerful, scoring 1.8 ± 0.1 , which is higher than the standard. Compound 3a and 5d also performed well, with a score that was comparable to the standard.

The findings reveal that the produced chemicals have potent antiangiogenic properties. The most active analogues 3a, 3d, 5a, 5b, 5c and 5d contain smaller groups as bridge substituents, such as COOH , COOCH_3 , or

COCl, whereas the least active analogues 6e, 6f, 6g, 6i, 6j and 6m have comparably big groups. Aromatic substituted compounds (6b, 6d, 6i, 6j and 6m) were the least active. So, compounds with the Piperidin-1-ylcarbonyl, carboxyl, methoxycarbonyl, and chlorocarbonyl moiety on the (Z)-1-phenyl-2-(4-nitrophenyl) ethene skeleton are among the most active in our investigation. The antiangiogenic action of the series investigated is influenced by the size of bridge substituents.

Table 2 Antiangiogenic activity of synthesized compounds in the CAM assay

Test compound	Concentration	Antiangiogenic score ^b ± sd (n = no. of experiment)
	(mg/pellet)	
3a	0.01	1.5 ± 0.1 (n =3)
3b	0.01	1.1 ± 0.1 (n =2)
3c	0.01	0.7 ± 0.1 (n =2)
3d	0.01	1.3 ± 0.1 (n =2)
4a	0.01	1.2 ± 0.1 (n =2)
4b	0.01	0.9 ± 0.1 (n =2)
4c	0.01	0.6 ± 0.2 (n =2)
4d	0.01	0.8 ± 0.1 (n =2)
5a	0.01	1.8 ± 0.1 (n =3)
5b	0.01	1.0 ± 0.1 (n =2)
5c	0.01	1.2 ± 0.1 (n =2)
5d	0.01	1.5 ± 0.1 (n =3)
6a	0.01	0.8 ± 0.1 (n =2)
6b	0.01	0.6 ± 0.2 (n =2)
6c	0.01	0.8 ± 0.1 (n =2)
6d	0.01	0.2 ± 0.3 (n =2)
6e	0.01	0.6 ± 0.1 (n =2)
6f	0.01	0.7 ± 0.1 (n =2)
6g	0.01	0.2 ± 0.4 (n =2)
6h	0.01	0.9 ± 0.1 (n =2)
6i	0.01	0.4 ± 0.2 (n =2)

6j	0.01	0.3 ± 0.3 (n =2)
6k	0.01	0.8 ± 0.1 (n =2)
6l	0.01	0.6 ± 0.1 (n =2)
6m	0.01	0.4 ± 0.1 (n =2)
6n	0.01	0.5 ± 0.1 (n =2)
Agarose pellet		0.1 ± 0.1 (n =10)
β-1,4-galactan sulphate (LuPS S5)	0.05	1.4 ± 0.1 (n =10)

^b0 = no or weak effect, 1 = medium effect, 2 = strong effect

Anticancer activity

Table 3 shows anticancer activity of screened compounds (3a, 3d, 5a, 5b, 5c, 5d) against of HCT-116 (colon cancer) and MCF-7 (breast cancer) cell lines by modified MTT assay method. Compound 5a showed maximum activity with % growth inhibition of 63.43 and 56.24 against HCT-116 and MCF-7 cell lines respectively. Compound 3a and 3d also showed more than 50% growth inhibition against HCT-116 cell lines. Results revealed that synthesized analogues are more active against HCT-116 (colon cancer) cell lines in comparison to MCF-7 (Breast cancer) cell lines. (Z)-1-phenyl-2-(4-nitrophenyl) ethene analogues showed significant growth inhibition of HCT-116 (colon cancer) and MCF-7 (breast cancer) cell lines.

Table 3 Anticancer activity of compounds against of HCT-116 (colon cancer) and MCF-7 (breast cancer) cell lines by modified MTT assay method

Test compound	Concentration (dose)	% Growth Inhibition	
		HCT-116	MCF-7
3a	10 μM	51.46	42.29
3d	10 μM	54.22	46.57
5a	10 μM	68.43	56.24
5b	10 μM	48.75	39.43
5c	10 μM	41.66	25.43
5d	10 μM	38.63	29.67

Conclusion

Antiangiogenic activity testing revealed that the majority of the synthesised compounds exhibited considerable antiangiogenic activity. Antiangiogenic action was discovered to be affected by the size of the bridge substituent (on the Ethylene Bridge). Compounds with fewer substituents were more active. The most appropriate groups were determined to be piperidin-1-ylcarbonylcarbonyl, carboxyl, methoxycarbonyl, and chlorocarbonyl. Compound 5a showed maximum anticancer activity with % growth inhibition of 63.43 and 56.24 against HCT-116 and MCF-7 cell lines respectively. Compound 3a and 3d also showed more than 50% growth inhibition against HCT-116 cell lines. Results revealed that synthesized analogues are more active against HCT-116 (colon cancer) cell lines in comparison to MCF-7 (Breast cancer) cell lines. (Z)-1-phenyl-2-(4-nitrophenyl) ethene analogues showed significant growth inhibition in anticancer screen. Finally, it's possible that further derivatization of these molecules will be pursued in the hopes of obtaining more selective and potent anticancer medicines.

References

1. A. D. Kinghorn, E. J. C. De Blanco, D. M. Lucas, H. L. Rakotondraibe, J. Orjala, D. D. Soejarto, N. H. Oberlies, C. J. Pearce, M. C. Wani, B. R. Stockwell, J.E. Burdette, S. M. Swanson, J. R. Fuchs, M. A. Phelps, L. Xu, X. Zhang and Y.Y. Shen, *Anticancer Research* 2016, 36, 5623-5638.
2. M. Turek, M. Krzyczmonik, P. Bałczewski, *Medicinal Chemistry* 2016, 12, 700-719.
3. (a) K.Z. Xiao, L.J. Xuan, J. Zhang, J.M. Xu, D.L. Bai, *Studies in Natural Products Chemistry*, Atta-ur-Rahman; Elsevier Science, B.V., Ed.; 2008, 34, 453-645. (b) Y. Zhou, J. Zheng, Y. Li, D.-P. Xu, S. Li, Y.-M. Chen and H.-B. Li, *Nutrients* 2016, 8, 515-550.
4. C. Rivière, A.D. Pawlus, J.M. Mérillon, *Natural Product Reports*, 2012, 29, 11, 1317- 1333.
5. T. Shen, X.N. Wang, H.X. Lou, *Natural Product Reports*, 2009, 26, 7, 916-935.
6. I. N. Ioffe, A. A. Granovsky, *J. Chem. Theory Comput.* 2013, 9, 4973-4990.
7. H. Rajak, P. Kumar Dewangan, V. Patel, D. Kumar Jain, A. Singh, R. Veerasamy, P. Chander Sharma, A. Dixit, *Current Pharmaceutical Design*, 2013, 19, 7, 1923-1955.
8. M. Cushman, D. Nagarathnam, D. Gopal, H. M. He, C. M. Lin, E. Hamel, *Journal of Medicinal Chemistry* 1992, 35, 2293-2306.
9. J. Jiang, C. Zheng, K. Zhu, J. Liu, N. Sun, C. Wang, H. Jiang, J. Zhu, C. Luo, and Y. Zhou, *Journal of Medicinal Chemistry* 2015, 58, 2538-2546.

10. a) M. Reinisalo, A. Karlund, A.Koskela, K. Kaarniranta, R. O. Karjalainen, *Oxidative Medicine and Cellular Longevity* 2015, 2015, 340-520; b) T. Hong, W. Jiang, H.-M. Dong, S.- X. QIU, Y. Lu, *Chinese Journal of Natural Medicines* 2015, 13(5) 0375–0382; c) S. Pervaiz, A.L. Holme, *Antioxid Redox Signal* 2009, 11, 2851-2897.
11. a) A. Csiszar, *Ann. N. Y. Acad. Sci.* 2011, 1215, 117-122. b) L. G. Carter, J. A. D’Orazio, K. J. Pearson, *Endocrine-Related Cancer* 2014, 21, 209-225.
12. S. Fulda, *Drug Discovery Today* 2010, 15, 757-765.
13. C. K. Singh, M. A. Ndiaye, N. Ahmad, *BiochimicaetBiophysicaActa*, 2015, 1852, 1178- 1185.
14. S. Molino, M. Dossena, D. Buonocore, F. Ferrari, L. Venturini, G. Ricevuti, M. Verri, *Life Sciences* 2016, 161, 69-77.
15. a) B. De Filippis, A. Giancristofaro, A. Ammazalorso, A. D’Angelo, M. Fantacuzzi, L. Giampietro, C. Maccallini, M. Petruzzelli, R. Amoroso, *European Journal of Medicinal Chemistry* 2011, 46, 4218-5224; b) B. De Filippis, M. Agamennone, A. Ammazalorso, I. Bruno, A. D’Angelo, M. Di Matteo, M. Fantacuzzi, L. Giampietro, A. Giancristofaro, C. Maccallini, R. Amoroso, *Med. Chem. Commun.* 2015, 6, 1513-1517; c) B. De Filippis, P. Linciano, A. Ammazalorso, C. Di Giovanni, M. Fantacuzzi, L. Giampietro, A. Laghezza, C. Maccallini, P. Tortorella, A. Lavecchia, F. Loiodice, R. Amoroso, *European Journal of Medicinal Chemistry*, 2015, 89, 817-825; d) L. Giampietro, A. D’Angelo, A. Giancristofaro, A. Ammazalorso, B. De Filippis, M. Fantacuzzi, P. Linciano, C. Maccallini, R. Amoroso, *Bioorganic & Medicinal Chemistry Letters* 2012, 22, 7662-7666; e) L. Giampietro, A. D’Angelo, A. Giancristofaro, A. Ammazalorso, B. De Filippis, M. Di Matteo, M. Fantacuzzi, P. Linciano, C. Maccallini, R. Amoroso, *Medicinal Chemistry* 2014, 10, 1, 59-65.
16. C. Li, J.-S. Fang, W.-W. Lian, X.-C. Pan , A.-L. Liu, and G.-H. Du, *ChemBiol Drug Des* 2015, 85, 427-438.
17. F.S. Aldawsari, R. P. Aguiar, L. A. M. Wiirzler, R. Aguayo-Ortiz, N. Aljuhani, R. K. N. Cuman, J. L. Medina-Franco, A. G. Siraki, C. A. Velázquez-Martínez, *Bioorganic & Medicinal Chemistry Letters* 2016, 26, 1411-1415.
18. a) E. Giacomini, S. Rupiani, L. Guidotti, M. Recanatini, M. Roberti, *Current Medicinal Chemistry*, 2016, 23(23), 2439- 2489; b) P. Marchetti, B. Pavan, D. Simoni, R. Baruchello, R. Rondanina, C. Mischianti, G. Feriottod, L. Ferraro, L.-C. Hsu, R. M. Lee, A. Dalpiaz, *European Journal of Pharmaceutical Sciences* 2016, 91, 50-63.
19. A. Chaudhary, S.N. Pandeya, P. Kumar, P.P. Sharma, S. Gupta, N. Soni, K.K. Verma, G. Bhardwaj, *Mini Reviews in Medicinal Chemistry*, 2007, 7(12), 1186-1205.
20. R. Hoffman, D. Paper, J. Donaldson, H. Vogl, *Brazilian Journal of Cancer*, 1996,73, 1183-1186.

21. M. R. Boyd, K. D. Paull, Drug Development Research, 1995, 34, 91-109.