Original Article

Synthesis and antiangiogenic activity of some novel analogues of 2-[(Z)-2-(4-Nitrophenyl) ethenyl]furan

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ABSTRACT

A series of 2-[(z)-2-(4-nitrophenyl)ethenyl]furan analogues were synthesized in order to obtain new compounds with potential antiangiogenic activity. Base catalysed condensation of p-nitrophenyl acetic acid (3) with furfuraldehyde (2), in the presence of triethylamine, yielded carboxylic acid derivative (4) which on esterification, with methanol, using a catalytic amount of H_2SO_4, gave corresponding ester derivative (5). Reaction of thionyl chloride with carboxylic acid derivative in refluxing benzene gave the corresponding acid chloride (6), which on subsequent reaction with appropriate amine gave compounds 7a-k. All compounds were evaluated for their antiangiogenic activity by chorioallantoic membrane (CAM) assay method. Compounds 6 and 7h showed pronounced antiangiogenic activity, however, they were less active than standard (β-1,4-galactan sulphate). All other compounds showed significant inhibition of angiogenesis when compared to control but they were much less active than standard. As some of 2-[(z)-2-(4-nitrophenyl)ethenyl]furan analogues showed significant antiangiogenic activity, this moiety may be further explored to find new antiangiogenic leads.

1. INTRODUCTION

Angiogenesis or neovascularization is a complex process involving the activation, adhesion, proliferation, and transmigration of endothelial cells from pre-existing blood vessels. It plays a critical role in normal physiological processes such as wound healing, but also in a number of pathological processes, for instance diabetes retinopathy, arthritis, and the growth of solid tumors. Due to the limited diffusion of oxygen and nutrients through tissue, tumours are dependent upon their ability to stimulate angiogenesis (the induction of new blood vessels) in order to grow beyond a size of ~1 mm³ [1-2]. Tumours also must continuously induce angiogenesis as they grow to compensate for the increasing number of cells the vessels must support, resulting in a level of endothelial proliferation not normally found in the adult tissues [3]. The resulting tumour vessels are abnormal in structure, often leaky, tortuous and lacking normal pericyte interaction [4-7]. The presence of these abnormal vessels, which are unique to the tumour, has led to the development of therapeutic strategies aimed at compromising the existing tumour blood vessels [3]. Therefore, angiogenesis is considered as a potential target for antitumor activity. Combretastatin A-4 (CA-4, figure 1) is currently under investigation as an angiogenesis inhibitor (antiangiogenic). It was isolated by Pettit and co-workers (1982) from the bark of the South African bush willow tree *Combretum caffrum* [8]. From the structure-activity relationship (SAR) point of view, CA-4 belongs to the class of natural compounds related to biphenyls and contains, as a key structural feature, the *cis*-stilbene motif. CA-4 exerts a potent cytotoxicity against a variety of human cancer cells including multi drug resistant (MDR) cancer cell lines [9-16], and also displays potent antitumor effect in a wide variety of preclinical tumor models [17-21] as well as substantial antivascular

(antiangiogenic) activity in tumor blood flow while causing no significant blood flow retention in normal tissues [22-28]. CA-4 does not show *in vivo* efficacy due to its poor pharmacokinetics and isomerization in inactive form [13].
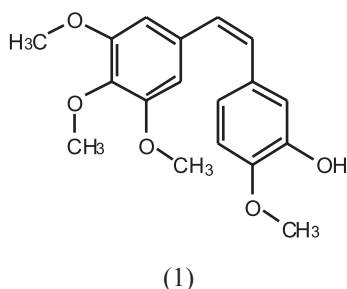

(1)

Fig. 1. Structural formula of CA-4.

Antivascular effect of CA-4 is related to its antitubulin activity. The cellular microtubule network plays a major role in maintaining cell shape, particularly in the case of neovasculature. CA-4 causes microtubules to rapidly depolymerize. As a result elongated endothelial cells round up, causing disruption of endothelial cell layer surrounding blood vessel and exposing of underlying basement membrane. This leads to blood vessel congestion and loss of blood flow, loss of oxygen and nutrient supply to tumor cells. Therefore, tumor cells undergo necrosis [12,28,29-30]. Some analogues, having furan ring in their structure, have been reported to possess potent antitubulin activity [31-32]. In view of strong anticancer/antivascular activity exhibited by CA-4 and some of furan analogues, we have synthesized a series of 2-[(*z*)-2-(4-nitrophenyl)ethenyl] furan analogues by replacing 3,4,5-trimethoxyphenyl ring of CA-4 by furan and by substituting ethylene bridge with various smaller and bulky groups, which yield active compound in various potent moieties, in the hope of obtaining additional potent antiangiogenic agents. In present series we have tested analogues having 4-nitro substituent on B-ring, while other substituents are planned to be tested in future course of work.

2. MATERIAL AND METHODS

All the chemicals used were laboratory grade and procured from Fisher Scientific, S.D. Fine Chemicals Ltd., CDH and Rankem. All compounds were purified by column chromatography and recrystallisation, and confirmatory establishment of structure was done by IR, ^{1}H NMR, Mass Spectroscopy and elemental analysis. Column chromatography was performed using silica gel (Qualigens, particle size 60-120 mm). Thin layer chromatography (TLC) plates (silica gel G) were used to confirm the purity of commercial reagents used, compounds synthesized and to monitor the reactions as well. All melting points were recorded on a DECIBEL digital melting point apparatus and are shown uncorrected. IR spectra were recorded on an 8400S SHIMADZU spectrometer (KBr Pellets). 1H NMR spectra were recorded on a dpx300 spectrometer using TMS as internal standard in DMSO. Mass spectras were recorded on an API 3000 LC/MS/

MS Q3 (SHIMADZU) spectrometer. Physical properties of the synthesized compounds are listed in Table 1, and scheme of synthesis is given in Fig. 2.
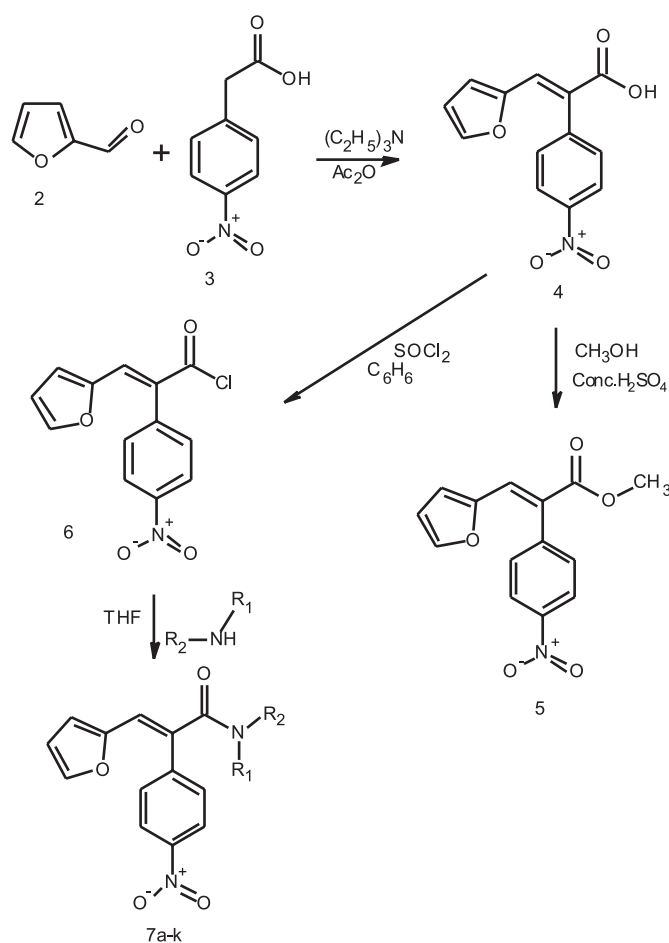

Fig. 2. Scheme of synthesis of compounds.

Procedure of synthesis of compound 4

A mixture of p-nitrophenyl acetic acid 3 (2 mmol), furfuraldehyde 2 (2 mmol), and triethylamine (0.5 ml) in acetic anhydride (5 ml) was refluxed for 12 hours, poured into hot saturated sodium carbonate solution (50 ml) and left ovenight. The mixture was extracted with diethyl ether (2×50 ml), and the ether extracts were discarded. The aquous solution was acidified with dilute HCl. The precipitated product was filtered with vaccum pump and dried. Product was subjected to column chromatography.

(2E)-3-(2-furyl)-2-(4-nitrophenyl)acrylic acid (4): Yield: 47%; Anal. Calcd: C, 60.24; H, 3.50; N, 5.40. Found: C, 60.25; H, 3.51; N, 5.42. FTIR (KBr): cm^{-1} 3041 and 858 (C-H), 3020 and 748 (Ar-H), 1704 (C=O), 1668 (C=C), 1596 and 1411 (COO$^-$), 1519 and 1342 (C-NO$_2$), 1234 and 1000 (C-O); ^{1}H NMR (DMSO): δ ppm 10.11 (1H, s), 8.25 (2H, d), 8.18 (1H, d), 7.86 (1H, s), 7.67 (1H, d), 7.46 (2H, d), 6.83 (1H, t); MS (TISI) 259.0 (M$^+$).

Procedure of synthesis of compound 5

Con centrated H_2SO_4 (0.5 ml) was added to a stirred solution of carboxylic acid 4 (0.5 mmol) in absolute methanol (20 ml), and the mixture was heated under reflux for 6 hours. About 90% of excess methanol was removed by evaporation, and the residue was poured into ice-water (300 ml). The product was extracted with diethyl ether (2×40 ml), and the combined extracts were washed with 2% NaOH solution (2×50 ml) followed by water (200 ml). Product was obtained from ether fraction. Product was purified by recrystallization from EtOAc-hexane.

Methyl (2E)-3-(2-furyl)-2-(4-nitrophenyl)acrylate (5): Yield: 59%; Anal. Calcd: C, 61.54; H, 4.06; N, 5.13. Found: C, 61.55; H, 4.07; N, 5.15. FTIR (KBr): cm^{-1} 3038 and 857 (C-H), 2924 and 1458 (CH_3), 1736 (C=O), 1689 (C=C), 1601 (C=C of Ar), 1522 and 1345 (C-NO_2), 1220, 1131 and 1018 (C-O), 748 (Ar-H); ^1H NMR (DMSO): δ ppm 8.26 (2H, d), 7.92 (1H, d), 7.70 (1H, s), 7.41-7.67 (3H, m), 6.87 (1H, t), 3.52 (3H, s); MS (TISI) 273.0 (M^+).

Procedure of synthesis of compound 6

A mixture of carboxylic acid 4 (0.5 mmol) and thionyl chloride (1ml) in benzene (10 ml) was refluxed for 6 hours. The excess thionyl chloride and benzene were removed under reduced pressure, and the residue was kept under vaccum for 30 minutes, and dried to give required product. Product was purified by recrystallization from EtOAc-hexane.

(2E)-3-(2-furyl)-2-(4-nitrophenyl)acryloyl chloride (6): Yield: 43%; Anal. Calcd: C, 56.23; H, 2.90; N, 5.04. Found: C, 56.22; H, 2.91; N, 5.07. FTIR (KBr): cm^{-1} 3041 and 858 (C-H), 3022 and 758 (Ar-H), 1798 (C=O), 1687 (C=C), 1607 (C=C of Ar), 1524 and 1345 (C-NO_2), 1258 and 1018 (C-O), 717 (C-Cl); ^1H NMR (DMSO): δ ppm 8.31 (1H, s), 8.26 (2H, d), 8.09 (1H, d), 7.72 (1H, d), 7.43 (2H, d), 6.90 (1H, t); MS (TISI) 277.0 (M^+).

Procedure of synthesis of compounds 7a-k

A solution of appropriate amine (0.5 mmol) in THF (5 ml) was added to a solution of acid chloride (prepared from 4 in 0.5 mmol scale, as described above) in THF (10 ml). The mixture was stirred for 3 hours. Solvents were romoved under reduced pressure, and the residue was poured onto ice (200 g). The product was extracted with diethyl ether (2×20 ml), washed with water and dried. Crude product was obtained from ether fraction. Product was purified by recrystallization from EtOAc-hexane.

(2E)-N-ethyl-3-(2-furyl)-2-(4-nitrophenyl)acrylamide (7a): Yield: 21%; Anal. Calcd: C, 62.93; H, 4.93; N, 9.79. Found: C, 62.92; H, 4. 91; N, 9.78. FTIR (KBr): cm^{-1} 3426 and 1559 (N-H), 3043 and 856 (C-H), 2893 (CH_3), 1678 (C=O), 1661 (C=C), 1600 (C=C of Ar), 1522 and 1342 (C-NO_2), 1465 (CH_2), 1238 and 1002 (C-O), 748 (Ar-H); ^1H NMR (DMSO): δ ppm 9.20 (1H, s), 8.24-8.34 (3H, m), 7.93 (1H, s), 7.58 (1H, d), 7.52 (2H, d), 6.88 (1H, t), 2.92 (2H, q), 1.17 (3H, t); MS (TISI) 286.1 (M^+).

(2E)-N-carbamothioyl-3-(furan-2-yl)-2-(4-nitrophenyl) prop-2-enamide (7b): Yield: 36%; Anal. Calcd: C, 55.43; H, 4.32; N, 13.85. Found: C, 55.42; H, 4.31; N, 13.89. FTIR (KBr):

cm^{-1} 3470, 3348 and 1601 (NH_2), 3438 (N-H), 3044 and 856 (C-H), 3023 and 750 (Ar-H), 1691 (C=O), 1665 (C=C), 1522 and 1344 (C-NO_2), 1236 and 1002 (C-O), 1119 (C=S); ^1H NMR (DMSO): δ ppm 9.56 (2H, s), 8.49 (1H, s), 8.28 (2H, d), 8.08 (1H, d), 7.79 (1H, s), 7.70 (1H, d), 7.39 (2H, d), 6.84 (1H, t); MS (TISI) 303.0 (M^+).

(2E)-N-(4-fluorophenyl)-3-(furan-2-yl)-2-(4-nitrophenyl) prop-2-enamide (7c): Yield: 23%; Anal. Calcd: C, 64.77; H, 3.72; N, 7.95. Found: C, 64.72; H, 3.71; N, 7.99. FTIR (KBr): cm^{-1} 3434 (N-H), 3047 and 857 (C-H), 3026 and 749 (Ar-H), 1689 (C=O), 1662 (C=C), 1599 (C=C of Ar), 1520 and 1345 (C-NO_2), 1236 and 1000 (C-O), 1123 (C-F); ^1H NMR (DMSO): δ ppm 9.61 (1H, s), 8.30 (2H, d), 8.08 (1H, d), 7.76 (3H, m), 7.66 (1H, s), 7.41 (2H, d), 7.22 (2H, d), 6.93 (1H, t); MS (TISI) 352.1 (M^+).

(2E)-3-(furan-2-yl)-N-(2-methylphenyl)-2-(4-nitrophenyl) prop-2-enamide (7d): Yield: 55%; Anal. Calcd: C, 68.96; H, 4.63; N, 8.04. Found: C, 68.94; H, 4.65; N, 8.05. FTIR (KBr): cm^{-1} 3427 (N-H), 3056 and 875 (C-H), 3029 and 750 (Ar-H), 2945 and 1461 (CH_3), 1684 (C=O), 1669 (C=C), 1597 (C=C of Ar), 1515 and 1340 (C-NO_2), 1244 and 1005 (C-O); ^1H NMR (DMSO): δ ppm 9.43 (1H, s), 8.28 (2H, d), 8.08 (1H, d), 7.79 (1H, s), 7.69 (1H, d), 7.04-7.46 (6H, m), 6.95 (1H, t), 2.31 (3H, s); MS (TISI) 348.1 (M^+).

(2E)-N-(2-chlorophenyl)-3-(furan-2-yl)-2-(4-nitrophenyl) prop-2-enamide (7e): Yield: 46%; Anal. Calcd: C, 61.88; H, 3.55; N, 7.60. Found: C, 61.87; H, 3.52; N, 7.61. FTIR (KBr): cm^{-1} 3436 (N-H), 3065 and 878 (C-H), 3030 and 748 (Ar-H), 1679 (C=O), 1665 (C=C), 1602 (C=C of Ar), 1524 and 1342 (C-NO_2), 1232 and 999 (C-O), 708 (C-Cl); ^1H NMR (DMSO): δ ppm 9.66 (1H, bs), 8.31 (2H, d), 8.06 (1H, d), 7.98 (1H, d), 7.74 (1H, s), 7.59-7.68 (2H, m), 7.45 (2H, d), 7.19-7.31 (2H, m), 6.84 (1H, t); MS (TISI) 368.0 (M^+).

(2E)-3-(furan-2-yl)-2-(4-nitrophenyl)-N-(pyridin-4-yl) prop-2-enamide (7f): Yield: 32%; Anal. Calcd: C, 64.47; H, 3.91; N, 12.53. Found: C, 64.48; H, 3.92; N, 12.55. FTIR (KBr): cm^{-1} 3444 (N-H), 3049 and 853 (C-H), 3027 and 749 (Ar-H), 1687 (C=O), 1661 (C=C), 1642 (C=N-C), 1600 (C=C of Ar), 1520 and 1345 (C-NO_2), 1234 and 1004 (C-O); ^1H NMR (DMSO): δ ppm 9.75 (1H, s), 8.48 (2H, d), 8.26 (2H, d), 8.06 (1H, d), 7.79 (1H, s), 7.71 (1H, d), 7.28-7.45 (4H, m), 6.97 (1H, t); MS (TISI) 335.1 (M^+).

(2E)-3-(furan-2-yl)-N-(naphthalen-1-yl)-2-(4-nitrophenyl) prop-2-enamide (7g): Yield: 16%; Anal. Calcd: C, 71.87; H, 4.20; N, 7.29. Found: C, 71.88; H, 4.22; N, 7.30. FTIR (KBr): cm^{-1} 3425 (N-H), 3066 and 763 (Ar-H), 3031 and 860 (C-H), 1684 (C=O), 1652 (C=C), 1599 (C=C of Ar), 1518 and 1345 (C-NO_2), 1240 and 1006 (C-O); ^1H NMR (DMSO): δ ppm 9.53 (1H, s), 8.31 (2H, d), 8.02-8.09 (3H, m), 7.79 (1H, s), 7.29-7.70 (7H, m), 7.02 (1H, d), 6.88 (1H, t); MS (TISI) 384.1 (M^+).

(2E)-3-(furan-2-yl)-2-(4-nitrophenyl)-1-(piperidin-1-yl) prop-2-en-1-one (7h): Yield: 35%; Anal. Calcd: C, 66.25; H, 5.56; N, 8.58. Found: C, 66.26; H, 5.57; N, 8.55. FTIR (KBr): cm^{-1} 3015 and 860 (C-H), 2958 and 1455 (CH_2), 1677 (C=O), 1644 (C=C), 1599 (C=C of Ar), 1526 and 1340 (C-NO_2), 1242

and 1006 (C-O), 1196 (NR$_3$), 748 (Ar-H); ^{1}H NMR (DMSO): δ ppm 8.27 (2H, d), 8.06 (1H, d), 7.79 (1H, s), 7.70 (1H, d), 7.44 (2H, d), 6.85 (1H, t), 3.57 (4H, t), 1.41-1.54 (6H, m); MS (TISI) 326.1 (M$^+$).

(2E)-3-(furan-2-yl)-2-(4-nitrophenyl)-1-(piperazin-1-yl) prop-2-en-1-one (7i): Yield: 41%; Anal. Calcd: C, 62.38; H, 5.23; N, 12.84. Found: C, 62.39; H, 5.22; N, 12.87. FTIR (KBr): cm^{-1} 3444 (N-H), 3037 and 854 (C-H), 3022 and 749 (Ar-H), 2955 and 1454 (CH$_2$), 1665 (C=O), 1598 (C=C of Ar), 1520 and 1345 (C-NO$_2$), 1247 and 1001 (C-O), 1195 (NR$_3$); ^{1}H NMR (DMSO): δ ppm 8.30 (2H, d), 8.05 (1H, d), 7.80 (1H, s), 7.69 (1H, d), 7.34 (2H, d), 6.86 (1H, t), 3.42 (4H, t), 2.82 (4H, t), 1.76 (1H, s); MS (TISI) 327.1 (M$^+$).

(2E)-3-(furan-2-yl)-N-(2-methoxyphenyl)-2-(4-nitrophenyl)prop-2-enamide (7j): Yield: 64%; Anal. Calcd: C, 65.93; H, 4.43; N, 7.69. Found: C, 65.96; H, 4.44; N, 7.70. FTIR

(KBr): cm^{-1} 3437 (N-H), 3036 and 855 (C-H), 3023 and 748 (Ar-H), 2945 and 1463 (CH$_3$), 1691 (C=O), 1661 (C=C), 1601 (C=C of Ar), 1518 and 1342 (C-NO$_2$), 1241 and 1009 (C-O); ^{1}H NMR (DMSO): δ ppm 9.81 (1H, s), 8.29 (2H, d), 8.08 (1H, d), 7.81 (1H, d), 7.72 (1H, s), 7.66 (1H, d), 7.40 (2H, d), 6.99-7.07 (3H, m), 6.88 (1H, t), 3.89 (3H, s); MS (TISI) 364.1 (M$^+$).

(2E)-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-3-(furan-2-yl)-2-(4-nitrophenyl) prop-2-enamide (7k): Yield: 52%; Anal. Calcd: C, 64.86; H, 4.54; N, 12.61. Found: C, 64.88; H, 4.53; N, 12.62. FTIR (KBr): cm^{-1} 3422 (N-H), 3033 and 854 (C-H), 3021, 748 and 704 (Ar-H), 2925 and 1471 (CH$_3$), 1713 and 1677 (C=O), 1661 (C=C), 1601 (C=C of Ar), 1518 and 1342 (C-NO$_2$), 1230 and 1002 (C-O), 1185 (NR$_3$); ^{1}H NMR (DMSO): δ ppm 9.58 (1H, s), 8.25 (2H, d), 8.08 (1H, d), 7.79 (1H, s), 7.66 (1H, d), 7.25-7.53 (6H, m), 6.89-6.96 (2H, m), 3.37 (3H, s), 2.46 (3H, s); MS (TISI) 444.1 (M$^+$).

Table 1. Physical data of synthesized compounds.
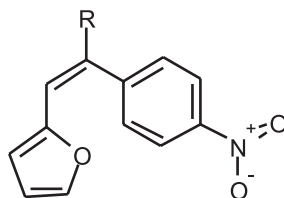
Compound No.	R	MP°C	R$_f$ value[a]	Recrystallization solvent	Molecular Formula
4	Carboxyl	169-171	0.600	EtOAc-Hexane (1:1)	C$_{13}$H$_9$NO$_5$
5	Methoxycarbonyl	198-200	0.808	EtOAc-Hexane (1:1)	C$_{14}$H$_{11}$NO$_5$
6	Chlorocarbonyl	119-121	0.900	EtOAc-Hexane (1:1)	C$_{13}$H$_8$ClNO$_4$
7a	Ethylcarbamoyl	129-131	0.566	EtOAc-Hexane (1:1)	C$_{15}$H$_{14}$N$_2$O$_4$
7b	Carbamothioylcarbamoyl	137-139	0.713	EtOAc-Hexane (1:1)	C$_{14}$H$_{11}$N$_3$O$_4$S
7c	4-fluorophenylcarbamoyl	124-126	0.661	EtOAc-Hexane (1:1)	C$_{19}$H$_{13}$FN$_2$O$_4$
7d	2-methylphenylcarbamoyl	116-118	0.721	EtOAc-Hexane (1:1)	C$_{20}$H$_{16}$N$_2$O$_4$
7e	2-chlorophenylcarbamoyl	100-102	0.682	EtOAc-Hexane (1:1)	C$_{19}$H$_{13}$ClN$_2$O$_4$
7f	pyridine-4-ylcarbamoyl	175-177	0.555	EtOAc-Hexane (1:1)	C$_{18}$H$_{13}$N$_3$O$_4$
7g	naphthalene-1-ylcarbamoyl	110-112	0.709	EtOAc-Hexane (1:1)	C$_{23}$H$_{16}$N$_2$O$_4$
7h	Piperidin-1-ylcarbonyl	150-152	0.425	EtOAc-Hexane (1:1)	C$_{18}$H$_{18}$N$_2$O$_4$
7i	Piperazin-1-ylcarbonyl	178-180	0.589	EtOAc-Hexane (1:1)	C$_{17}$H$_{17}$N$_3$O$_4$
7j	2-methoxyphenylcarbamoyl	112-114	0.540	EtOAc-Hexane (1:1)	C$_{20}$H$_{16}$N$_2$O$_5$
7k	Aminoantipyrinylcarbonyl	134-136	0.737	EtOAc-Hexane (1:1)	C$_{24}$H$_{20}$N$_4$O$_5$

a The solvent system used for TLC was cyclohexane:carbon tetrachloride:methanol in ratio 10:10:1.

3. PHARMACOLOGY

CAM assay is routinely used as a preliminary method to determine antiangiogenic effect of a compound. This assay is based upon the formation of a chorioallantoic membrane, in which neovascularization takes place, in fertilized chicken eggs at a certain stage of the development of the embryo. Agarose pellets impregnated with the test compound are placed onto the vascular membrane of opened eggs, and the influence on angiogenesis is evaluated [33]. For assay purpose the fertile chicken eggs were procured from Kalchina hatchery, Modinagar.

Antiangiogenesis study by chorioallantoic membrane (CAM) assay

Twelve eggs were used per experiment to test one compound as a given dose. The eggs were fertilized at 37^{0}C and 80% relative humidity in ideal conditions. The shells of eggs were cleaned with 70% EtOH to avoid infections. After 72 hrs 8-10 ml of albumin was removed with a syringe at the lower side of the egg, and the hole was sealed with tape. Subsequently the upper part of the shell was removed, and the eggs were covered with a plastic film and incubated for another 72 hrs. At this point of

time, when the diameter of CAM is between 1.8 and 2.6 cm, the pellets containing the test substances were placed on the CAM. Test substances were dissolved or suspended in a 2.5% agarose solution. After gel formation, the volume of agarose gel corresponding to the dose of the test compound to be applied to the CAM was taken by means of a micropipette for viscous solutions. Therefore the agarose pellets do not have a uniform size. The half-cone-shaped agarose pellets are fixed because they slightly sink into the CAM. After 24 hrs the antiangiogenic effect was measured after addition of cream as a contrast fluid, by means of a stereomicroscope, by observing the avascular zone surrounding the pellet. Antiangiogenic activity is expressed as a score where 0 = no or weak effect (size of capillary free zone is less than the pellet), 1 = medium effect (capillary free zone is as large as the pellet), and 2 = strong effect (capillary free zone is at least twice as large as the pellet). Also membrane irritation and embryotoxicity can be evaluated. β-1,4-galactan sulfate (LuPS S5) with an average molecular weight of 20000 was used as positive control [34] and an agarose pellet as a blank.

4. RESULTS AND DISCUSSION

In present work we synthesized some novel 2-[(z)-2-(4-nitrophenyl)ethenyl]furan analogues. Compounds were synthesized by base catalysed condensation of p-nitrophenyl
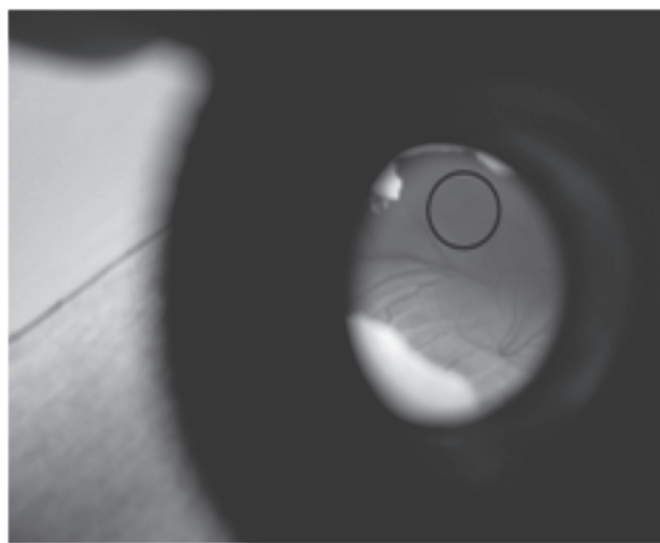
Fig. 3. Figure showing capillary free zone for compound 7h in CAM assay (photograph is taken through a dissecting microscope).

acetic acid with furfuraldehyde, in the presence of triethylamine, followed by esterification or reaction with thionyl chloride, followed by reaction with appropriate amine to give 2-[(z)-2-(4-nitrophenyl)ethenyl]furan analogues. Spectral data of all the synthesized compounds were found in full agreement with the proposed structures. The antiaogenic activity of the synthesized compounds is listed in table 2. All the compounds were tested at a dose of 10μg/pellet, corresponding to approximately 30nmol/pellet, because at higher dose most of compounds showed a toxic effect. Compounds 6 and 7h showed an antiangiogenic score of more than 1. All other compounds

showed antiangiogenic score less than 1. All compounds showed significant inhibition of angiogenesis when compared to control (agarose pellet) but only two analogues (6 and 7h) had activity closer to that of standard. No analogue was found to have activity greater than the standard. Compound 7h was found to be most potent with a score of 1.3 ± 0.1 (capillary free zone for compound 7h is shown in Figure 3).

5. CONCLUSIONS

The result of present study shows that synthesized compounds have significant antiangiogenic activity. The analogues having smaller groups like COOH, COOCH₃ or COCl as bridge substituents as in compounds 4, 5 and 6, were more active than the analogues having comparatively bulkier groups. Potent activity of compound 7h is exception in this regard. Compounds 7c-g, having aromatic substituents, were least active. So, we can say that compound possessing the Piperidin-1-ylcarbonyl, carboxyl, methoxycarbonyl and chlorocarbonyl moiety on 2-[(z)-2-(4-nitrophenyl)ethenyl]furan skeleton are among the most active compounds in our study. The present study yielded two significantly antiangiogenic compounds having chlorocarbonyl/piperidin-1-ylcarbonyl moiety on 2-[(z)-2-(4-nitrophenyl)ethenyl]furan skeleton. Further exploration of this moiety may lead to finding of novel antiangiogenic leads.

Table 2. Antiangiogenic activity of synthesized compounds in the CAM assay.

Test compound	Concentration		Antiangiogenic score[b] ± sd (n = no. of experiment)
	(μg/pellet)	nmol/pellet	
4	10	39	0.8 ± 0.1 (n =3)
5	10	37	0.9 ± 0.1 (n =2)
6	10	36	1.1 ± 0.1 (n =3)
7a	10	37	0.8 ± 0.1 (n =2)
7b	10	32	0.6 ± 0.2 (n =2)
7c	10	28	0.2 ± 0.3 (n =2)
7d	10	29	0.5 ± 0.1 (n =2)
7e	10	27	0.3 ± 0.1 (n =2)
7f	10	30	0.4 ± 0.1 (n =2)
7g	10	26	0.2 ± 0.1 (n =2)
7h	10	31	1.3 ± 0.1 (n =2)
7i	10	31	0.9 ± 0.1 (n =2)
7j	10	27	0.6 ± 0.1 (n =2)
7k	10	23	0.2 ± 0.1 (n =2)
Agarose pellet			0.1 ± 0.1 (n =10)
β-1,4-galactansulphate (LuPS S5)	50	2.5	1.4 ± 0.1 (n =10)

b Antiangiogenic score was calculated as diameter of capillary free zone divided by diameter of the pellet of the compound.

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