ORIGINAL RESEARCH ARTICLES

SYNTHESIS AND BIOLOGICAL EVALUATION OF 1-SUBSTITUTED-2-THIENYL-5-(4-CHLOROPHENYL) PYRAZOLINE DERIVATIVES AS ANTIPROLIFERATIVE AGENTS

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ABSTRACT

Cancer is not a single disease, but a large group of diseases characterized by uncontrolled, rapid and pathological proliferation of abnormally transformed cells. Pyrazoline is a five-membered heterocyclic ring having two adjacent nitrogen atoms within the ring. It has only one endocyclic double bond and is basic in nature. The present study involves synthesis of 1-substituted-2-thienyl-5-(4-chlorophenyl) pyrazoline derivatives. The synthesized compounds were subjected to anticancer screening against SK-OV-3 cells line to determine the growth inhibitory effects of the compounds. Amongst all the derivatives in series (6a-j), the pyrazoline derivatives exhibited potent anticancer activity. All synthesized compounds possessed good to moderate anticancer activity. Compounds 6b and 6c at concentration 80 µg/mL possessed % control growth inhibition comparable to standard drug andriamycin. The order for the % control growth inhibition of SK-OV-3 was found to be 6h> 6j> 6f> 6i> 6e> 6g> 6d> 6a. All the compounds inhibited 50 % of the cell growth at the conc. <10 µg/mL. Compounds 6f and 6g inhibited total cell growth at the conc. <10 µg/mL and 65.9 µg/mL, respectively. The structures of the synthesized compounds were established by IR and NMR spectral studies.

Keywords: Pyrazoline, Endocyclic, Anticancer, SK-OV-3, Andriamycin

INTRODUCTION

Cancer is not a single disease, but a large group of diseases characterized by uncontrolled, rapid and pathological proliferation of abnormally transformed cells. Despite recent advances in cancer therapy, cancer is still the second leading cause of death after cardiovascular disorders throughout the world. Resistance to chemotherapeutic agents remains a key challenge in the fight against cancer. Generally, anticancer drugs destroy normal cells as well as cancer cells and often cause serious adverse effects[1-4]. In 2016, an estimated 1,685,210 new cases of cancer had been diagnosed in the United States and 595,690 people had died from the disease. The most common cancers in 2016 were breast cancer, lung and bronchus cancer, prostate cancer, colon and rectum cancer, bladder cancer, melanoma of the skin, non-Hodgkin lymphoma, thyroid cancer, kidney and renal pelvis cancer, leukemia, endometrial cancer and pancreatic cancer. The number of new cases of cancer (cancer incidence) is 454.8 per 100,000 men and women per year (based on 2008-2012 cases). The number of cancer deaths (cancer mortality) is 171.2 per 100,000 men and women per year (based on 2008-2012 deaths). Cancer mortality is higher among men than women (207.9 per 100,000 men and 145.4 per 100,000 women). It is highest in African American men (261.5 per 100,000) and lowest in Asian/Pacific islander women (91.2 per 100,000) (based on 2008-2012 deaths). The number of people living beyond a cancer diagnosis reached nearly 14.5 million in 2014 and is expected to rise to almost 19 million by 2024. National expenditures for cancer care in the United States totaled nearly $125 billion in 2010 and could reach $156 billion in 2020. Around one third of deaths from cancer are due to the 5 leading behavioral and dietary risks: high body mass index, low fruit and vegetable intake, lack of physical activity, tobacco use and alcohol use. Tobacco use is the most important risk factor for cancer and is responsible for approximately

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22% of cancer deaths. The design of new compounds to deal with the resistance problem has become one of the most important goals of anticancer research today[5].

Pyrazoline is heterocyclic having two adjacent nitrogen atoms within in ring. It has only one endocyclic double bond and is basic in nature[6]. They are also known as dihydropyrazoles and their chemistry is closely related to pyrazoles. Among its various derivatives, 2-pyrazolines seem to be frequently studied pyrazoline type compounds[7]. 2-Pyrazolines can be considered to have a cyclic hydrazine moiety [8]. It plays a crucial role in the development of theory in heterocyclic chemistry and is also extensively used as useful synthons in organic synthesis[9]. 2-pyrazolines display a broad spectrum of potential pharmacological activities and are present in a number of pharmacologically active molecules such as phenazone/amidopyrene/ methampyrone (analgesic and antipyretic), azolid/tandearil (anti-inflammatory), indoxacarb (insecticidal) and anturane (uricosuric)[10]. Pyrazoline derivatives were found to have potential antipyretic-analgesic, tranquillizing muscle relaxant, psychoanaleptic, antiepileptic, antidepressant, anti-inflammatory, insecticidal, antimicrobial and antihypotensive activities[11]. Their derivatives were also found to exhibit cytotoxic activity, inhibitory activity of platelet aggregation, herbicidal activity and cannabinoid CB_1-receptor modulation. Pyrazoline interest extended to dyes and dye couplers too. These heterocyclic compounds are widely occurring in nature in the form of alkaloids, vitamins, pigments and as constituents of plant and animal cell[12]. Several pyrazolines act as anticancer agents[13, 14] and tubulin assembly inhibitors[15]. Pyrazoloacridine (PZA) is a new anticancer drug currently undergoing phase II clinical trials[16,18]. Axitinib (AG013736) is a vascular endothelial growth factor receptor (VEGFR) inhibitor used in clinical treatment[19, 20]. Doramapimod (BIRB-796) is a selective *p*38α mitogen-activated protein kinase (MAPK) inhibitor undergoing phase III clinical trials [21, 22]. 3-(5'-Hydroxymethyl-2'-furyl)-1-benzyl indazole (YC-1) is a hypoxia-inducible factor (HIF)-1 inhibitor as well as a VEGF inhibitor [23, 24]. The compounds containing pyrazole nucleus like sulphenazole, celecoxib and analgin are well established in the market[25].

MATERIALS AND METHODS

The commercial chemicals employed for the present work were purchased from Spectrochem and Loba Chem. All the solvents used for the reaction were of LR grade and purified before use in different reactions. Thin layer chromatography was carried on pre coated (Merck 60F_{254}) and self-prepared silica gel coated plates for monitoring the reaction. The solvent system used for developing the chromatogram was petroleum ether: ethyl acetate in variable ratios. UV chambers were used for visualization of TLC spots. The identification and characterization of the compounds were carried out by determining melting points on a melting point apparatus by capillary method and were uncorrected. All the IR spectra of the synthesized compounds were recorded on Bruker alpha-E FTIR-ATR. ^{1}H NMR spectra were recorded on Bruker Avance II (400MHz) spectrometer using $CDCl_3$ as solvent at SAIF, Punjab University, Chandigarh.

Synthesis of 3-(4-chlorophenyl)-1-(thiophen-2-yl) prop-2-en-1-one (3)

A mixture of 2-acetylthiophene (0.04 M) (1) and 4-chlorobenzaldehyde (0.04M) (2) and 10% aqueous sodium hydroxide (10 mL) in ethanol (30 mL) was stirred at room temperature for 6-8 h. The reaction mixture (3) was poured into crushed ice. The precipitated solid was filtered, washed with water and dried. The product was crystallized from ethanol. State: off-white powder, Yield: 70.7%, M. Pt: 120-122°C; R_f 0.66 (Petroleum ether: Ethylacetate, 7:3V/V)

Synthesis of 5-(4-Chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazole (4)

A mixture of 3-(4-chlorophenyl)-1-(thiophen-2-yl) prop-2-en-1-one (0.01 Mol) (3) and hydrazine hydrate (0.02 M) in ethanol (30 mL) was refluxed for 5 h. The reaction mixture (4) was cooled and kept at 0 °C overnight. The resulting solid was filtered and dried. The product was crystallized from ethanol. State: brown powder, Yield: 53.7%, M. Pt: 72-75°C, R_f 0.70 (Petroleum ether : Ethylacetate, 7:3V/V)

Synthesis of 1-(chloroacetyl)-3-(2-thienyl)-5-(4-chlorophenyl)-2-pyrazoline (5)

5-(4-Chlorophenyl)-3-(2-thienyl)-2-pyrazoline (0.007 M) (4) and triethylamine (0.007M) were dissolved in toluene (30 mL). The reaction mixture (5) was cooled in an ice bath and chloroacetyl chloride (0.007M) was added drop wise with constant stirring. The mixture thus obtained was further agitated for 1 h at room temperature. The solvent was evaporated to dryness under reduced pressure. The residue was washed with water and the product was recrystallized from ethanol. State: Dark brown powder, Yield: 77.8%, M. Pt: 60-62°C, R_f 0.61 (Petroleum ether: Ethylacetate, 7:3 V/V)

Synthesis of 1-substituted-(5-(4-chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydropyrazol-1-yl)ethanone (6a-j)

A mixture of 1-(chloroacetyl)-3-(2-thienyl)-5-(4-chlorophenyl)-2-pyrazoline (0.005M) (5) and substituted aromatic amine (0.005 M) was treated in acetone (30 mL) at room temperature for 8 h. The solvent was evaporated; the residue was washed with water and recrystallized from ethanol (6a-j).

2-(1*H*-Benzoimidazol-2-ylamino)-1-(5-(4-chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydropyrazol-1-yl)ethanone (6a)

State: Dark grey powder, Yield: 56.64%, M. Pt: 190-193°C, R_f 0.55 (Petroleum ether: Ethyl acetate, 7:3); **IR (ν, cm^{-1}),** 3052 (Ar-C-H), 1661 (C=O), 726 (Ar-C-Cl), 3317 (N-H), 1529 (C=N), 1016 (C-S-C), 1238 (C-N), 1466 (Ar-C=C); **^{1}HNMR (CDCl$_3$),** 1.37-1.41 (t, *J* = 7.32 Hz, 1H, CH_2-pyrazoline), 2.04 (s, 1H, CH_2-pyrazoline), 4.53-4.54 (d, *J* = 6.12 Hz, 1H, CH), 5.03-5.07 (d, *J* = 15.8 Hz, 1H, N-H), 5.52-5.55 (m, 1H, N-H), 7.08-7.87 (m. 11H, Ar-H).

1-(5-(4-Chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydropyrazol-1-yl)-2-(6-methoxy benzothiazol-2-ylamino) ethanone (6b)

State: Brown powder, Yield: 66.54%, M. Pt: 85-87°C, R_f 0.70 (Petroleum ether: Ethyl acetate, 7:3); **IR (ν, cm^{-1}),** 2930 (Ar-C-H), 1661 (C=O), 709 (Ar-C-Cl), 3086 (N-H), 1537 (C=N), 1020 (C-S-C), 1462 (Ar-C=C); **^{1}HNMR (CDCl$_3$),** 1.34-1.37 (t, *J* = 7.24 Hz, 2H, Ar-pyrazoline), 3.70-3.83 (m, 2H, CH_2, OCH_3), 4.49-4.58 (m, 2H, CH, N-H), 7.07-7.75 (m, 10H, Ar-H).

1-(5-(4-Chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydropyrazol-1-yl)-2-(4 nitrophenyl amino)ethanone (6c)

State: Grey powder, Yield: 52.71%, M. Pt: 110-112°C, R_f 0.76 (Petroleum ether: Ethyl acetate, 7:3); **IR (ν, cm^{-1}),** 3078 (Ar-C-H), 1635 (C=O), 707 (Ar-C-Cl), 3474 (N-H), 1015 (C-S-C), 1590 (Ar-NO_2), 1484 (Ar-C=C), 1298 (C-N); **^{1}HNMR (CDCl$_3$),** 1.29-1.36 (m,1H,CH_2-pyrazoline), 2.55 (s, 1H, CH_2-pyrazoline), 3.69-3.85 (m, 1H, CH_2), 4.06-4.07 (d, *J*= 4.72 Hz, 1H, N-H), 4.38-4.58 (m, 1H, CH), 6.31-6.75 (m, 2H, Ar-H), 7.05-7.99 (m, 9H, Ar-H).

1-(5-(4-Chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydropyrazol-1-yl)-2-(phenylamino) ethanone (6d)

State: Brown powder, Yield: 64.08%, M. Pt: 140-142°C, R_f 0.70 (Petroleum ether: Ethyl acetate, 7:3); **IR (ν cm^{-1}),** 2805 (Ar-C-H), 1654 (C=O), 742 (Ar-C-Cl), 3380 (N-H), 1019 (C-S-C), 1456 (Ar-C=C), 1595 (C=N); **^{1}HNMR (CDCl$_3$),** 1.19-1.25 (m,1H,CH_2-pyrazoline), 2.56 (s, 1H, CH_2-pyrazoline), 3.68-3.82 (m, 1H, CH_2), 4.29-4.39 (m,1H, N-H), 4.52-4.54 (d, *J*= 8.56 Hz, 1H, CH), 6.65-6.78 (m, 3H, Ar-H), 7.06-7.69 (m, 9H, Ar-H).

1-(5-(4-Chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydropyrazol-1-yl)-2-(4-chloro phenyl amino) ethanone (6e)

State: Dark red powder, Yield: 85.31%, M. Pt: 133-135°C, R_f 0.77 (Petroleum ether: Ethyl acetate, 7:3); **IR (ν cm^{-1}),** 2813 (Ar-C-H), 1648 (C=O), 709 (Ar-C-Cl), 3388 (N-H), 1015 (C-S-C), 1484 (Ar-C=C), 1591 (C=N); **^{1}HNMR (CDCl$_3$),** 1.37-1.40 (t,*J*= 7.34 Hz, 2H,CH_2-pyrazoline), 4.48-4.53 (m, 1H, CH), 5.51-5.54 (m, 1H, N-H), 7.09-7.27 (m, 11H, Ar-H).

1-(5-(4-Chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydropyrazol-1-yl)-2-(3-chloro phenyl amino) ethanone (6f)

State: Dark brown powder, Yield: 66.26%, M. Pt: 137-139°C, R_f 0.69 (Petroleum ether: Ethyl acetate, 7:3); **IR (ν cm^{-1}),** 2787 (Ar-C-H), 1659 (C=O), 672 (Ar-C-Cl), 3395 (N-H), 1014 (C-S-C), 1473 (Ar-C=C), 1515 (C=N); **^{1}HNMR (CDCl$_3$),** 1.37-1.40 (t, *J*= 7.06 Hz, 1H,CH_2-pyrazoline), 2.57 (s, 1H, CH_2-pyrazoline), 3.77-3.85 (m, 1H, CH_2), 4.25-4.36 (m, 1H, N-H), 4.49-4.58 (m, 1H, CH), 6.55-6.57 (m, 2H, Ar-H), 6.65-6.70 (m, 1H, Ar-H), 7.05-7.51 (m, 8H, Ar-H).

1-(5-(4-Chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydropyrazol-1-yl)-2-(5-phenyl-1,3,4 -thiadiazol-2-ylamino)ethanone (6g)

State: Yellow powder, Yield: 60%, M. Pt: 144-146°C, R_f 0.75 (Petroleum ether: Ethyl acetate, 7:3); **IR (ν cm^{-1}),** 3093 (Ar-C-H), 1662 (C=O), 705 (Ar-C-Cl), 3454 (N-H), 1014 (C-S-C), 1449 (Ar-C=C), 1593 (C=N); **^{1}HNMR (CDCl$_3$),** 2.16-2.17 (d, *J*= 2.44 Hz, 1H,CH_2-pyrazoline), 2.62 (s, 1H, CH_2-pyrazoline), 3.74-3.82 (m, 1H, CH_2), 4.17 (s, 1H, N-H), 4.48-4.57 (m, 1H, CH), 7.05-7.85 (m, 12H, Ar-H).

2-(5-(4-Chlorophenyl)-1,3,4-thiadiazol-2-ylamino)-1-(5-(4-chlorophenyl)-3-(thiophen-2-yl) 4, 5-dihydropyrazol-1-yl)ethanone (6h)

State: Dark brown powder, Yield: 70.9%, M. Pt: 117-119°C, R_f 0.73 (Petroleum ether: Ethyl acetate, 7:3); **IR (ν cm^{-1}),** 3084 (Ar-C-H), 1658 (C=O), 703 (Ar-C-Cl), 1011 (C-S-C), 1483 (Ar-C=C), 1589 (C=N); **^{1}H NMR**

($CDCl_3$), 1.34-1.38 (t,*J*= 7.32 Hz, 1H,CH_2-pyrazoline), 2.04 (s, 1H, CH_2-pyrazoline), 3.75-3.83 (m, 1H, CH_2), 4.06 (m, 1H, N-H), 4.49-4.58 (m, 1H, CH), 7.06-7.96 (m, 11H, Ar-H).

1-(5-(4-Chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydropyrazol-1-yl)-2-(5-(4-nitro phenyl)-1,3,4-thiadiazol-2-ylamino)ethanone (6i)

State: Grey powder, Yield: 56.66%, M. Pt: 157-160°C, R_f 0.65 (Petroleum ether: Ethyl acetate, 7:3); **IR (ν, cm^{-1}),** 3085 (Ar-C-H), 1655 (C=O), 707 (Ar-C-Cl), 1008 (C-S-C), 1483 (Ar-C=C), 1592 (Ar-NO_2)); **^{1}H NMR ($CDCl_3$),** 1.34-1.37 (t, *J*= 7.32 Hz, 1H,CH_2-pyrazoline), 2.16 (s, 1H, CH_2-pyrazoline), 3.75-3.83 (m, 1H, CH_2), 4.06 (m, 1H, N-H), 4.49-4.58 (m, 1H, CH), 7.04-7.87 (m, 9H, Ar-H), 8.18-8.21 (m, 2H, Ar-H).

2-(5-(2-Chlorophenyl)-1,3,4-thiadiazol-2-ylamino)-1-(5-(4-chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydropyrazol-1-yl)ethanone (6j)

State: Brown powder, Yield: 81.50%, Melting point: 112-115°C, R_f 0.76 (Petroleum ether: Ethyl acetate, 7:3); **IR (ν, cm^{-1}),** 3072 (Ar-C-H), 1660 (C=O), 705 (Ar-C-Cl), 1035 (C-S-C), 1409 (Ar-C=C), 1587 (C=N); **^{1}H NMR ($CDCl_3$),** 1.33-1.37(t,*J*= 7.30 Hz, 1H,CH_2-pyrazoline), 2.16 (s, 1H, CH_2-pyrazoline), 3.76-3.84 (m, 1H, CH_2), 4.06 (m, 1H, N-H), 4.49-4.59 (m, 1H, CH), 7.05-7.88 (m, 11H, Ar-H).

ANTICANCER EVALUATION

Reagents and Conditions

a) 2-Acetylthiophene, 4-chlorobenzaldehyde, aqueous sodium hydroxide (10%), ethanol, stirring, 8h, rt **b)** hydrazine hydrate, ethanol, reflux, 5h **c)** chloroacetyl chloride, triethylamine, toluene, stirring, 1h, rt **d)** acetone, stirring, 8h, rt.

In vitro anti-cancer screening

The anticancer screening of all the synthesized compounds was conducted against ovarian cancer cells line (SK-OV-3) to determine the growth inhibitory effects of the compounds. Source of cell line was NCI, USA. Vehicle used for testing was dimethylsulfoxide (DMSO). *In vitro* testing was done using SRB assay protocol; each derivative was tested at 4 dose levels (10 µg/mL, 20 µg/mL, 40 µg/mL, 80 µg/mL).

Using the seven absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth was calculated at each of the drug concentrations levels. Percentage growth inhibition was calculated as:

[(Ti-Tz)/(C-Tz)] x 100 for concentrations for which Ti>/=Tz

[(Ti-Tz)/Tz] x 100 for concentrations for which Ti<Tz.

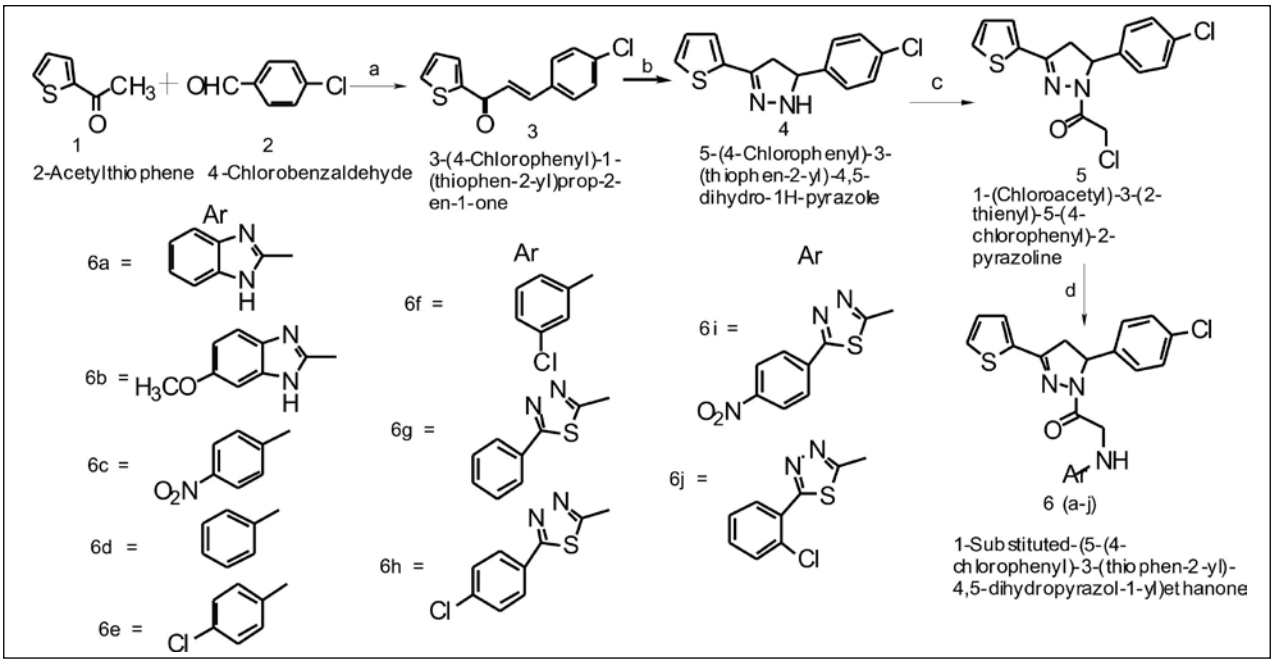

Fig. 1: Reaction scheme for the synthesis of 1-substituted-2-thienyl-5-(4-chlorophenyl) pyrazoline derivatives

Three dose response parameters GI_{50}, total growth inhibition (TGI) and LC_{50} were calculated for every compound.

RESULTS AND DISCUSSION

As per the proposed protocol (Fig. 1) the synthesis of various 1-substituted-2-thienyl-5-(4-chlorophenyl) pyrazoline derivatives was carried out. The yield of the said derivatives was found to be in range of 52-85%. The melting points of compounds ranged from 85-195°C and are uncorrected. R_f 0.55-0.77 were observed values between using different solvents and detecting systems (Table I). The IR spectra of the final derivatives exhibited the absorption band at 1670-1641 cm^{-1} which confirmed the presence of C=O stretch, 2983-2920 cm^{-1} confirmed the presence of aromatic C-H stretch, 1005-1107 cm^{-1} confirmed the presence of C-S-C group, 1595 cm^{-1} confirmed the presence of –C=N stretch, 1600-1475cm^{-1} confirmed the presence of C=C stretch, 785-540 cm^{-1}confirmed the presence of C-Cl group, 3500-3100 cm^{-1}

Table I: Physical Characteristics of synthesized compounds (6a-j)

Compound No.	Molecular Formula	Colour	Melting point (°C)	R_f^*	Yield (%)
6a	$C_{22}H_{18}ClN_5OS$	Dark grey	190-193	0.55	56.6
6b	$C_{23}H_{19}ClN_4O_2S_2$	Brown	85-87	0.70	66.5
6c	$C_{21}H_{17}ClN_4O_3S$	Grey	110-112	0.76	52.7
6d	$C_{21}H_{18}ClN_3OS$	Brown	140-142	0.70	64.0
6e	$C_{21}H_{17}Cl_2N_3OS$	Dark red	133-135	0.77	85.3
6f	$C_{21}H_{17}Cl_2N_3OS$	Dark brown	137-139	0.69	66.2
6g	$C_{23}H_{18}ClN_5OS_2$	Yellow	144-146	0.75	60.0
6h	$C_{23}H_{17}Cl_2N_5OS_2$	Dark brown	117-119	0.73	70.9
6i	$C_{23}H_{17}ClN_6O_3S_2$	Grey	157-160	0.65	56.6
6j	$C_{23}H_{17}Cl_2N_5OS_2$	Brown	112-115	0.76	81.5

R_f = Petroleum ether: Ethylacetate (7:3)

Table II: *In vitro* percentage control growth of SK-OV-3 cell line at different molar drug concentrations

C. No.	Drug Concentrations (µg/ml)															
	(Experiment 1)				(Experiment 2)				(Experiment 3)				Average % control growth			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
6a	-16.0	-45.6	-40.1	5.6	36.8	-22.9	-32.9	8.4	47.8	-11.5	-6.9	19.7	22.8	-26.7	-26.6	11.2
6b	-26.2	-31.7	-29.8	-17.9	-18.3	-15.9	-40.1	-27.0	-20.1	-18.1	-31.5	-23.2	-21.5	-21.9	-33.8	-22.7
6c	-7.1	-19.8	-26.8	-23.6	-2.6	-4.6	-33.9	-24.1	0.8	0.1	-28.8	-28.2	-3.0	-8.1	-29.9	-25.3
6d	54.2	22.3	-5.4	22.7	63.4	24.6	-5.2	12.7	51.7	25.4	-12.0	-7.3	56.5	24.1	-7.6	9.4
6e	-11.4	-12.8	-13.3	-1.1	-7.7	-8.7	-17.8	-9.8	-15.0	-17.8	-21.8	-12.5	-11.4	-13.1	-17.6	-7.8
6f	-6.8	-5.6	74.0	-8.0	-4.4	-2.1	63.7	-8.8	-5.6	-6.6	38.1	-13.0	-5.6	-4.8	58.6	-9.9
6g	45.6	23.2	-3.7	-1.3	37.5	41.2	0.4	-0.2	41.3	26.1	-4.1	-2.0	41.5	30.2	-2.5	-1.1
6h	-19.1	-19.5	-39.2	-38.4	0.8	43.3	39.5	13.3	-18.1	-37.3	-41.2	-38.9	-12.1	-4.5	-13.6	-21.3
6i	-26.7	-37.7	-32.1	-25.2	7.1	35.4	38.6	25.5	-24.4	-37.0	-38.0	-28.0	-14.7	-13.1	-10.5	-9.2
6j	-26.4	-32.1	-36.1	-33.1	1.0	27.2	29.6	9.4	-23.9	-33.5	-41.2	-37.3	-16.4	-12.8	-15.9	-20.3
ADR	-57.3	-60.4	-57.3	-31.6	-50.8	-49.8	-40.9	-17.6	-60.7	-61.9	-60.2	-34.4	-56.3	-57.3	-52.8	-27.8

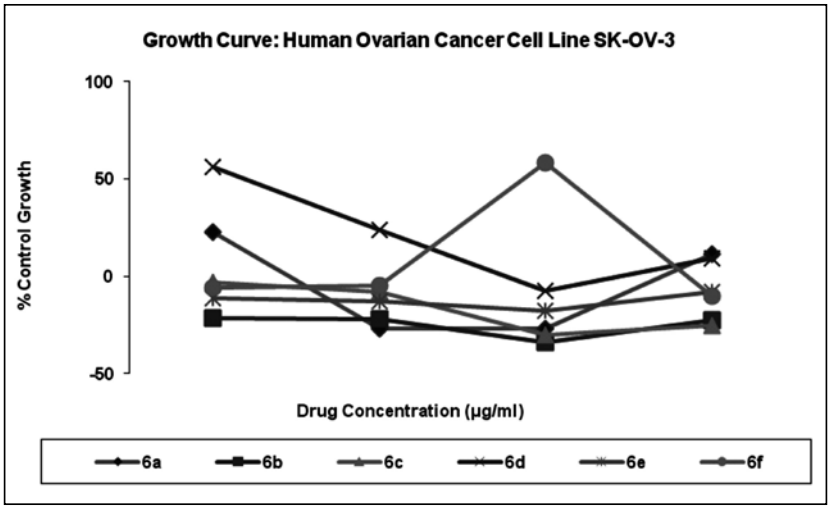

Fig. 2: Graph showing % control growth against the drug concentrations (6a-6f) and standard andriamycin

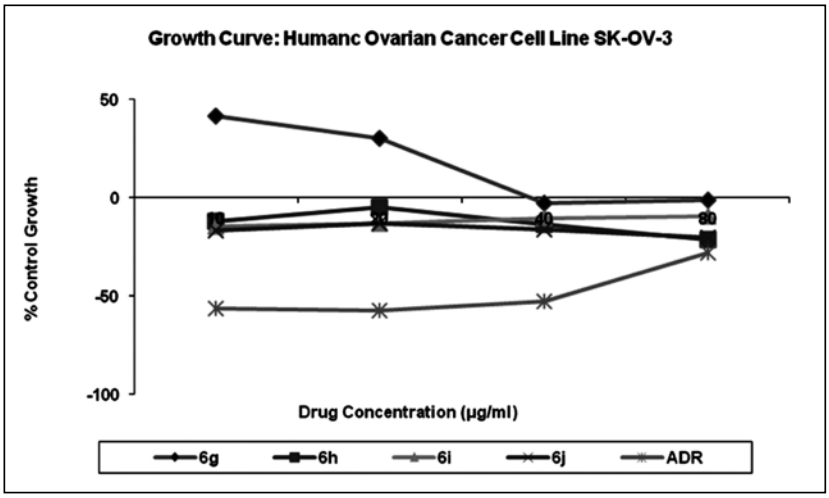

Fig. 3: Graph showing % control growth against the drug concentrations (6g-6j) and standard andriamycin

Table III: TGI, LC_{50} and GI_{50} of the synthesized compounds against SK-OV-3 cell line

SK-OV-3	LC_{50}	TGI	GI_{50}*
6a	NE	NE	<10
6b	NE	NE	<10
6c	NE	NE	<10
6d	NE	NE	<10
6e	NE	NE	<10
6f	NE	<10	<10
6g	NE	65.9	<10
6h	NE	NE	<10
6i	NE	NE	<10
6j	NE	NE	<10
ADR	34.16	<10	<10

[1]confirmed the presence of N-H stretch. [1]H NMR spectra had multiplet in region δ 6.31-7.87 ppm and indicated the presence of aromatic protons, multiplet at δ 4.49-4.58 ppm showed one proton of N-H group, singlet at δ 2.16 ppm showed one proton of CH_2 of pyrazoline, triplet at δ 1.37-1.41 ppm indicated the presence of CH_2 of pyrazoline.

Anticancer Drug Screening

All the synthesized compounds were screened against ovarian cancer cells line (SK-OV-3) to determine the growth inhibitory effects of the compounds. *In vitro* testing was done using SRB assay protocol; each derivative was tested at 4 dose levels (10 µg/mL, 20 µg/mL, 40 µg/mL, 80 µg/mL).

The synthesized 1-substituted-(5-(4-chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydropyrazol-1-yl)ethanones exhibited encouraging anticancer results. The compound **6b** and **6c** at concentration 80 µg/mL possessed higher % control growth inhibition comparable to standard drug Andriamycin. The order for the % control growth inhibition of SK-OV-3 was found to be **6h> 6j> 6f> 6i> 6e> 6g> 6d> 6a** as shown in Table II.

All the compounds inhibited 50% of the cell growth at the conc. <10 µg/mL. The compound **6f** and **6g** inhibited the total cell growth at the conc. <10 and 65.9 µg/mL respectively (Table III and Fig. 2, 3). The better activity of 6c can be attributed to the presence of electron withdrawing substituent at position 4 of the aromatic phenyl ring. Similarly the compound 6b had 6-methoxy benzothiazole substitution. The other compounds like **6h, 6j, 6f, 6i, 6e** and **6g** also possessed various substitutions like chlorophenyl and substituted thiadiazole. The substitution position also affected the activity in the order 4-Cl> 2-Cl> 3-Cl>unsubstituted. Further the activity can be improved/ explored by incorporating substituents of different nature and studying the SAR.

CONCLUSION

In conclusion, a total of ten 1-substituted-2-thienyl-5-(4-chlorophenyl)pyrazoline derivatives 6a-6j, have been

synthesized and evaluated for their anti-cancer activity. Among these synthesized heterocycles, the higher activity of 6c can be attributed to the presence of electron withdrawing substituent at position 4 of the aromatic phenyl ring. Similarly, the compound 6b had 6-methoxy benzothiazole substitution. The other compounds like 6h, 6j, 6f, 6i, 6e and 6g also possessed various substitutions like chloro phenyl and substituted thiadiazole. The substitution position also affected the activity in the order 4-Cl> 2-Cl> 3-Cl>unsubstituted. Further, the activity can be improved/ explored by incorporating substituent of different nature and studying the SAR.

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