Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 1, 2016

Original Article

NOVEL PYRAZOLINES: SYNTHESIS AND EVALUATION OF THEIR DERIVATIVES WITH ANTICANCER AND ANTI-INFLAMMATORY ACTIVITIES

L. SIVA SANKER REDDY*[1], M. BHAGAVAN RAJU[2], C. SRIDHAR[3]

[1]Dept. of Pharmaceutical Chemistry, Creative Educational Society's College of Pharmacy, N. H-7, Chinnatekur, Kurnool, Andhra Pradesh 518218, [2]Gland Institute of Pharmaceutical Sciences, Kothapet, Shivampet, Medak, Telangana 502313, [3]Sri Padmavathi School of Pharmaceutical Sciences, Tirupathi, Andhra Pradesh 517503
Email: shiva_s_rl@yahoo.co.in

Received: 01 Jul 2015 Revised and Accepted: 25 Nov 2015

ABSTRACT

Objective: Synthesis of novel pyrazolines (P2-P4 & P7-P9) from the chalcones (C2-C10) obtained by condensing different aldehydes with 2-acetyl-5-bromothiophene and evaluates them for *in vitro* anticancer and anti-inflammatory activities.

Methods: The synthesized pyrazolines and chalcones were screened for anticancer activity against human breast cancer cell lines-MCF-7 and MDA-MB-468 in the range of 100 nm to 100 µm. Inhibition of bovine albumin denaturation and heat-induced hemolysis *in vitro* methods were followed to screen for anti-inflammatory activity. The structures of synthesized compounds were confirmed based on the IR, ^{1}H NMR and mass spectral data.

Results: Among the synthesized compounds, methoxy trisubstituted pyrazoline derivative (P6) exhibited an interesting profile of anticancer activity against MCF-7 cell line with GI_{50}<0.1 µ M. similar to that of the standard drug doxorubicin. Compounds C8, P8, P3 have moderate anti-inflammatory activity in bovine denaturation and heat induced hemolytic method.

Conclusion: Novel pyrazolines and chalcones were synthesized and evaluated for anticancer and anti-inflammatory activity. The methoxy containing compounds one of which P6 found to be active against MCF-7 breast cancer cell line. The chloro-substituted compounds found to show anti-inflammatory activity.

Keywords: Pyrazoline, Chalcone, MCF-7, MDA-MB-468, Anti-inflammatory, Anticancer.

INTRODUCTION

For centuries, cancer has been prevailing as most serious disease and its incidence is rising day-to-day in the world. Cancer treatment usually falls into the category of surgery, radiation and chemotherapy. Despite of all these treatments, cancer is still continuing as uncontrollable disease and exploring for new approaches in anticancer therapy. Chemotherapy is generally used to treat cancer that has spread or metastasized because the medicines travel throughout the entire body. Recently, several substituted thiophenes and pyrazoles have been reported for anticancer activity. [1-4]. Pyrazoles substituted with another heterocyclic compound such as thiophene resulted in compounds with improved anti-proliferative activity against a number of solid and hematological tumors. [5] Even some prescribed drugs like omeprazole (proton pump inhibitor), eprosartan (angiotensin II receptor antagonist) and lore diplons (anxiolytic agent) have pyrazole ring connected with another heterocyclic moiety. Thiophene nucleus is also an important heterocyclic ring which is part of some of the drugs like raloxifene (osteoporosis), olanzapine (antipsychotic), and clopidogrel (antiplatelet agent). Thus, we were interested in synthesizing thiophene substituted pyrazolines and look for their anti-proliferative activity.

As a part of our research work, we synthesized a series of 1-(5-bromothiophen-2-yl)-3-(phenyl) prop-2-en-1-one and 1-(3-(5-bromo-thiophene-2-yl)-5-(aryl)-4,5-dihydropyrazole-1-yl) ethanone and tested biologically. The method followed for the synthesis of the final compounds is in accordance with the literature [6]. Later, the anticancer activity of compounds was reported by screening against human breast cancer cell lines MCF-7 and MDA-MB-468 and *in vitro* anti-inflammatory activity was done by inhibition of bovine albumin denaturation method and heat induced hemolytic method.

MATERIALS AND METHODS

Chemistry

Melting points of the compounds were determined using open capillary melting point apparatus and were reported uncorrected. Ultraviolet, visible spectroscopic analysis has been carried out in UV-visible double beam spectrophotometer (LAB INDIA 3000+), IR spectra was recorded by a KBr pellet method using a bruker FTIR ALPHA transmission mode spectrophotometer. The ^{1}H NMR spectra were recorded in DMSO-d6 by NMR 300MHZ spectrometers using tetramethyl silane as an internal standard. All the chemicals and solvents used in this study were of analytical grade (S. D. FINE Chem. Limited, Mumbai). Reaction progress was checked by TLC in a solvent-vapor-saturated chamber on glass plates coated with Silica Gel GF_{254} followed by visualization under UV light (254 nm). The solvent system used for thin layer chromatography was n-hexane: ethyl acetate (8:2).

Preparation of chalcones

0.01 Mol (2.05g) of 2-acetyl-5-bromothiophene taken in a 100 ml round bottom flask containing 20 ml of ethanol, to that equimolar quantity of substituted benzaldehydes added. The contents of the flask were stirred continuously using a magnetic stirrer, and the temperature was maintained below 20 ° C. Then 0.1 ml of 40% KOH was added drop by drop to the flask. The reaction was monitored by using a precoated TLC plate. After completion of the reaction, the contents of the flask were neutralized with dilute HCl to get precipitates of chalcones & filtered, washed with cold ethanol, dried and recrystallized from ethanol.

Preparation of 2-pyrazolines

0.002 moles of chalcone, 0.008 mole of hydrazine hydrate were taken in a 100 ml round bottom flask containing 30 ml of glacial acetic acid and refluxed for 70 h at 140 °C. The reaction mixture was monitored by using a precoated TLC plate. After completion of the reaction the content of the flask was poured into the crushed ice to get brown precipitate. The precipitate was dried and purified by column chromatography. Different gradients of ethyl acetate: petroleum ether, i.e. 2%, 4%, 6%, 8%, 10% and 12% was used to elute the pure compound successively. The eluent containing the compound was collected separately and evaporated to get the pure compound.

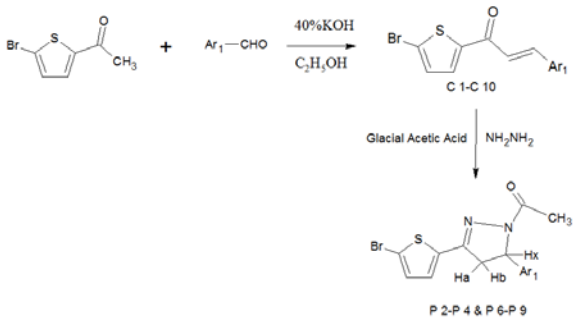
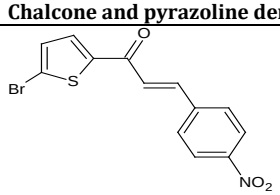
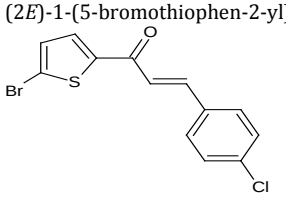
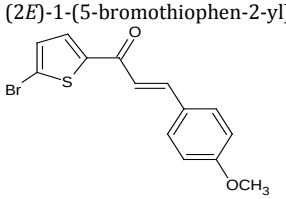
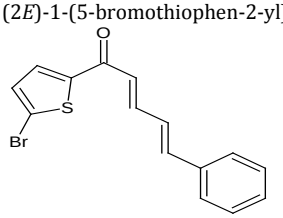
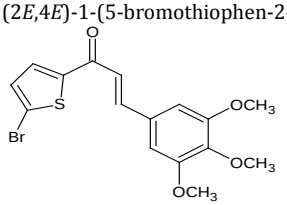
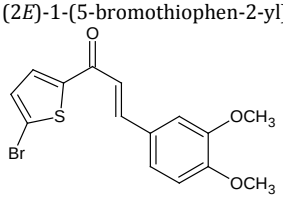
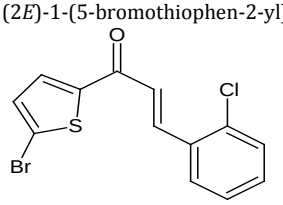
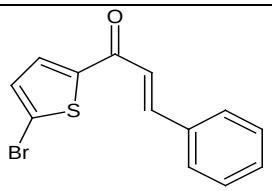


Table 1: List of synthesized compounds

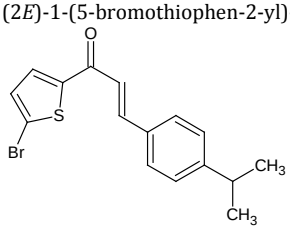
Sample code	Chalcone and pyrazoline derivative
C2	 (2*E*)-1-(5-bromothiophen-2-yl)-3-(4-nitrophenyl)prop-2-en-1-one
C3	 (2*E*)-1-(5-bromothiophen-2-yl)-3-(4-chlorophenyl)prop-2-en-1-one
C4	 (2*E*)-1-(5-bromothiophen-2-yl)-3-(4-methoxyphenyl)prop-2-en-1-one
C5	 (2*E*,4*E*)-1-(5-bromothiophen-2-yl)-5-phenylpenta-2,4-dien-1-one
C6	 (2*E*)-1-(5-bromothiophen-2-yl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one
C7	 (2*E*)-1-(5-bromothiophen-2-yl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one
C8	 (2*E*)-1-(5-bromothiophen-2-yl)-3-(2-chlorophenyl)prop-2-en-1-one

C9



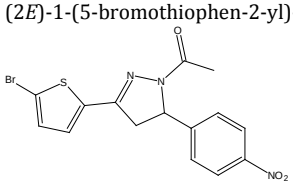
(2*E*)-1-(5-bromothiophen-2-yl)-3-phenylprop-2-en-1-one

C10



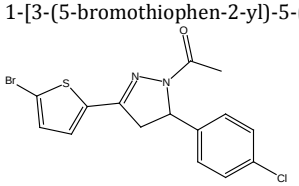
(2*E*)-1-(5-bromothiophen-2-yl)-3-[4-(propan-2-yl)phenyl]prop-2-en-1-one

P2



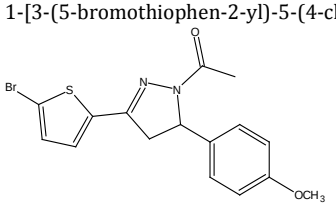
1-[3-(5-bromothiophen-2-yl)-5-(4-nitrophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]ethanone

P3



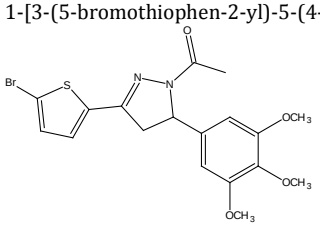
1-[3-(5-bromothiophen-2-yl)-5-(4-chlorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]ethanone

P4



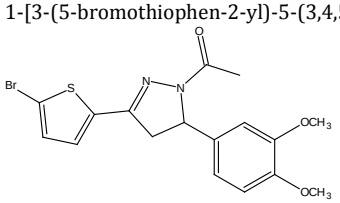
1-[3-(5-bromothiophen-2-yl)-5-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]ethanone

P6



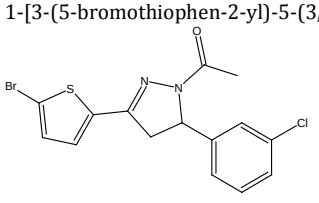
1-[3-(5-bromothiophen-2-yl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]ethanone

P7



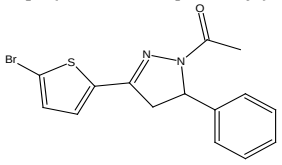
1-[3-(5-bromothiophen-2-yl)-5-(3,4-dimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]ethanone

P8



1-[3-(5-bromothiophen-2-yl)-5-(3-chlorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]ethanone

P9



1-[3-(5-bromothiophen-2-yl)-5-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl]ethanone

***In vitro* anticancer activity by SRB assay** [18]

The effect of synthesized compounds on cell growth was determined on two human tumor cells MCF-7 & MDA-MB-468. The sulforhodamine B (SRB) assay is used for cell density determination, based on the measurement of cellular protein content. The method described here has been optimized for the toxicity screening of compounds to adherent cells in a 96-well format. After an incubation period, cell monolayers are fixed with 10% (Wt/Vol) trichloroacetic acid and stained for 30 min with 0.4% (Wt/Vol) SRB dissolved in 1% acetic acid after which the excess dye was removed by repeatedly washing with 1% (Vol/Vol) acetic acid. The protein-bound dye was dissolved in 10 mM tris base solution for OD determination at 564 nm using a microplate reader.

- Appropriate positive controls were run in each experiment, and each experiment was repeated thrice.
- Results are in terms of

GI_{50} (concentration of the compound that produces 50% inhibition of the cells),

TGI (concentration of the compound that produces total inhibition of the cells) and

LC_{50} (concentration of the compound that kills 50% of the cells)

- Compounds with $GI_{50} \leq 1\mu M$ was considered as active ones.
- Doxorubicin was taken as a positive control.

***In vitro* anti-inflammatory activity [19-21]**

Inhibition of bovine albumin denaturation method

To 2 ml of various concentrations of test or standard solutions, 2.8 ml of normal saline (pH=7.4) and 0.2 ml of 1% bovine albumin solution was added. Simultaneously blank samples were prepared for each concentration without the addition of 1% bovine albumin solution and an equal volume of normal saline (pH 7.4) was added to each blank sample. To 4.8 ml of normal saline (pH 7.4), 0.2 ml of 1% bovine albumin solution was added and used as a control. The test/standard samples were incubated for 15 min at 70 ° C. Then the tubes were cooled under running tap water and then absorbance was recorded at 660 nm. % inhibition of denaturation of bovine albumin was calculated using the formula,

$$\% \text{ Inhibition} = [\![(A - A1) \div A]\!] \times 100$$

Where A=absorbance of the control,

A1= absorbance of the test/standard

Heat-induced hemolytic method

To 1 ml of various concentrations of test or standard solutions, 1 ml of 1% RBC's suspension was added. Simultaneously blank samples were prepared for each concentration without the addition of 1% RBC's solution and an equal amount of normal saline was added to each blank sample. An equal amount of 1% RBC's solution and normal saline was added and was used as a control.

All these samples were taken into centrifuge tubes and incubated in a water bath at 56 °C for 30 min. The tubes were cooled under running tap water and then centrifuged at 2500 rpm for 15 min and absorbance of the supernatant was taken at 560 nm. % inhibition was calculated using formula:

$$\% \text{ Inhibition} = [\![(A - A1) \div A]\!] \times 100$$

Where A=absorbance of the control, A1= absorbance of the test/standard.

IC_{50} values

IC_{50} was calculated using GraphPad prism software

Statistical analysis

All the data were expressed as mean±SEM. Statistical significance was tested by using one-way ANOVA followed by the Turkey's test using a computer-based fitness program (Graph pad prism 5)

RESULTS AND DISCUSSION

Synthesis

Claisen-Schmidt condensation reaction between 2-acetyl-5-bromothiophene and different substituted aldehydes catalysed by 40% KOH gave chalcones (C2-C10). The obtained chalcones were cyclised in the presence of glacial acetic acid to give 2-pyrazoliones (P2-P4 & P7-P9) (Scheme-I). All the synthesized compounds were characterized by FT IR, ^{1}NMR and mass spectroscopic data.

In the IR spectra of 1-(5-bromothiophen-2-yl)-3-(phenyl)prop-2-en-1-one (C2-C10) characteristic absorption due to a carbonyl group appears in the range of 1655-1630 cm^{-1}. The olefinic double (C=C) appears in the range of1593-1522 cm^{-1}. The ^{1}HNMR spectra, the two olefinic protons (CH=CH) appears as a doublet in the region of δ 6.9-7.9 ppm and trans type of geometrical isomerism can be confirmed due to *J*>14. The thiophene protons seen as doublets were distinguished from other aromatic protons based on the *J* value which is 4. Aromatic protons of the benzene appear as a complex multiple in the range of δ 6.8-8.3 ppm.

The IR spectra of 1-(3-(5-bromothiophene-2-yl)-5-(aryl)-4, 5-dihydropyrazole-1-yl) ethanone (P2-P4 &P6-P9), the characteristic absorption due to a carbonyl group appears in the range of 1673-1645 cm^{-1}. The absorption of C-Br stretching appears in the range of 580-591 cm^{-1}. ^{1}H NMR data showed Ha, Hb, Hx type of coupling due to spin coupling of CH_2 protons with CH proton of the pyrazoline nucleus with doublet of doublets around δ 3.099 ppm (1H, dd, Ha), δ 3.811 ppm (1H, dd, Hb), and δ 5.646 ppm (1H, dd, Hx) respectively, with coupling constants(J_{ab}=17.6, J_{ax}=5.2, J_{bx}=11.6). The thiophene protons were in the range of δ 7.03 to 6.9 ppm as a doublet with *J* value of 4. The phenyl protons were lying in the region of δ 6.3 to 8.2 ppm with *J* values 7-9 depending on the type of substitution on the phenyl ring. The acetyl protons on the pyrazoline nucleus were present as a single and had δ value of 2.3 to 2.4 ppm. Thus, all the protons of pyrazoline compounds were accounted.

Spectral data of compounds

Compound C 2:(2E)-1-(5-bromothiophen-2-yl)-3-(4-nitrophenyl)prop-2-en-1-one

IR (KBr)V_{max} in cm^{-1}**:**C=O str =1649.16, C=C str =1586.86, Ar-H str =3076.73, =C-H str = 2924.13,Ar-N-O str =1516.82, C-Br str = 670.70; ^{1}H NMR ($CDCl_3$) in δ (ppm): 7.197 (d, 1H, C4 of thiophene*J*=4), 7.638 (d, 1H, C3 of thiophene *J*=4), 7.39 & 7.868 (d, 2H,-CH=CH-trans *J*=15.6), 7.79 & 8.29 (d, 4H,Ar-H*J*=8.8& J=8.4).

Compound C 3:(2E)-1-(5-bromothiophen-2-yl)-3-(4-chlorophenyl) prop-2-en-1-one

IR (KBr)V_{max} in cm^{-1}**:** C=O str =1650.08, C=C str =1593.94, Ar-H str =3072.28 cm^{-1}, Ar-C=C str =1491.88, =C-H str = 2924.94, C-Clstr = 771.88; ^{1}H NMR ($CDCl_3$) in δ (ppm): 7.16 (d, 1H, C4 of thiophene*J*=4), 7.30 (d, 1H, C3 of thiophene), 7.805 (d, 1H,-CH=CH-trans *J*=15.6), 7.6 (m, 1H,-CH=CH-trans *J*=17.6), 7.42 (d, 2H, orthoAr-H *J*=8.4) & 7.56 (d, 2H Meta Ar-H *J*=8.4)

Compound C 4:(2E)-1-(5-bromothiophen-2-yl)-3-(4-methoxyphenyl)prop-2-en-1-one

IR (KBr)V_{max} in cm^{-1}: Aliphatic C-H str = 2838.82, C=O str =1645.48, C=C str =1587.27, Ar-H str =3084.58, Ar-C=C str =1510.30, =C-H str = 3004.19, C-O-C str = 1302.44, 1031.93;^{1}H NMR ($CDCl_3$) in δ (ppm): 7.15 (d, 1H, C4 of thiophene *J*=4),7.575 (d, 1H, C3 of thiophene), 7.83 (d, 1H,-CH=CH-trans *J*=15.6), 7.218 (d 1H,-CH=CH-trans *J*=15.6), 7.606 (m, 2H,orthoAr-H *J*=8.8) & 6.952 (d, 2H, meta Ar-H *J*=8.4) 3.86(s, 3H,para Ar-OCH_3).

Compound C 5:(2*E*, 4E)-1-(5-bromothiophen-2-yl)-5-phenyl-penta-2, 4-dien-1-one

IR (KBr)V_{max} in cm^{-1}: C=O str =1634.88, C=C str =1572.08, Ar-H str =3105.88, Ar-C=C str =1572.08, 1445.91, =C-H str = 3025.17, C-Br str = 686.29;^{1}H NMR ($CDCl_3$) in δ (ppm): 7.13 (d, 1H, C4 of thiophene*J*=4),7.52 (m, 1H C3 of thiophene), 6.906 (d, 1H,-CH=CH-trans *J*=14.8), 7.65 (m, 1H,-CH=CH-trans *J*=15.48), 6.98 (m, 1H,-

CH=CH-trans), 7.03 (m, 1H,-CH=CH-trans) 7.516 (m, 2H,orthoAr-H *J*=8.4) & 7.314 to 7.402 (m, 3H, meta & paraAr-H).

Compound C 6:(2E)-1-(5-bromothiophen-2-yl)-3-(3, 4, 5-trimethoxyphenyl) prop-2-en-1-one

IR (KBr) Vmax in cm^{-1}: Aliphatic C-H str = 2938.37,C=O str =1645.24, C=C str =1522.50, Ar-H str =3113.22, Ar-C=C str =1499.61, =C-H str = 3004.74, C-O-C str = 1214.49, C-Br str = 681.35;^{1}H NMR ($CDCl_3$) in δ (ppm):7.2 (m,1H, C4 of thiophene*J*=4),7.618 (d, 1H, C3 of thiophene), 7.15 (m, 1H,-CH=CH-), 7.79 (d, 1H,-CH=CH-trans *J*=15.6), 6.851 (s, 2H,orthoAr-H), 3.926 (s, 6H, meta (Ar-OCH_3)$_2$), 3.906 (s, 3H, meta Ar-OCH_3).

Compound C 7:(2E)-1-(5-bromothiophen-2-yl)-3-(3,4-dimethoxyphenyl) prop-2-en-1-one

IR (KBr) V_{max} in cm^{-1}**:** Aliphatic C-H str = 2834.62, C=O str =1639.66, C=C str =1570.53, Ar-H str =3079.72, Ar-C=C str =1511.08, =C-H str = 2933.22, C-O-C str = 1254.11, C-Br str = 591.60 cm^{-1}; ^{1}H NMR ($CDCl_3$) in δ (ppm): 7.197 (s, 1H, C4 of thiophene*J*=4), 7.6 (d, 1H, C3 of thiophene), 7.245 (m, 1H,-CH=CH-), 7.815 (d, 1H,-CH=CH-trans *J*=15.2), 6.89 (d, 1H, meta Ar-H), 3.95 (s, 6H, meta, para (Ar-OCH_3)$_2$).

Compound C 8:(2E)-1-(5-bromothiophen-2-yl)-3-(2-chlorophenyl) prop-2-en-1-one

IR (KBr) V_{max} in cm^{-1}: C=O str =1655.14, C=C str =1593.94, Ar-H str =3092.02, Ar-C=C str =1471.55, =C-H str = 2622.64, C-Clstr = 775.32, C-Br str = 689.31; ^{1}H NMR ($CDCl_3$) in δ (ppm):7.167 d (1H C4 of thiophene*J*=4),7.601 d (1H C3 of thiophene), 7.089 m (1H,-CH=CH-), 8.236 (d, 1H,-CH=CH-trans *J*=15.6), 7.735 (dd, 1H orthoAr-H),7.466 (dd, 1H, meta Ar-H), 7.319 to 7. 372 (m, 2H, meta, paraAr-H).

Compound C 9: (2*E*)-1-(5-bromothiophen-2-yl)-3-phenylprop-2-en-1-one

IR (KBr) V_{max} in cm^{-1}: C=O str =1649.12, C=C str =1593.26, Ar-H str =3074.07, Ar-C=C str =1521.14, =C-H str = 3027.33, C-Br str = 685.59;^{1}H NMR ($CDCl_3$) in δ (ppm): 7.161 (d, 1H, C4 of thiophene*J*=4),7.60 (d, 1H, C3 of thiophene*J*=4), 7.06 (m, 1H,-CH=CH-), 7.863 (d, 1H,-CH=CH-trans *J*=15.6), 7.646 (m, 2H,orthoAr-H), 7.419 to 7. 434 (m, 3H, 2 meta, 1paraAr-H).

Compound C 10: (2*E*)-1-(5-bromothiophen-2-yl)-3-[4-(propan-2-yl) phenyl] prop-2-en-1-one

IR (KBr) V_{max} in cm^{-1}: Aliphatic C-H str = 2868.78, C=O str =1640.69, C=C str =1588.90, Ar-H str =3078.10, =C-H str = 2957.41, CH_3 bending= 1412.45;^{1}H NMR ($CDCl_3$) in δ (ppm): 7.157 (d, 1H, C4 of thiophene*J*=4),7.585 (m, 1H, C3 of thiophene), 7.275(m, 1H,-CH=CH-), 7.853 (d, 1H,-CH=CH-trans *J*=15.6), 7.559 (m, 2H, orthoAr-H), 7.297 (m, 2H, meta Ar-H), 2.95 (septet, 1H, CH of isopropyl), 1.282 & 1.265 (6H–(CH_3)$_2$).

Compound P 2: 1-[3-(5-bromothiophen-2-yl)-5-(4-nitrophenyl)-4, 5-dihydro-1*H*-pyrazol-1-yl]ethanone

IR (KBr) V_{max} in cm^{-1}: Aliphatic C-H str = 2925.85, C=O str =1666.37, Ar-C=C str =1605.79 cm^{-1}, 1514.99, Ar-H str =3107.29, Ar-N-O str =1514.99,1216.40, C-Br str = 587.26; ^{1}H NMR ($CDCl_3$) in δ (ppm): 2.37(s, 3H,-CH_3,), 3.099 (dd, 1H, Ha), 3.811 (dd, 1H, Hb), 5.646 (dd, 1H, Hx), 8.21 (d, 2H,Ar-H, *J*=8.4 HZ), 7.40 (d, 2H, Ar-H, *J*=8.4), 7.036 to 6.928 (dd, 2H, thiopheneH), (J_{ab}=17.6, J_{ax}=5.2, J_{bx}=11.6)

Table 2: Physico-chemical characterization data of synthesized chalcones of scheme I

Code	Molecular formula	Molecular weight	Melting point (ºC)	% yield (%)	R_f value*	Colour
C2	$C_{13}H_8BrNO_3S$	338.17	193-196	72	0.62	Yellow flakes
C3	$C_{13}H_8BrClOS$	327.62	135-138	67	0.64	Creamish white Amorphous
C4	$C_{14}H_{11}BrO_2S$	323.20	128-130	82	0.70	Cream
C5	$C_{15}H_{11}BrOS$	319.21	128-130	86	0.68	Light yellow Crystalline needles
C6	$C_{16}H_{15}BrO_4S$	383.25	140-143	82	0.59	Yellow; Crystalline needles
C7	$C_{15}H_{13}BrO_3S$	353.23	103-106	64	0.62	Dark Yellow; Crystalline needles
C8	$C_{13}H_8BrClOS$	327.62	123-126	60	0.67	Cream; Amorphous
C9	$C_{13}H_9BrOS$	293.17	70-73	63	0.58	Light yellow; Crystals
C10	$C_{16}H_{15}BrOS$	335.25	78-80	60	0.62	Cream; Amorphous
P2	$C_{15}H_{12}BrN_3O_3S$	394.24	143-145	71	0.56	Creamy crystals
P3	$C_{15}H_{12}BrClN_2OS$	383.69	188-189	68	0.58	White crystals
P4	$C_{16}H_{15}BrN_2O_2S$	379.27	159-160	95	0.60	Creamy crystals
P6	$C_{18}H_{19}BrN_2O_4S$	439.32	90-92	78	0.64	Yellow crystals
P7	$C_{17}H_{17}BrN_2O_3S$	409.29	116-118	79	0.58	Creamy crystals
P8	$C_{15}H_{12}BrClN_2OS$	383.69	180-183	86	0.56	Light brown crystals
P9	$C_{15}H_{13}BrN_2OS$	349.24	200-202	76	0.58	Dark brown crystals

*n-Hexane: Ethyl acetate (8:2)

Compound P 3: 1-[3-(5-bromothiophen-2-yl)-5-(4-chlorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]ethanone

IR (KBr) V_{max} in cm^{-1}: Ali C-H str = 2929.09, C=O str =1645.67, Ar-C=C str =1524.49, Ar-H str =3060.76, C-Br str = 580.79, C-Clstr = 626.89; ^{1}H NMR ($CDCl_3$) in δ (ppm): 2.35(s, 3H,-CH_3,), 3.07 (dd, 1H, Ha), 3.73 (dd, 1H, Hb), 5.55 (dd, 1H,Hx), 7.30 (d, 2H, Ar-H, *J*=8.4 HZ), 7.16 (d, 2H, Ar-H, *J*=8.4), 6.921 (s, 1H, C-2 H of thiophene), 7.024 (s, 1H, C-3 H of thiophene),(J_{ab}=17.6, J_{ax}=4.8, J_{bx}=11.6); ESI-MS 385 $(M+H)^+$.

Compound P 4: 1-[3-(5-bromothiophen-2-yl)-5-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]ethanone

IR (KBr) V_{max} in cm^{-1}: Aliphatic C-H str = 2840.59, C=O str =1657.18, Ar-C=C str =1514.32, Ar-H str =3064.73, C-Br str = 582.28, Ar-C-O-C str = 1248.50, 1028.05 cm^{-1}; ^{1}H NMR ($CDCl_3$) in δ (ppm): 2.34(s, 3H,-CH_3,), 3.1 (dd, 1H, Ha), 3.703 (dd, 1H, Hb), 3.77 (s, 3H, p-Ar-OCH_3) 5.55 (dd, 1H, Hx), 7.154 (d, 2H, Ar-H, *J*=8.4), 6.855 (d, 2H, Ar-H, *J*=8.4), 6.921 (s, 1H, C-2 H of thiophene), 7.019 (s, 1H, C-3 H of thiophene), (J_{ab}=17.6, J_{ax}=4.8, J_{bx}=11.6); ESI-MS 381 $(M+H)^+$.

Compound P 6: 1-[3-(5-bromothiophen-2-yl)-5-(3, 4, 5-trimethoxyphenyl)-4, 5-dihydro-1*H*-pyrazol-1-yl]ethanone

IR (KBr) V_{max} in cm^{-1}:Aliphatic C-H str = 2826.52, C=O str =1664.80, Ar-C=C str =1506.84, Ar-H str =3085.12, C-Br str = 591.83, Ar-C-O-C str = 1237.66, 1006.90; ^{1}H NMR ($CDCl_3$) in δ (ppm): 2.38(s, 3H,-CH_3,), 3.1 (dd, 1H, Ha), 3.7 (dd, 1H, Hb), 3.80 (s, 3H, p-Ar-OCH_3), 3.826 (s, 6H, m-Ar-(OCH_3)$_2$), 5.521 (dd, 1H, Hx), 7.154 (d, 2H, Ar-H, *J*=8.4 HZ), 6.399 (d, 2H, Ar-H, *J*=8.4), 6.927 (s, 1H, C-2 H of thiophene), 7.027 (s, 1H, C-3 H of thiophene), (J_{ab}=17.6, J_{ax}=4.8, J_{bx}=11.6); ESI-MS 441 $(M+H)^+$.

Compound P 7: 1-[3-(5-bromothiophen-2-yl)-5-(3,4-dimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]ethanone

IR (KBr) V_{max} in cm^{-1}: Aliphatic C-H str = 2834.71, C=O str =1657.64, Ar-C=C str =1518.62, Ar-H str =2999.84, C-Br str = 578.98, Ar-C-O-C str = 1258.43, 1025.12; ^{1}H NMR ($CDCl_3$) in δ (ppm): 2.36(s, 3H,-CH_3,), 3.11 (dd, 1H, Ha), 3.71 (dd, 1H, Hb), 3.83 (s, 3H, p-Ar-OCH_3), 3.85 (s, 3H, m-Ar-(OCH_3), 5.54 (dd, 1H, Hx), 6.76 (d, 2H, Ar-H, *J*=9.6 HZ), 6.81

(d, 1H, Ar-H, *J*=8), 6.92 (s, 1H, C-2 H of thiophene), 7.025 (s, 1H, C-3 H of thiophene), (J_{ab}=17.6, J_{ax}=4.4, J_{bx}=11.6); ESI-MS 411 $(M+H)^+$.

Compound P 8: 1-[3-(5-bromothiophen-2-yl)-5-(3-chloro-phenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]ethanone

IR (KBr) V_{max} in cm^{-1}: Aliphatic C-H str = 2927.73, C=O str =1673.45, Ar-C=C str =1530.85, Ar-H str =3325.17, C-Clstr = 635.24; ^1H NMR ($CDCl_3$) in δ (ppm): 2.42(s, 3H,-CH_3,), 3.01 (dd, 1H, Ha), 3.83 (dd, 1H, Hb), 5.92 (dd, 1H, Hx), 7.22 (m, 2H, Ar-H, *J*=4.4 HZ), 7.05 (m,1H, p, Ar-H, *J*=9.2), 7.406 (m, 1H,meta, Ar-H, *J*=9.2), 7.003 to 6.909 (dd, 2H, thiopheneH), (J_{ab}=17.6, J_{ax}=4.8, J_{bx}=11.6); ESI-MS 385 $(M+H)^+$.

Compound P 9: 1-[3-(5-bromothiophen-2-yl)-5-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl]ethanone

IR (KBr) V_{max} in cm^{-1}: C=O str =1659.68, Ar-C=C str =1452.02, Ar-H str =3085.56, C-Br str = 577.14; ^1H NMR ($CDCl_3$) in δ (ppm):2.36(s, 3H,-CH_3,), 3.11 (dd, 1H, Ha), 3.73 (dd, 1H, Hb), 5.59 (dd, 1H, Hx), 6.91 (s, 1H, C-2 H of thiophene), 7.016 (s, 1H, C-3 H of thiophene), (J_{ab}=17.6, J_{ax}=4.8, J_{bx}=11.6), 7.2 (s,1H, p-Ar-H,), 7.21 (d, 2H, ortho-Ar-H, *J*=7.2), 7.3 (2H, m, meta-Ar-H, *J*=7.2); ESI-MS 351 $(M+H)^+$.

Biological activities

The synthesized compounds were evaluated for their anticancer activity in selected human breast cancer cell lines MCF-7 and MDA-MB-468. IC_{50} values, defined as the concentration corresponding to 50% growth inhibition were based on concentration and exponential cell growth curves as shown in fig. 1-4. The compounds that exhibit $GI_{50} \leq 1$ µM are considered to be active. All the compounds exhibited significant anticancer activity with GI_{50} values ranging from<0.1 to>100 µM, while the positive control doxorubicin demonstrated the GI_{50}<0.1µM in the cell lines employed. Compound P6 exhibits an interesting profile of anticancer activity with MCF-7 cell line with GI_{50}<0.1 µM. Even the activity of P6 in MDA-MB-468 cell line was also found to be GI_{50} = 36.6 µM, which is the best activity when compared to all other compounds.

The promising results shown by compound P6 on both cell lines suggest that it has potent broad-spectrum anticancer activity. However, compounds P2-P4 and P7-P9 also expressed significant activity values of GI_{50}<100 µM in MCF-7 breast cancer cell lines. Whereas, in anticancer activity assay against MDA-MB-468 cell lines compound P8 shown GI_{50} value 57.2 µM and rest all shown GI_{50}>100 µM. C6 and P6 having tri-methoxy substitution showed GI_{50} (µ molar concentration of drug/compound causing 50% inhibition of the cell growth) value of 28.6 µM and<0.1 µM respectively against MCF-7 breast cancer cell lines. C7 having dimethoxy substitution and P6 having tri-methoxy substitution has GI_{50} value of 16.7 µM and 36.6 µM respectively against MDA-MB-468 breast cancer cell lines. Doxorubicin was the standard drug which has GI_{50} value of<0.1 µM. The other compounds have GI_{50} value greater than 16 µM. Concentrations of the test material used were 10, 20, 40 and 80µg/ml.

The *in vitro* anti-inflammatory activity was performed by inhibition of bovine albumin denaturation method and heat induced hemolytic method. The inhibitory activity of the compounds was compared with the control and the significance factor "p" was less than 0.001 for all the compounds. The compound with chloro group as a substituent showed the highest inhibition activity suggesting that electron donating groups may aid the activity (table 3-6). The inhibitory activity of the compounds was compared with the control and the significance factor "p" was less than 0.001 for all the compounds.

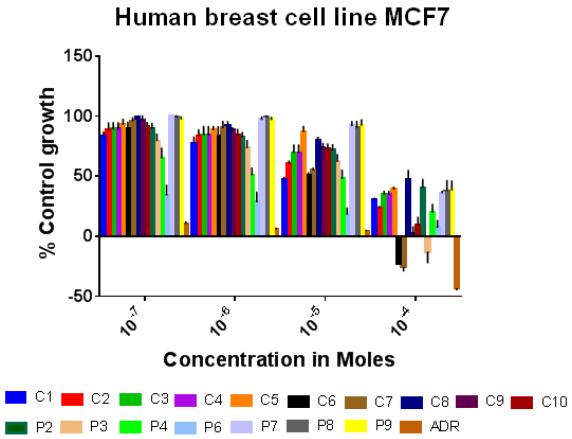


Fig. 1: % Control growth in human breast cancer cell line MCF-7

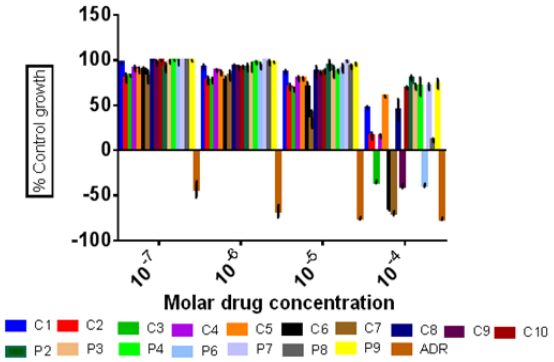


Fig. 2:% Control growth in human breast cancer cell line MDA-MB-468

Table 3: Bovine albumin denaturation method

Conc. (µg/ml)	% Inhibition±SEM*									
	Diclofenac sodium	C2	C3	C4	C5	C6	C7	C8	C9	C10
20	75.3± 0.364	31.9±0.190	31.2± 0.503	41.1± 0.049	33.9± 0.607	40.1± 0.429	37.1± 0.283	44.8± 0.024	40.2± 0.301	30.8± 0.239
40	81.4± 0.234	34.0± 0.424	47.0± 0.432	41.9± 0.291	49.0± 0.602	42.6± 0.793	31.0± 0.541	64.7± 0.023	43.8± 0.207	32.9± 0.266
60	86.0± 0.321	44.1± 0.560	40.1± 0.233	32.9± 0.502	22.5± 0.103	32.2± 0.670	21.4± 0.580	32.6± 0.435	33.2± 0.328	33.7± 0.024
80	94.0± 0.423	35.3± 0.457	42.6± 0.342	55.3± 0.649	44.2± 0.547	42.4± 0.368	22.8± 0.798	44.60± 0.672	41.7± 0.721	24.8± 0.640
100	96.4± 0.624	59.2± 0.628	54.7± 0.235	51.8± 0.129	45.4± 0.694	60.3± 0.117	30.7± 0.402	69.5± 0.264	45.5± 0.024	40.7± 0.452
120	98.0± 0.245	35.2± 0.610	45.2± 0.166	53.3± 0.712	37.5± 0.590	35.2± 0.064	40.5± 0.142	62.6± 0.264	39.9± 0.429	32.1± 0.126
IC_{50} (µg/ml)	7.873	76.40	68.0	62.80	156.62	46.28	241.6	46.249	98.0	78.92

*All the values are average of three readings, mean±SEM, SEM = Standard Error Mean IC_{50}= Half maximal inhibitory concentration.

Table 4: Bovine albumin denaturation method

Conc. (µg/ml)	% Inhibition±SEM*							
	Diclofenac sodium	P2	P3	P4	P6	P7	P8	P9
20	75.3±	42.8±	36.2±	41.8±	40.9±	49.7±	33.1±	58.3±
	0.364	0.439	0.563	0.299	0.767	0.024	0.238	0.444
40	81.4±	48.0±	39.0±	45.1±	41.8±	44.7±	43.0±	56.0±
	0.234	0.004	0.420	0.216	0.627	0.730	0.121	0.043
60	86.0±	40.2±	30.2±	31.5±	33.2±	39.1±	25.5±	40.9±
	0.321	0.830	0.038	0.215	0.359	0.525	0.668	0.534
80	94.0±	42.0±	46.0±	39.0±	30.6±	39.8±	52.20±	54.0±
	0.423	0.968	0.890	0.846	0.642	0.25	0.684	0.842
100	96.4±	32.2±	32.2±	57.8±	45.4±	60.3±	30.7±	69.5±
	0.624	0.558	0.558	0.129	0.694	0.117	0.402	0.264
120	98.0±	56.6±	56.6±	50.3±	39.5±	36.5±	42.5±	60.6±
	0.245	0.665	0.665	0.672	0.580	0.054	0.342	0.284
IC_{50} (µg/ml)	7.873	63.89	48.69	59.90	189.50	65.24	39.42	43.89

*All the values are the average of three readings, mean±SEM, SEM = Standard Error Mean, IC_{50}= Half maximal inhibitory concentration

Table 5: Heat-induced hemolytic method

Conc. (µg/ml)	% Inhibition±SEM*									
	Diclofenac sodium	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**	**C10**
20	74.8±	34.0±	33.2±	45.1±	38.4±	44.6±	34.8±	46.8±	42.2±	36.6±
	0.282	0.424	0.303	0.949	0.670	0.480	0.203	0.824	0.310	0.294
40	78.2±	44.0±	57.6±	51.9±	48.8±	44.8±	35.0±	54.8±	48.6±	36.2±
	0.644	0.544	0.430	0.491	0.260	0.736	0.468	0.823	0.446	0.863
60	89.0±	43.3±	44.1±	36.8±	32.8±	38.8±	25.7±	34.6±	38.8±	34.2±
	0.482	0.260	0.346	0.684	0.468	0.127	0.452	0.428	0.645	0.465
80	91.2±	38.6±	44.9±	55.3±	46.8±	43.8±	42.1±	54.90±	48.2±	44.6±
	0.514	0.46	0.264	0.399	0.647	0.640	0.579	0.820	0.530	0.602
100	93.2±	57.6±	59.8±	52.3±	42.8±	50.6±	40.7±	62.6±	42.6±	48.6±
	0.321	0.268	0.548	0.560	0.680	0.946	0.682	0.246	0.240	0.252
120	94.4±	36.7±	44.2±	54.5±	37.4±	38.2±	46.±6	52.4±	36.3±	38.6±
	0.821	0.262	0.257	0.625	0.856	0.664	0.220	0.462	0.626	0.582
IC_{50} (µg/ml)	8.624	62.46	67.24	68.63	166.20	66.68	221.8	42.86	110.0	76.46

*All the values are the average of three readings, mean±SEM, SEM = Standard Error Mean IC_{50}= Half maximal inhibitory concentration

Table 6: Heat-induced hemolytic method

Conc. (µg/ml)	% Inhibition±SEM*							
	Diclofenac sodium	**P2**	**P3**	**P4**	**P6**	**P7**	**P8**	**P9**
20	74.8±	48.4±	46.2±	51.4±	48.6±	48.6±	38.8±	54.5±
	0.282	0.494	0.425	0.299	0.434	0.243	0.224	0.484
40	78.2±	46.8±	49.2±	42.1±	47.8±	48.3±	44.8±	52.0±
	0.644	0.404	0.402	0.616	0.060	0.630	0.251	0.482
60	89.0±	46.6±	38.2±	30.6±	38.9±	36.4±	35.8±	44.6±
	0.482	0.80	0.083	0.624	0.569	0.456	0.688	0.584
80	91.2±	46.0±	45.0±	39.0±	38.6±	38.8±	32.80±	58.8±
	0.514	0.680	0.870	0.044	0.240	0.425	0.824	0.242
100	93.2±	36.4±	36.8±	52.0±	48.3±	58.4±	44.6±	59.2±
	0.321	0.588	0.458	0.290	0.734	0.868	0.259	0.242
120	94.4±	52.6±	52.6±	56.3±	38.5±	39.0±	46.9±	64.2±
	0.821	0.550	0.465	0.58	0.880	0.654	0.802	0.740
IC_{50} (µg/ml)	8.624	64.90	56.60	68. 0	141.0	59.20	176.04	68.38

*All the values are average of three readings, mean±SEM, SEM = Standard Error Mean IC_{50}= Half maximal inhibitory concentration

CONCLUSION

The synthesis of chalcones and condensing them to form pyrazolines was done according to the reported methods. The synthesized chalcones and pyrazolines were screened for anti-tumor activity against human breast cancer cell lines-MCF-7 and MDA-MB-468. Compound P6 was found to be an active agent against human breast cancer cell lines-MCF7. The same compound showed anticancer activity against human breast cancer cell lines MDA-MB-468 but not as active as against cancer cell lines-MCF. The anti-inflammatory activity also established in all the synthesized compounds shown significant inhibition.

The compounds C8, P3 and P8 shown moderate anti-inflammatory activity. It is found that the compounds with chloro substitution have *in vitro* anti-inflammatory activity.

ACKNOWLEDGEMENT

The authors are thankful to the head of the department of chemistry, Creative educational society's college of pharmacy, Kurnool, India, for providing laboratory facilities to carry out the research work. We also thank LailaImpex, R&D center, Vijayawada for analysing the compounds for ^{1}H NMR and Mass. We are greatful to ACTREC-Mumbai for performing the anti-cancer activity.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Jose LG, Chad ES, Tanja W, Reto B, Farial AT, David W, Todd B, *et al*. Synthesis and antiparasitic evaluation of bis-2,5-[4-guanidinophenyl] thiophenes. Eur J Med Chem 2007:42; 552-7.
2. Kristina S, Marijeta K, Ivo P, Lidija S, Krešimir P, Grace KZ. Synthesis, photochemical synthesis, DNA binding and antitumor evaluation of novel cyano-and amidino-substituted derivatives of naphtho-furans, naphtho-thiophenes, thieno-benzofurans, benzo-dithiophenes and their acyclic precursors. Eur J Med Chem 2006:41;925-39.
3. Sayed MR, Thoraya AF, Magda AA, Mohamed MA, Mohamed RA. New pyrazoles incorporating pyrazolyl pyrazole moiety: Synthesis, anti-HCV and antitumor activity. Eur J Med Chem 2010:45;1042-50.
4. Amol VG, Chetan P, Sahebrao SR, Bhausaheb KK. Synthesis and antimicrobial activity of novel fluorine-containing 4-(substituted-2-hydroxybenzoyl)-1H-pyrazoles and pyrazolylbenzo [d]oxazoles. Bioorg Med Chem Lett 2010:20;5572-6.
5. Steve P, Walter B, Richard JB, David EC, Peter HC, Hazel JD, et al. Identification and optimization of a series of substituted 5-(1H-pyrazol-3-yl)-thiophene-2-hydroxamic acids as potent histone deacetylase (HDAC) inhibitors. Bioorg Med Chem Lett 2007:17;370-5.
6. John RG, Francesc PB, Brett MF, Pei Yong S, David MF. Identification of compounds with anti-west nile virus activity. J Med Chem 2006:49;2127-37.
7. Mallikarjuna RJ, Sreeramulu LK, Ravindranath G, Nagaraja Reddy K, Hanumanthurayudu G, Nageswara Reddy A, *et al.* Synthesis and biological screening of some pyridine and pyrrole derivatives of pyrazolo [3, 4-c] pyrazoles. J Chem Pharm Res 2012;4:272-8.
8. Johnson M, Younglove B, Lee L, LeBlanc R, Holt H, Hills P, *et al.* Design, synthesis, and biological testing of pyrazoline derivatives of combretastatin-A4. Bioorg Med Chem Lett 2007;17:5897-901.
9. Shaharyar M, Ali MA, Siddiqui AA. Synthesis, structural activity relationship and anti-tubercular activity of novel pyrazoline derivatives. Eur J Med Chem 2007;42:268-75.
10. Ozdemir Z, Kandilci HB, Gümüşel B, Caliş U, Bilgin AA. Synthesis and studies on antidepressant and anticonvulsant activities of some 3-(2-furyl)-pyrazoline derivatives. Eur J Med Chem 2007;42:373-9.
11. Joshi RS, Mandhane PG, Diwakar SD, Dabhade SK, Gill CH. Synthesis, analgesic and anti-inflammatory activities of some novel pyrazolines derivatives. Bioorg Med Chem Lett 2010;20:3721-5.
12. Shaharyar M, Abdullah MM, Bakht MA, Majeed J. Pyrazoline bearing benzimidazoles: search for anticancer agents. Eur J Med Chem 2010;45:114-9.
13. Go ML, Wu X, Liu XL. Chalcones: an update on cytotoxic and chemoprotective properties. Curr Med Chem 2005;12:481-99.
14. Shaharyar M, Abdullah MM, Bakht MA, Majeed J. Pyrazoline bearing benzimidazoles: search for anticancer agents. Eur J Med Chem 2010;45:114-9.
15. Anto RJ, Sukumaran K, Kuttan G, Rao MN, Subbaraju V, Kuttan R. Anticancer and antioxidant activity of synthetic chalcones and related compounds. Cancer Lett 1995;97:33-7.
16. Sugiura K. Effect of various compounds on the Ehrlich ascites carcinoma. Cancer Res 1953;13:431-41.
17. Alba M, Jairo Q, Rodrigo A, Manuel N, Justo C, BraulioI. Synthesis and *in vitro* antitumor activity of a novel series of 2-pyrazoline derivatives bearing the 4-aryloxy-7-chloroquinoline. Fragment Molecular Orbital 2014;19:18656-75.
18. Ngameni B, Kuete V, Ambassa P, Justin K, Marlyse ML. Synthesis and evaluation of anticancer activity of o-allyl chalcone derivatives. Med Chem 2013;3:233-7.
19. Joshi RS, Mandhane PG, Diwakar SD, Dabhade SK, Gill CH. Synthesis, analgesis, and anti-inflammatory of some novel pyrazoline derivatives. Bioorg Med Chem Lett 2010;20:3721-5.
20. Chandra S, Priyanka C, Protapaditya D, Bhattacharya S. Evaluation of *in vitro* anti-inflammatory activity of coffee against the denaturation of protein. Asian Pac J Trop Biomed 2012;3:178-80.
21. Jainey PJ, Bhat IK. Antitumor, analgesic, and anti-inflammatory activities of synthesized pyrazolines. J Young Pharm 2012;4:82-7.