



## Antitumor, Analgesic, and Anti-inflammatory Activities of Synthesized Pyrazolines

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### ABSTRACT

Nitrogen heterocyclic compounds such as pyrazolines have been found to possess a broad spectrum of biological activities such as anticancer, antitubercular, anti-inflammatory, analgesic, and antidepressant activities. Pyrazoline derivatives IV, V (a–e) have been synthesized from the intermediate chalcones III (a–h) by cyclizing with phenyl hydrazine and hydrazine hydrate. The structures of these compounds were confirmed by IR, NMR, and mass spectroscopy. Biological studies of the synthesized compounds showed promising antitumor, analgesic, and anti-inflammatory activities. The compounds were tested for their *in vitro* antitumor activity against EAC tumor cell lines. Compounds IVa and IVb showed the highest cytotoxicity of 80% at a 200 µg mL concentration. Among the tested compounds, IVa and Vd seem to be more effective analgesic agents. Compounds IVc, IVd, and Ve are found to be the most effective anti-inflammatory agents. Thus the results show that synthesized compounds possess antitumor, analgesic, and anti-inflammatory activity. It was observed that the test compounds with electron withdrawing groups (halogens) on the aromatic ring favors antitumor, analgesic, and anti-inflammatory activity.

**Key words:** Analgesic, anti-inflammatory activity, antitumor, chalcones, pyrazolines

### INTRODUCTION

Recently, different authors worldwide have reported antitumor, antiproliferative, or anticancer potential of thiophene,<sup>[1]</sup> and pyrazoline derivatives.<sup>[2]</sup> This gave us immense confidence to carry out work on pyrazoline which possesses antitubercular,<sup>[3]</sup> antidepressant,<sup>[4]</sup> anticonvulsant,<sup>[4]</sup>

antitumor,<sup>[5]</sup> anti-inflammatory,<sup>[6]</sup> analgesic,<sup>[6]</sup> and anticancer<sup>[7]</sup> activities. Pain directly related to cancer or caused by treatments for cancer is a highly prevalent clinical problem. Therefore, analgesics and anti-inflammatory drugs are prescribed simultaneously along with cancer chemotherapeutics, in normal practice. Due to great potential of both the moieties, synthesis of pyrazoline bearing thiophene [IVa–d, Va–d] was carried out to evaluate antitumor, anti-inflammatory, and analgesic potential.

Pyrazolines are synthesized from the intermediate chalcones by the condensation of 2-acetyl thiophene with substituted benzaldehydes. Chalcones are of great interest as compounds exhibiting antimalarial,<sup>[8]</sup> anticancer,<sup>[9]</sup> antioxidant,<sup>[9]</sup> analgesic,<sup>[10]</sup> and anti-inflammatory<sup>[10]</sup> activities.

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The drug development program has employed testing in a few well characterized transplantable animal tumor systems. Simple *in vitro* assays shorten the testing program. Here the method is trypan blue exclusion and the tumor cell lines are Ehrlich Ascites Carcinoma (EAC).<sup>[11]</sup>

## MATERIALS AND METHODS

Melting points were determined by the capillary method and were uncorrected. The IR spectra are recorded by using a Shimadzu Perkin Ekmer 8201 PC IR Spectrometer and using a thin film on potassium bromide pellets techniques and frequencies are expressed in  $\text{cm}^{-1}$ . The PMR spectra were recorded on a Bruker Avance II 400 NMR spectrometer. All spectra were obtained in  $\text{CDCl}_3$  and Dimethyl sulphoxide (DMSO). Chemical shift values are reported as values in ppm relative to TMS ( $\delta=0$ ) as an internal standard. The FAB mass spectra were recorded on a JEOL SX-102/DA-6000 Mass spectrometer using Argon/Xenon (6 kV, 10 Ma) as the FAB gas. All the animal experiments were approved by institutional animal ethical committee (IAEC).

### General procedure for synthesis of chalcones

A mixture of 2-acetyl thiophene (0.01 mol) and substituted benzaldehydes (0.01 mol) in ethanol (20 ml) were stirred together for 24 h, in the presence of 20% NaOH (4 ml). The mixture was poured into crushed ice and acidified with 5% HCl. The product (substituted chalcones) obtained was filtered, washed with water, and re-crystallized from suitable solvents [Table 1].

#### *3-(4-Fluorophenyl)-1-(thiophen-2-yl) prop-2-en-1-one*

IR (KBr  $\text{cm}^{-1}$ ): 1648.9 (CO), 1596.4 (aliphatic C=C), 3067.7 (C-H), 1516 (aromatic C=C), 1216.2 (C-F);  $^1\text{H NMR}$  ( $\delta$  ppm): 7.79 (d, 1H, =CH), 7.18 (d, 1H, =CH), 7.52–7.87 (m, 7H, Ar-H); Mass (m/z): 232.

#### *3-(4-Chlorophenyl)-1-(thiophen-2-yl)prop-2-en-1-one*

IR (KBr  $\text{cm}^{-1}$ ): 1672.8 (CO) group, 1611.3 (aliphatic C=C), 2924.1 (C-H), 1533.7 (aromatic C=C), 765.3 (C-Cl);  $^1\text{H NMR}$  ( $\delta$  ppm): 6.97–6.99 (d, 1H, =CH), 6.71–6.72 (d, 1H, =CH), 7.53–7.89 (m, 7H, Ar-H); Mass (m/z): 248.

#### *3-(4-Methylphenyl)-1-(thiophen-2-yl) prop-2-en-1-one*

IR (KBr  $\text{cm}^{-1}$ ): 1683.5 (CO), 1609.7 (aliphatic C=C), 2987.3 (C-H), 1523.5 (aromatic C=C), 765.3 (C-Cl);  $^1\text{H NMR}$  ( $\delta$  ppm): 7.12–7.14 (d, 1H, =CH), 7.17–7.19 (d, 1H, =CH), 7.51–7.899 (m, 7H, Ar-H); Mass (m/z): 228.

### General procedure for synthesis of pyrazolines

A mixture of substituted chalcones (0.01 mol) in 20 ml of ethanol and phenyl hydrazine, hydrazine hydrate (0.01 mol) were added and refluxed for 5–8 h and 16–20 h, respectively, in the presence of few drops of pyridine as catalyst. After the completion of the reaction, the reaction mixture was poured into 250 ml of ice cold water. The solid separated is filtered and washed with cold water. The separated compound is recrystallized by using methanol/ethyl acetate. Ethyl acetate: acetone (9:1) is the solvent system for TLC [Table 2].

#### *5-(4-Fluorophenyl)-1-phenyl-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole*

IR (KBr  $\text{cm}^{-1}$ ): 3431 (C-H), 1646.8 (C=N), 1324.3 (C-N), 1594.7 (C=C), 1224.9 (C-F);  $^1\text{H NMR}$  ( $\delta$  ppm): 3.064–3.124 (dd, 1H, Ha), 3.786–3.859 (dd, 1H, Hb), 5.213–5.261 (dd, 1H, Hc), 6.76–7.31 (m, 12H, Ar-H); Mass (m/z): 322.

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

IR (KBr  $\text{cm}^{-1}$ ): 3039.6 (C-H), 1635.6 (C=N), 1336.7 (C-N), 1522.5 (C=C), 703.8 (C-Cl);  $^1\text{H NMR}$  ( $\delta$  ppm): 3.049–3.109 (dd, 1H, Ha), 3.779–3.852 (dd, 1H, Hb), 5.190–5.239 (dd, 1H, Hc), 6.769–7.308 (m, 12H, Ar-H); Mass (m/z): ( $M^+$ ) 338, ( $M^+ + 2$ ) 340.

#### *5-(4-Fluorophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole*

IR (KBr  $\text{cm}^{-1}$ ): 3239.2 (C-H), 1546.3 (C=N), 1499.33

**Table 1: Physical data of substituted chalcone derivatives**

Chalcones	R	Molecular formula	MP (°C)	% Yield
IV	4-Cl	$\text{C}_{13}\text{H}_9\text{ClOS}$	80–82	78
IVb	4-F	$\text{C}_{13}\text{H}_9\text{FOS}$	83–85	75
IVc	4-OH	$\text{C}_{13}\text{H}_{10}\text{O}_2\text{S}$	104–106	62
IVd	4-N(CH <sub>3</sub> ) <sub>2</sub>	$\text{C}_{15}\text{H}_{15}\text{NOS}$	87–89	68
Va	4-CH <sub>3</sub>	$\text{C}_{14}\text{H}_{12}\text{OS}$	96–98	77
Vb	3-OH	$\text{C}_{13}\text{H}_{10}\text{O}_2\text{S}$	88–90	68
Vc	3-Cl	$\text{C}_{13}\text{H}_9\text{ClOS}$	92–95	63
Vd	3-NO <sub>2</sub>	$\text{C}_{13}\text{H}_9\text{NO}_3\text{S}$	111–112	68

**Table 2: Physical data of the synthesized pyrazolines**

Pyrazolines	R	Molecular formula	MP (°C)	% Yield
IV	4-Cl	$\text{C}_{19}\text{H}_{15}\text{ClN}_2\text{S}$	153–155	69
IVb	4-F	$\text{C}_{19}\text{H}_{15}\text{FN}_2\text{S}$	158–160	71
IVc	4-CH <sub>3</sub>	$\text{C}_{20}\text{H}_{18}\text{N}_2\text{S}$	106–108	55
IVd	3-NO <sub>2</sub>	$\text{C}_{19}\text{H}_{15}\text{N}_3\text{O}_2\text{S}$	175–177	49
Va	p-Cl	$\text{C}_{13}\text{H}_{11}\text{ClN}_2\text{S}$	171–174	68
Vb	p-F	$\text{C}_{13}\text{H}_{11}\text{FN}_2\text{S}$	159–161	65
Vc	p-CH <sub>3</sub>	$\text{C}_{14}\text{H}_{14}\text{N}_2\text{S}$	88–90	43
Vd	m-NO <sub>2</sub>	$\text{C}_{13}\text{H}_{11}\text{N}_3\text{O}_2\text{S}$	145–147	51

(C–N), 1523.9 (C=C), 1197.3 (C–F); <sup>1</sup>H NMR (δ ppm): 3.064–3.124 (dd, 1H, Ha), 3.786–3.859 (dd, 1H, Hb), 5.213–5.261 (dd, 1H, Hc), 6.76–7.31 (m, 12H, Ar–H); Mass (m/z): 246.

*5-(4-Chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole*  
 IR (KBr cm<sup>-1</sup>): 3141.1 (C–H), 1689.6 (C=N), 1421.3 (C–N), 1583.9 (C=C), 737.2 (C–Cl); <sup>1</sup>H NMR (δ ppm): 3.106–3.198 (dd, 1H, Ha), 3.657–3.748 (dd, 1H, Hb), 5.891–5.931 (dd, 1H, Hc), 6.331–7.05 (m, 12H, Ar–H); Mass (m/z): (M<sup>+</sup>) 262, (M<sup>+</sup>+2) 264.

### Antitumor activity

The synthesized compounds were tested for their cytotoxicity *in vitro*, in comparison with 5-fluorouracil as a reference drug, against EAC cells. EAC cells (1 × 10<sup>6</sup>) were incubated with synthesized compounds at various concentrations of 25, 50, 100, and 200 µg/ml, in 1 ml phosphate buffered saline (incorporated with 10 µL DMSO) at 37°C for 3 h. Viable cells were counted in a hemocytometer using the trypan blue dye exclusion method.<sup>[12]</sup> Experiments were carried out in triplicate, and results are reported in Table 3.

### Pharmacological screening

#### Animals

Adult female albino rats were used for acute toxicity studies. The acute toxicity test was carried out according to the organization for economic co-operation and development (OECD) guidelines to establish the effective dose of the test compounds after obtaining ethical clearance from ethics committee of K.S. Hegde Medical Academy, Deralakatte, Mangalore, India (Ethical Clearance Reg. No. KSHEMA/AEC/093/2009).

#### Acute toxicity

Adult female albino rats weighing 150–230 g were grouped into six groups of six animals each, starved for 24 h. On the day of the experiment, animals were

**Table 3: Antitumor activities of synthesized pyrazolines**

Pyrazolines	No. of dead cells (%) at different concentrations (µg/ml)			
	50	100	200	250
Control				
IV	20	45	68	78
IVb	26	28	58	65
IVd	16	20	27	43
Va	10	15	42	55
Vb	14	17	22	32
5-Fluorouracil	45	80	100	100

orally administered different compounds to different groups with an increasing dose of 10, 20, 100, 200, 1000, and 2000 mg/kg body weight. Then, the animals were observed continuously for 3 h concerning the general behavioral, neurological, and autonomic profiles, then, every 30 min for the next 3 h, and finally for the next 24 h or until death.

#### Analgesic activity

The analgesic activity of the test compounds was carried out *in vivo* by the tail immersion method.<sup>[13]</sup> Pentazocine (10 mg/kg) was administered as standard for comparison and test compounds at a dose level of 50 mg/kg were administered orally. The lower portion of the tail was immersed in the thermostatic organ bath in which water is maintained at exactly 55°C. Within few seconds the rat reacts by withdrawing the tail. The reaction times 0, 30, 60, 90, and 120 min after the treatment of the test substance were noted. Increased or decreased in reaction time of the test substance was then compared with standard drug treated and solvent treated. The results are given in Table 4.

Percentage increase in reaction time = [(R<sub>t</sub>/R<sub>c</sub>)-1] × 100, where R<sub>t</sub> is mean reaction time of the treated group and R<sub>c</sub> is mean reaction time of control group.

#### Anti-inflammatory activity

The anti-inflammatory of the test compounds was carried out using the carrageenan-induced rat paw edema inhibition method according to Winter *et al.*<sup>[14]</sup> Acute inflammation was produced by subplantar injection of 0.1 ml of 1% suspension of carrageenan in the right hind paw of the rats, 30 min after oral administration of the drugs. The paw volume was measured plethysmometrically (IITC digital plethysmograph IITC-520) at 1, 2, 3, and

**Table 4: Analgesic activities of synthesized pyrazolines**

Treatment	Reaction time in sec at time (min)			
	30	60	90	120
Control	2.27 ± 0.03	2.65 ± 0.02	2.83 ± 0.03	2.75 ± 0.04
Pentazocine	5.21 ± 0.04**	7.34 ± 0.02**	8.7 ± 0.10**	5.38 ± 0.09**
IV	4.8 ± 0.04**	6.9 ± 0.02**	8.33 ± 0.05**	5.9 ± 0.03*
IVb	4.3 ± 0.04**	6.2 ± 0.05**	7.9 ± 0.03**	5.3 ± 0.05**
IVc	2.38 ± 0.02	2.99 ± 0.03*	3.99 ± 0.04*	3.87 ± 0.02*
IVd	2.22 ± 0.03	2.9 ± 0.04*	3.10 ± 0.04*	2.92 ± 0.04*
IVe	4.5 ± 0.02**	6.8 ± 0.04**	8.0 ± 0.03**	6.8 ± 0.04**
Va	4.6 ± 0.04**	6.4 ± 0.03**	7.9 ± 0.03**	5.67 ± 0.03**
Vb	3.75 ± 0.03**	6.03 ± 0.03**	7.77 ± 0.04**	4.9 ± 0.03**
Vc	2.39 ± 0.02	3.24 ± 0.03*	3.95 ± 0.03*	3.5 ± 0.03*
Vd	4.76 ± 0.03**	6.13 ± 0.03**	8.12 ± 0.02**	5.15 ± 0.03**
Ve	4.29 ± 0.03**	6.45 ± 0.03**	8.2 ± 0.03	5.58 ± 0.04*

Values are expressed as mean ± SEM of six animals in each group. \*Statistically significant (P ≤ 0.05). \*\*Statistically significant (P ≤ 0.001)

4 h after the carrageenan injection. Indomethacin was used as the standard drug at a dose level of 10 mg/kg. The percentage inhibition of edema was calculated using the formula,

$$\% \text{ Inhibition} = (1 - V_t / V_c) \times 100,$$

where  $V_t$  and  $V_c$  is the edema volume in treated and control groups, respectively. The results are summarized in Table 5.

## RESULTS AND DISCUSSION

The sequence of the reactions employed for the development of novel pyrazoline derivatives is outlined in Scheme 1. Chalcones III (a–h) were synthesized by the condensation of 2-acetyl thiophene I with various substituted benzaldehydes II in the presence of NaOH. This chalcones on cyclization with phenyl hydrazine and hydrazine hydrate gave pyrazolines IV, V (a–e), respectively, in the presence of pyridine as catalyst.

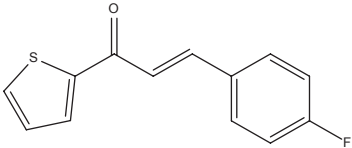
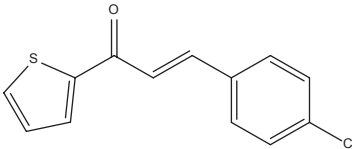
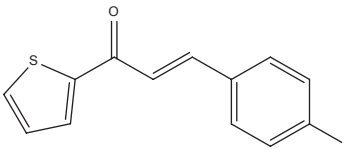
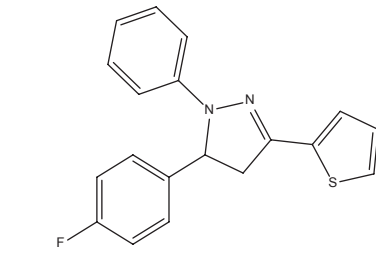
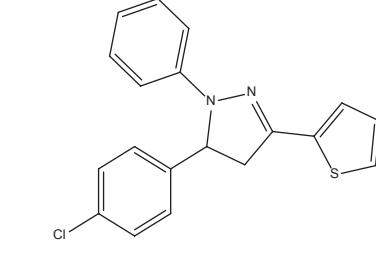
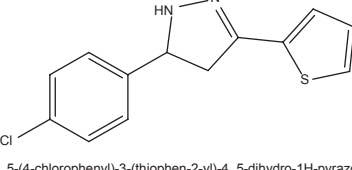
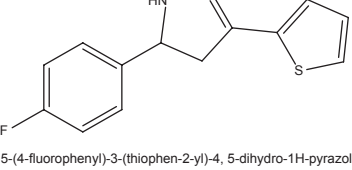
The structures of newly synthesized compounds are well supported by spectral data such as IR, NMR, and Mass spectral analysis. The formation of title compounds IV, V (a–e) is indicated by the disappearance of peak due to C=O of the intermediate chalcones and the presence of peaks due to C=N of the pyrazoline ring in IR spectra as given above. Further, in their  $^1\text{H}$  NMR spectrum, the appearance of a signal at  $\delta$  3.064–3.124 (dd, 1H, Ha), 3.786–3.859 (dd, 1H, Hb), 5.213–5.261 (dd, 1H, Hc), confirms the presence of the pyrazoline ring.

All the tested compounds showed antitumor effects and are reported. Compounds IVa and IVb showed the highest

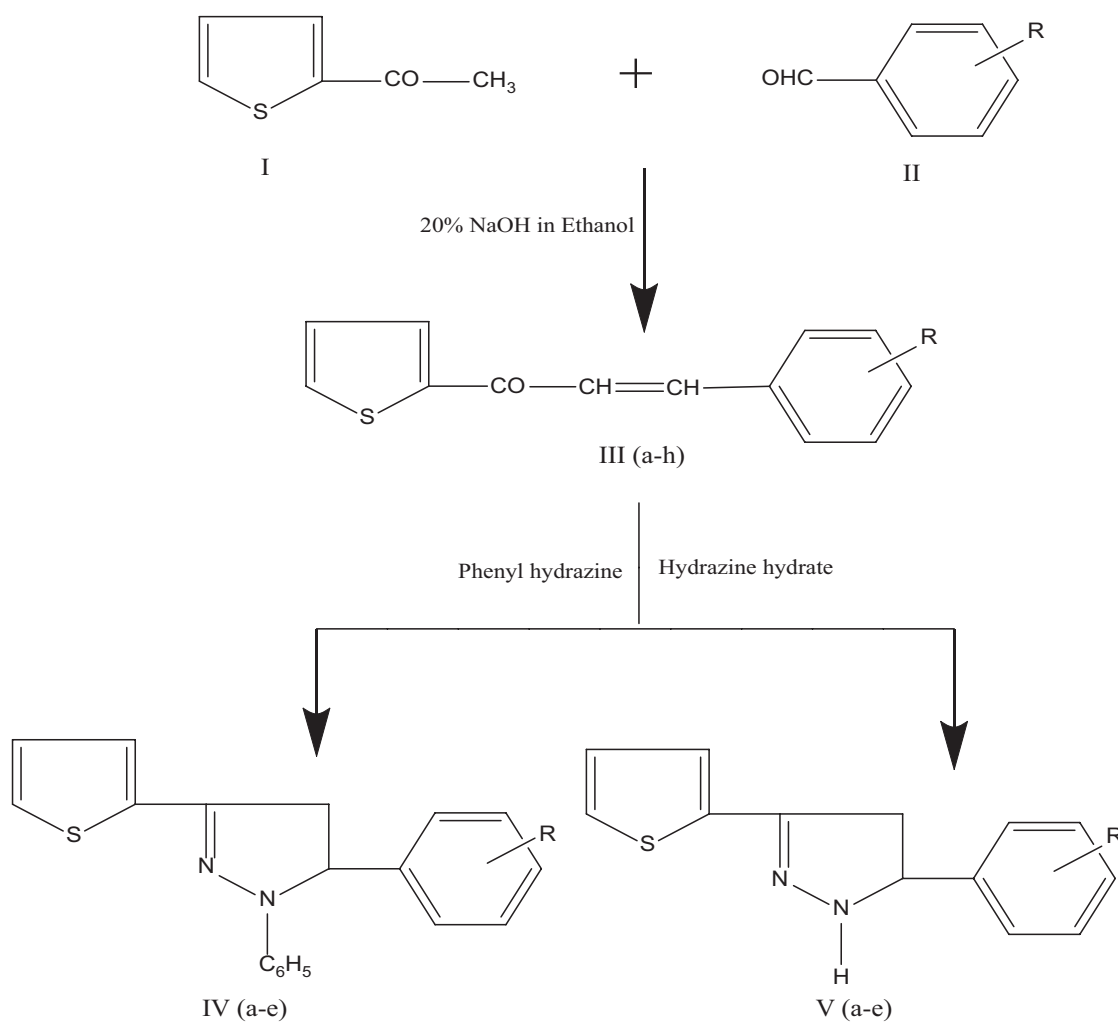
**Table 5: Anti-inflammatory activities of synthesized pyrazolines**

Treatment	Mean paw volume at different time intervals			
	1 h	2 h	3 h	4 h
Control	0.46 ± 0.01	0.68 ± 0.01	0.79 ± 0.01	0.88 ± 0.01
Indomethacin	0.22 ± 0.01**	0.20 ± 0.01**	0.19 ± 0.01**	0.11 ± 0.01**
IV	0.39 ± 0.02**	0.35 ± 0.02**	0.34 ± 0.02**	0.21 ± 0.01**
IVb	0.45 ± 0.02*	0.38 ± 0.01*	0.31 ± 0.03*	0.29 ± 0.02*
IVc	0.23 ± 0.01**	0.21 ± 0.01**	0.20 ± 0.02**	0.18 ± 0.02**
IVd	0.28 ± 0.01*	0.25 ± 0.02*	0.21 ± 0.02*	0.15 ± 0.01*
IVe	0.41 ± 0.01*	0.37 ± 0.02*	0.32 ± 0.03*	0.27 ± 0.02*
Va	0.35 ± 0.02**	0.30 ± 0.02**	0.28 ± 0.01**	0.19 ± 0.01**
Vb	0.42 ± 0.01*	0.39 ± 0.03*	0.30 ± 0.01*	0.25 ± 0.01*
Vc	0.38 ± 0.01**	0.35 ± 0.02**	0.27 ± 0.02**	0.20 ± 0.02**
Vd	0.47 ± 0.01*	0.43 ± 0.02*	0.38 ± 0.01*	0.31 ± 0.01*
Ve	0.29 ± 0.02**	0.26 ± 0.01**	0.23 ± 0.02**	0.17 ± 0.01**

Values are expressed as mean ± SEM of six animals in each group. \*Statistically significant ( $P \leq 0.05$ ). \*\*Statistically significant ( $P \leq 0.001$ )

Structures of synthesized compounds	IR spectra
 3-(4-fluorophenyl)-1-(thiophen-2-yl) prop-2-en-1-one	1648.9 (CO), 1596.4 (aliphatic C = C), 3067.7 (C–H), 1516 (aromatic C = C), 1216.2 (C–F)
 3-(4-chlorophenyl)-1-(thiophen-2-yl) prop-2-en-1-one	1672.8 (CO) group, 1611.3 (aliphatic C = C), 2924.1 (C–H), 1533.7 (aromatic C = C), 765.3 (C–Cl)
 3-(4-methylphenyl)-1-(thiophen-2-yl) prop-2-en-1-one	1683.5 (CO), 1609.7 (aliphatic C = C), 2987.3 (C–H), 1523.5 (aromatic C = C)
 5-(4-fluorophenyl)-1-phenyl-3-(thiophen-2-yl)-4, 5-dihydro-1H-pyrazole	3431 (C–H), 1646.8 (C = N), 1324.3 (C–N), 1594.7 (C = C), 1224.9 (C–F)
 5-(4-chlorophenyl)-1-phenyl-3-(thiophen-2-yl)-4, 5-dihydro-1H-pyrazole	3039.6 (C–H), 1635.6 (C = N), 1336.7 (C–N), 1522.5 (C = C), 703.8 (C–Cl)
 5-(4-chlorophenyl)-3-(thiophen-2-yl)-4, 5-dihydro-1H-pyrazole	3239.2 (C–H), 1546.3 (C = N), 1499.33 (C–N), 1523.9 (C = C), 1197.3 (C–F)
 5-(4-fluorophenyl)-3-(thiophen-2-yl)-4, 5-dihydro-1H-pyrazole	3141.1 (C–H), 1689.6 (C = N), 1421.3 (C–N), 1583.9 (C = C), 737.2 (C–Cl)

cytotoxicity of 80% at a 200  $\mu\text{g}$  mL concentration. The cytotoxicity activity was expressed as percentage of dead cells. Among the tested compounds, IVa and Vd seem to be more effective analgesic agents. The analgesic activity was expressed as percentage increase in reaction time.



**Scheme 1:** Synthesis of pyrazolines from the intermediate chalcones. I, 2-Acetyl thiophene; II, substituted benzaldehydes; III, substituted chalcones; IV and V, substituted pyrazolines. R = Cl, F, CH<sub>3</sub>, OH, NO<sub>2</sub>.

Compounds IVc, IVd, and Ve are found to be the most effective anti-inflammatory agents. The results indicate the percentage inhibition of inflammatory.

## CONCLUSION

Thus the results show that synthesized compounds possess antitumor, analgesic, and anti-inflammatory activities. Their synthesis was simple with satisfactory yields. It was observed that the test compounds with electron withdrawing groups (halogens) on the aromatic ring favors antitumor, analgesic, and anti-inflammatory activities. However, further studies are required to establish the exact mechanism of action.

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