



## MICROWAVE ASSISTED SYNTHESIS AND ANTI-INFLAMMATORY ACTIVITY OF 3, 5-DIARYL SUBSTITUTED -2-PYRAZOLINES

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### ABSTRACT

An efficient, rapid, eco-friendly and cost effective microwave-assisted protocol using fewer amounts of solvents was developed for the synthesis of 3, 5-diaryl/heteryl substituted -2-pyrazolines IV by reaction of substituted prop-2-ene-1-ones III with hydrazine hydrate in acetic acid. A considerable enhancement in the reaction rate has been observed, with better yields. The structures of the synthesized compounds were confirmed by elemental analysis and spectral studies. The newly synthesized compounds were evaluated for their anti-inflammatory activity and have shown significant anti-inflammatory activity.

**Key words:** Pyrazolines, Prop-2-ene-1-ones, Anti-inflammatory

### INTRODUCTION

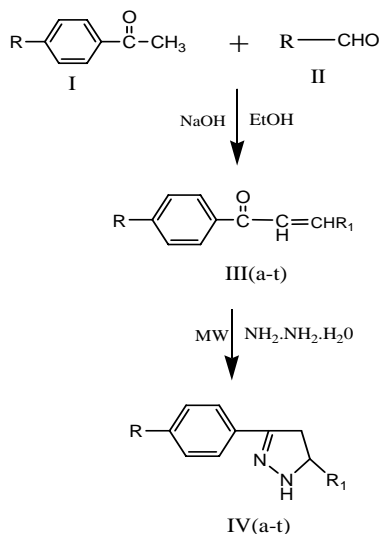
Pyrazolines have been reported to show a broad spectrum of biological activities including antibacterial,<sup>1</sup> antifungal,<sup>2</sup> anti-inflammatory,<sup>3</sup> analgesic,<sup>4</sup> antipyretic,<sup>5</sup> insecticidal<sup>5</sup> diuretic,<sup>6</sup> cardiovascular<sup>7</sup> and antidepressant activities<sup>8</sup>. Nitrogen containing heterocyclic compound like pyrazoline has received considerable attention in recent years due to their biological activities such as anticonvulsant<sup>9</sup> and antidiabetic<sup>10</sup>. The pyrazoline function is quite stable and has inspired chemists to utilize this stable fragment in bioactive moieties to synthesize new compounds possessing biological activities. High speed microwave assisted chemistry is being utilized in recent years successfully in various fields of synthetic organic chemistry<sup>11</sup> including cycloaddition reactions<sup>12</sup>, heterocycle synthesis<sup>13</sup>, rapid preparation of radiolabelled materials<sup>14</sup>, Transition metal catalyzed processes<sup>15</sup>, solvent free reaction<sup>16</sup> and phase transfer catalysis<sup>17-19</sup>. Literature reviews prove the synthetic utility of MORE chemistry in routine organic synthesis.<sup>20-22</sup> It can be termed as 'e-chemistry' because it is easy, effective, economical and eco-friendly and is believed to be a step towards green chemistry. Under the framework of "Green Chemistry" we were, therefore, interested in developing a rapid, microwave-assisted protocol for the synthesis of 3,5 diaryl substituted-2-pyrazolines. In continuation to our earlier work on synthesis of bioactive heterocycles, we report here a simple

microwave protocol for synthesis of 3, 5-diaryl substituted 2-pyrazolines IV (a-t) from substituted prop-2-ene-1-ones and hydrazine hydrate.

### MATERIALS AND METHODS

Melting points were taken in open capillaries and are uncorrected. The IR spectra were taken on FTIR, PMR spectra were recorded on Bruker (200 MHz) spectrophotometer using TMS as an internal standard and Mass spectra were taken on ESI-Esquire 3000 Bruker Daltonics instrument. All the chemicals were obtained from Merck Make, while the reagents and solvents were of analytical grade purity. The reactions were carried out conventionally and by using green methods. Microwave assisted synthesis was performed on synthetic Microwave of CATA using power of 700 watts. The purity of synthesized compounds has been checked by TLC.

The desired prop-2-ene-1-ones<sup>23</sup> were synthesized by Claisen Schmidt condensation by reacting, equimolar quantities of substituted acetophenones with various substituted aromatic aldehydes in the presence of alkali. Their structures were established by elemental and spectral study. The title compounds 3,5 diaryl substituted 2-pyrazolines IV (a-t) were prepared by condensation of different prop-2-ene-1-ones III (a-t) with hydrazine hydrate, acetic acid in ethanol as a solvent. (Scheme 1). Their structures were confirmed by spectral analysis.



Scheme-1

## General procedure for the preparation of 1,3-prop-2-ene-1-one

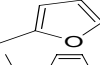
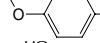
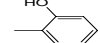
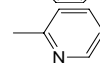
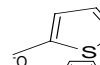
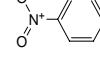
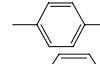
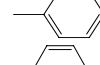
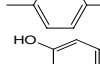
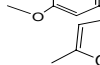
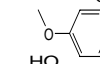
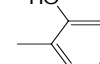
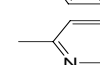
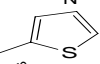
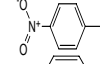
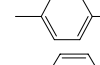
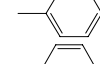
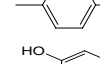
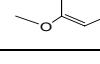
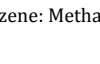
### 1. Conventional Method (Claisen-Schmidt method)

Equimolar quantities of substituted acetophenones (0.01mole) and substituted aromatic aldehydes (0.01 mole) were dissolved in ethanol (30ml) and ice-cold solution of NaOH (15ml, 40%) was added in portion keeping the temperature below 10°C with continuous stirring. The reaction mixture was corked and kept at room temperature for overnight. It was then acidified with dilute HCl and poured over crushed ice with continuous stirring. The product obtained was filtered, washed with cold water, dried and recrystallised from ethanol. The yield and MP were recorded. These compounds gave red coloration with conc. H<sub>2</sub>SO<sub>4</sub> indicating the presence of  $\alpha$ ,  $\beta$ -unsaturated carbonyl system.

### 2. Microwave- assisted synthesis (Solution phase MWI)

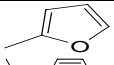
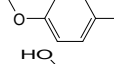
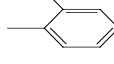
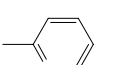
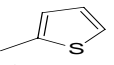
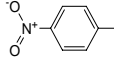
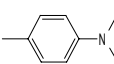
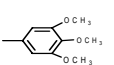
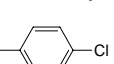
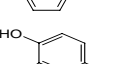
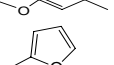
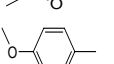
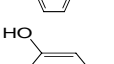
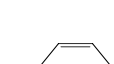
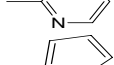
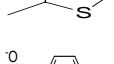
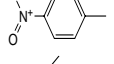
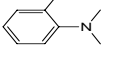
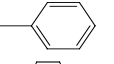
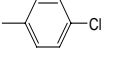
Equimolar quantities of substituted acetophenone and substituted aromatic aldehydes in ethanol (10ml) and NaOH (4ml, 40%) were taken in Erlenmeyer's flask. The reaction mixture was irradiated in a microwave oven for 5-7 min. at H power level (700W) with short interruption of 30sec, to avoid the excessive evaporation of solvent and to record the temperature. All the temperatures recorded were in the range of 74 -85°C. This protocol was repeated in overall heating time. On completion of reaction (TLC) the reaction mixture was cooled at room temp, acidified with dilute HCL. The product was separated, filtered, washed with cold water, dried and recrystallised from ethanol. Similarly, all other compounds of the series (III a-t) were synthesized. Their physical constants are given in Table 1.

**Table 1: Characterization Data of prop-2-ene-1-ones (IIIa -IIIt)**

Comp. No.	R	R <sub>1</sub>	Yield (%)	M.P(°C)	Mol. Formula	Mol. Weight	R <sub>f</sub>
III a	H		62	190	C <sub>13</sub> H <sub>10</sub> O <sub>2</sub>	198.22	0.64
III b	H		74	75	C <sub>16</sub> H <sub>14</sub> O <sub>2</sub>	238.28	0.68
III c	H		83	65	C <sub>15</sub> H <sub>12</sub> O <sub>2</sub>	224.25	0.82
III d	H		76	124	C <sub>14</sub> H <sub>11</sub> NO	209	0.68
III e	H		68	72	C <sub>13</sub> H <sub>10</sub> OS	214	0.76
III f	H		77	120	C <sub>15</sub> H <sub>11</sub> NO <sub>3</sub>	253.25	0.82
III g	H		65	220	C <sub>17</sub> H <sub>17</sub> NO	190	0.86
III h	H		76	190	C <sub>15</sub> H <sub>12</sub> O	208	0.75
III i	H		92	190	C <sub>15</sub> H <sub>11</sub> ClO	242	0.82
III j	H		84	190	C <sub>16</sub> H <sub>14</sub> O <sub>3</sub>	254	0.79
III k	OH		62	112	C <sub>13</sub> H <sub>10</sub> O <sub>2</sub>	214	0.85
III l	OH		62	112	C <sub>16</sub> H <sub>14</sub> O <sub>3</sub>	254	0.85
III m	OH		74	123	C <sub>15</sub> H <sub>12</sub> O <sub>3</sub>	240	0.69
III n	OH		69	114	C <sub>14</sub> H <sub>11</sub> NO <sub>2</sub>	225	0.87
III o	OH		42	94	C <sub>13</sub> H <sub>10</sub> O <sub>2</sub> S	240	0.67
III p	OH		74	98	C <sub>15</sub> H <sub>11</sub> NO <sub>4</sub>	269	0.84
III q	OH		82	112	C <sub>17</sub> H <sub>17</sub> NO	206	0.82
III r	OH		62	128	C <sub>15</sub> H <sub>12</sub> O	224	0.68
III s	OH		82	154	C <sub>15</sub> H <sub>11</sub> ClO <sub>2</sub>	58	0.87
III t	OH		76	175	C <sub>16</sub> H <sub>14</sub> O <sub>3</sub>	270	0.78

Mobile Phase for TLC: Benzene: Methanol (9:1)

Table 2: Characterization data of 3,5- di aryl substituted 2-pyrazolines (IV a-IV t)

Comp. No.	R	R <sub>1</sub>	Mol. Formula	Time (min.)	Yield (%)	M.P(°C)	R <sub>f</sub>
IV a	H		C <sub>13</sub> H <sub>12</sub> N <sub>2</sub> O	6	72	240	0.56
IV b	H		C <sub>16</sub> H <sub>16</sub> N <sub>2</sub> O	7	83	175	0.62
IV c	H		C <sub>15</sub> H <sub>14</sub> N <sub>2</sub> O	6	79	192	0.82
IV d	H		C <sub>14</sub> H <sub>13</sub> N <sub>3</sub>	5	86	184	0.45
IV e	H		C <sub>13</sub> H <sub>12</sub> N <sub>2</sub> S	5	91	210	0.64
IV f	H		C <sub>15</sub> H <sub>13</sub> N <sub>3</sub> O <sub>2</sub>	7	95	225	0.49
IV g	H		C <sub>17</sub> H <sub>19</sub> N <sub>3</sub>	6	82	220	0.86
IV h	H		C <sub>15</sub> H <sub>14</sub> N <sub>2</sub>	4	79	198	0.64
IV i	H		C <sub>15</sub> H <sub>13</sub> ClN <sub>2</sub>	5	83	149	0.76
IV j	H		C <sub>16</sub> H <sub>16</sub> N <sub>2</sub> O <sub>2</sub>	7	92	210	0.68
IV k	OH		C <sub>13</sub> H <sub>12</sub> N <sub>2</sub> O <sub>2</sub>	6	85	275	0.45
IV l	OH		C <sub>16</sub> H <sub>16</sub> N <sub>2</sub> O <sub>2</sub>	7	94	255	0.52
IV m	OH		C <sub>15</sub> H <sub>14</sub> N <sub>2</sub> O <sub>2</sub>	6	92	194	0.68
IV n	OH		C <sub>14</sub> H <sub>13</sub> N <sub>3</sub> O	5	89	215	0.54
IV o	OH		C <sub>13</sub> H <sub>12</sub> N <sub>2</sub> SO	4	84	245	0.6
IV p	OH		C <sub>15</sub> H <sub>13</sub> N <sub>3</sub> O <sub>3</sub>	7	79	198	0.84
IV q	OH		C <sub>17</sub> H <sub>19</sub> N <sub>3</sub> O	6	86	265	0.79
IV r	OH		C <sub>15</sub> H <sub>14</sub> N <sub>2</sub> O	6	92	281	0.64
IV s	OH		C <sub>15</sub> H <sub>13</sub> ClN <sub>2</sub> O	5	97	234	0.66
IV t	OH		C <sub>16</sub> H <sub>16</sub> N <sub>2</sub> O <sub>3</sub>	7	84	45	0.68

Mobile Phase for TLC: Benzene: Methanol (9:1)

### Spectroscopic data of synthesized compounds

The infra red spectra of prepared chalcones (III a-t) showed a carbonyl absorption in the region 1655 – 1665  $\text{cm}^{-1}$  which is the characteristic of the  $\alpha$ ,  $\beta$  – unsaturated carbonyl group as well as an olefinic C = C band in the region 1604 – 1611.

The  $^1\text{H}$  NMR spectra showed the olefinic protons H- $\beta$  and H- $\alpha$  as two doublets in the regions at  $\delta$  6.57 - 6.72 and  $\delta$  7.25 -7.62 ppm, respectively. The aromatic protons appeared as multiplets at  $\delta$  6.85 – 8.05. Mass m/e (III a) M +1 199.

### General procedure for the preparation of 3,5 diaryl substituted 2. Pyrazolines

#### Conventional Synthesis

##### Procedure

A mixture of substituted prop-2-ene -1-one (chalcone) (0.01 mole), hydrazine hydrate (0.6 gm, 0.012 mole) and acetic acid (20ml) in methanol was refluxed for six hours. The reaction mixture was cooled and poured over ice water. The solid separated was filtered, washed with water and crystallized from ethanol. The completion of the reaction was monitored by TLC.

#### Microwave assisted synthesis (Solution phase MWI)

##### Procedure

A mixture of substituted prop-2-en-1-one (chalcone) (0.01 mole), hydrazine hydrate (0.6 gm, 0.012 mole) and acetic acid (4 ml) in methanol (10 ml) were taken in Erlenmeyer's flask. The reaction mixture was irradiated in a microwave oven for 7-15min. at H power level (700W) with short interruption of 30 sec, to avoid the excessive evaporation of solvent and to record temperature of the reaction. All the reactions were carried out at 80-85 °C. This protocol was repeated in overall heating time. On completion of reaction (TLC) the reaction mixture was cooled at room temp and poured over ice water. The product separated was filtered, washed with cold water, dried and recrystallised from ethanol.

Similarly, all other compounds of the series were synthesized. Their physical constants, yield and anti-inflammatory activity are recorded in Table 2 and Table 3.

**Spectroscopic data of synthesized compounds:** IR (KBr  $\text{cm}^{-1}$ ): 3450 (Ar-OH), 3355(N-H), 2923.9 (C-H str), 2968(C-H asym), 2850 (C-H sym), 1650 (C=N str), 1548.7 (C=C str).

$^1\text{H}$  NMR (DMSO)  $\delta$  ppm: 1.8-2.1(d, 2H, methylene of pyrazoline), 3.6-3.8(m, 9H, methoxy); 4.5-5.5 (d, 1H, methine of pyrazoline); 6.2-7.5 (m, 7H, Ar-H); 7.1(s,1H,NH of pyrazoline.)

Mass m/e (IV a): M +1 =213.

### Pharmacological screening

All the newly synthesized compounds were screened for anti-inflammatory activity by Carrageenan- induced rat hind paw oedema<sup>23</sup>

#### Anti Inflammatory activity

Carrageenan- induced rat hind paw oedema

The method of Winter et al (Winter et al 1962) was used with slight modification. The apparatus used for the measurement of rat paw volume was that of Butle et al, modified by Singh and Gosh. The animals were divided into different groups each containing 4 animals one group served as control, another group served as a standard (Indomethacin) and the rest of the groups were used for the test drugs.

The rats were dosed orally at 100 mg/kg body weight, including the control and indomethacin. Test compounds and standard drug were suspended in 0.5 % sodium carboxy methylcellulose mucilage, which was used as vehicle. For the control group a solution of 1% of carrageenan was used as anti-inflammatory agent. The Pharmacological screening was conducted with CPCSEA Reg. no. 221/CPCSEA 2000.

### RESULTS AND DISCUSSION

As a result of our studies related to the development of synthetic protocols using microwave irradiation, we report here a novel and easy access to 3,5-diaryl substituted 2-pyrazolines using a one-pot procedure and demonstrate its superiority over previously reported classical heating methods. All the synthesized compounds were characterized on the basis of their spectral analysis (FTIR, Mass, PMR). The anti-inflammatory activity of synthesized compounds was studied using Carrageenan-induced hind paw edema model in albino rats. In this study indomethacin was used as standard drug. The results are shown in the Table 3. All the synthesized pyrazoline derivatives except a few have shown significant activity. Among these compound no. IV a (R=H, R<sub>1</sub> = furan); IV b (R =H, R<sub>1</sub>= methoxy phenyl); IV e(R= -H, R<sub>1</sub> = thiophene); and IV g (R= -H, R<sub>1</sub> =p-amino phenyl) IV h (R= -H, R<sub>1</sub> =trimethoxy phenyl) IV j (R= -H, R<sub>1</sub> =3-methoxy 2-hydroxy phenyl) IV m (R= -OH, R<sub>1</sub> =2-hydroxy phenyl) were found to show equivalent potency with the standard drug (Figure 1).

Table 3: Anti-inflammatory activity of 3,5-diaryl substituted- 2-pyrazolines (IVa-IV t)

Compound Code	Paw Edema (in ml) (Mean $\pm$ SE)			
	0hr	1hr	2hr	4hr
Control	3.75 $\pm$ 0.023	4.69 $\pm$ 0.075	5.10 $\pm$ 0.21	4.65 $\pm$ 0.23
Std. Indomethacin	3.77 $\pm$ 0.143	3.85 $\pm$ 0.183	3.75 $\pm$ 0.17	3.75 $\pm$ 0.26**
IV a	3.69 $\pm$ 0.15	4.57 $\pm$ 0.24 (91%) (6.38%)	4.78 $\pm$ 0.19 (98%) (19.25%)	3.80 $\pm$ 0.35* (97.77%) (87.77%)
IV b	3.59 $\pm$ 0.14	4.73 $\pm$ 0.12 (21.27%)	4.10 $\pm$ 0.15 (62.10%)	3.64 $\pm$ 0.24** (82.22%)
IV c	3.71 $\pm$ 0.06	4.74 $\pm$ 0.19 (9.57%)	4.79 $\pm$ 0.11 (20%)	3.75 $\pm$ 0.07* (50%)
IV d	3.79 $\pm$ 0.13	4.64 $\pm$ 0.09 (9.57%)	3.74 $\pm$ 0.09** (103.7 %)	3.89 $\pm$ 0.11** (80%)

IV e	3.52±0.11	4.35±0.17 (11.70%)	4.50±0.13 (27.4%)	3.85±0.10* (93%)
IV f	3.75±0.07	4.20±0.14 (52.12%)	4.41±0.52 (51.11%)	3.87±0.24** (83%)
IV g	3.64±0.11	4.40±0.37 (19.14%)	4.59±0.41 (29.62%)	3.75±0.18* (87.7%)
IV h	3.57±0.12	4.41±0.51 (1.63%)	4.39±0.34 (39.25%)	3.75±0.36* (88.8%)
IV i	3.69±0.15	4.58±0.24 (5.31%)	4.74±0.19 (22.22%)	3.80±0.35* (81.8%)
IV j	3.59±0.14	4.70±0.12 (13.82%)	4.10±0.15 (62.22%)	3.82±0.24** (91.3%)
IV k	3.72±0.06	4.74±0.19 (8.51%)	4.77±0.11 (22.22%)	3.76±0.07* (50%)
IV l	3.80±0.13	4.63±0.09 (11.70%)	3.72±0.09** (105.9%)	3.84±0.11** (50%)
IV m	3.54±0.11	4.40±0.17 (8.51%)	4.57±0.13 (23.70%)	3.77±0.10* (91.3%)
IV n	3.74±0.07	4.25±0.14 (45.74%)	4.40±0.52 (51.11%)	3.81±0.24** (71.4%)
IV o	3.67±0.11	4.47±0.37 (14.89%)	4.54±0.41 (35.55%)	3.71±0.18* (50%)
IV p	3.54±0.12	4.50±0.51 (2.12%)	4.41±0.34 (35.55%)	3.65±0.36* (81.8%)
IV q	3.65±0.15	4.55±0.24 (4.25%)	4.79±0.19 (15.55%)	3.75±0.35* (80%)
IV r	3.51±0.14	4.72±0.12 (3.19%)	4.10±0.15 (56.29%)	3.62±0.24** (81.8%)
IV s	3.76±0.06	4.74±0.19 (4.25%)	4.77±0.11 (25.18%)	3.82±0.07* (66.6%)
IV t	3.79±0.13	4.63±0.09 (10.63%)	3.74±0.09** (103.07%)	3.82±0.11** (33.3%)

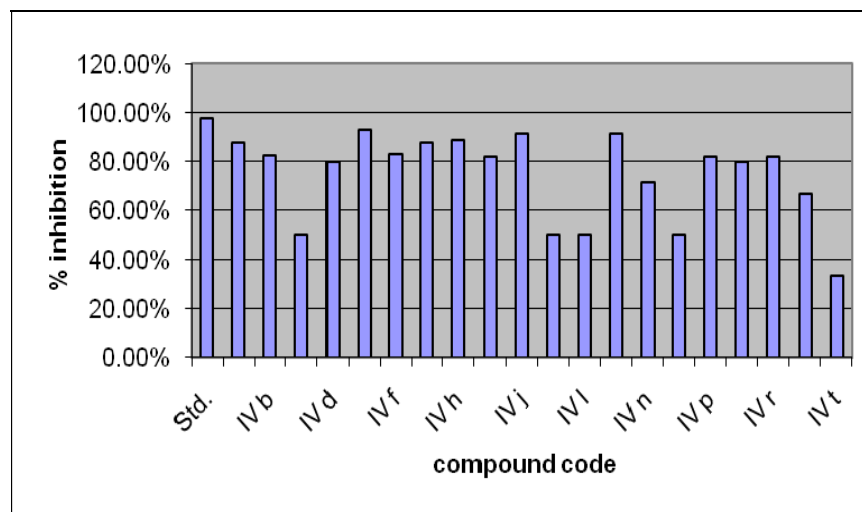


Fig. 1: % inhibition after 3 hr

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