

# Synthesis, Antibacterial and Antifungal Evaluation of Novel Pyrazoline Derivatives

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**ABSTRACT:** A new series of chalcones (**2a-j**) were prepared by reacting substituted aldehydes and substituted ketones in alcohol medium in presence of NaOH. The chalcones underwent selective cyclization with guanicol hydrazide (**1**) in glacial acetic acid medium to yield the title compounds 1,3,5-trisubstituted pyrazolines (**3a-j**). The new compounds were characterized on the basis of <sup>1</sup>H-NMR, IR and mass spectral data. All the newly synthesized compounds were evaluated for their *in-vitro* antibacterial and antifungal activities. Some of the tested compounds **3a** and **3e** showed good activity against bacterial strains and compounds **3d** and **3h** revealed good activity against fungal strains.

**Key words:** Chalcones, pyrazolines, antibacterial activity, antifungal activity, guanicol hydrazide

## INTRODUCTION

Heterocyclic compounds are extremely significant to life and are present in living cells in different forms. These compounds serve as the starting materials for the manufacture of variety of a drugs. Heteroatoms commonly present in these compounds are oxygen, nitrogen, sulphur like simple carbocyclic compounds. Heterocyclic compounds are abundantly available in nature.

Pyrazoline is a five-membered heterocyclic compound having two adjacent nitrogen atoms within the ring. It has only one endocyclic double bond and is basic in nature. The dihydro derivative of pyrazole is known as pyrazoline. Depending on the position of the double bond, it can exist in three separate forms: 1-pyrazoline, 2-pyrazoline and 3-pyrazoline.<sup>1</sup>

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 (insecticide) and anturane (uricosuric). Changes in their structure have offered a high degree of diversity that has proven useful for the development of new therapeutic agents having improved potency and lesser toxicity.

Chalcones are 1,3-diphenyl-2-propene-1-one, in which two aromatic rings are linked by a three carbon  $\alpha$ ,  $\beta$ -unsaturated carbonyl system. These are abundant in edible plants and are considered to be the precursors of flavonoids and isoflavonoids.<sup>2</sup> Chalcones are synthesized by Claisen-Schmidt condensation of aldehydes and ketones by base catalyzed or acid catalyzed followed by dehydration to yield chalcones.<sup>3</sup>

Chalcones are popular intermediates for synthesizing various heterocyclic compounds. The compounds with the backbone of chalcones have been reported to possess various biological activities such as antimicrobial<sup>4</sup>, anti-inflammatory<sup>5</sup>, antimalarial<sup>6</sup>, molluscicidal<sup>7</sup>, anti-mycobacterail<sup>8</sup> etc. Similarly pyrazolines are reported to possess

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antimicrobial<sup>9</sup>, anti-inflammatory<sup>10</sup>, anti-tubercular<sup>11</sup>, antibacterial<sup>12</sup>, antidepressant<sup>13</sup>, etc preperetes.

With this in mind and in continuation of our work on pyrazolines<sup>14-17</sup>, we envisage the design and synthesis of a combination of guanicol hydrazide with heterocyclic moiety like pyrazoline, to obtain therapeutically active antibacterial and antifungal agents.

## MATERIALS AND METHODS

Laboratory grade chemicals and reagents were used to synthesize all the reported compounds. The IR spectra were recorded by using Bruker IR spectrometer using a thin film on KBr pellet technique and frequencies are expressed in  $\text{cm}^{-1}$ .  $^1\text{H-NMR}$  spectra were recorded on Bruker Avance-II 400 MHz NMR spectrometer. All spectra were obtained in  $\text{CDCl}_3$  and DMSO. Chemical shift values are reported in ppm relative to TMS ( $\delta=0$ ) as internal standard. Mass spectra were recorded on ESI. Melting points were determined by open capillary method and are uncorrected. Precoated silica gel plates (Merck, Silica gel 60 F<sub>254</sub>) were used for analytical TLC and visualized by exposure to iodine

vapor and UV radiations. ethyl acetate : acetone (1:9) was as mobile phase.

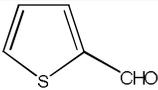
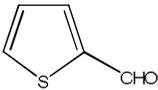
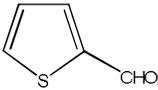
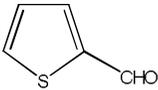
**Synthesis of guanicol hydrazide<sup>18</sup> (1).** To a solution of guanicol ethyl ester (0.01 mol) in ethanol (25 ml), hydrazine hydrate (0.02 ml) was added and refluxed for about 24 h. The reaction mixture was allowed to cool. The white crystals which are separated, were filtered, dried and recrystallized from ethanol.

% Yield: 65%, mp: 62-64°C.

IR (KBr)  $\nu$  ( $\text{cm}^{-1}$ ): 1536(C=C), 1662(C=O), 2837 (OCH<sub>2</sub>), 3069 (C-H), 3327 (NH).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  3.88 (s, OCH<sub>3</sub>, 3H), 3.94 (bs, NH<sub>2</sub>, 2H), 4.61 (s, OCH<sub>2</sub>, 2H), 6.84-7.04 (m, Ar-H, 4H), 8.28 (s, CONH, 1H).

**General procedure for the synthesis of chalcones (2a-j).** To a solution of substituted aromatic aldehydes (0.01 mol) and substituted ketones (0.01 mol) in ethanol (25 ml), a solution of NaOH (6 ml, 40%) was added. The reaction mixture was stirred at room temp for a period of 24 hr, diluted with water (100 ml) and acidified with dil. HCl. The product obtained was filtered, washed with water, dried and recrystallized from ethanol. The physical properties of compounds (2a-j) are given in Table 1.

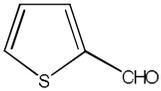
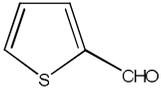
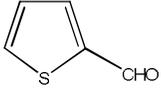
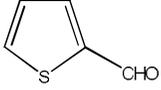
Table 1. Physical properties of chalcones (2a-j).

Compd.	Ar-CHO	Ar <sup>1</sup> -COCH <sub>3</sub>	Molecular formula	MP (°C)	Yield (%)
3a		4-Cl	C <sub>13</sub> H <sub>9</sub> ClOS	142-44	64
3b		4-OH	C <sub>13</sub> H <sub>10</sub> O <sub>2</sub> S	168-70	76
3c		4-Br	C <sub>13</sub> H <sub>9</sub> BrOS	118-20	65
3d		4-OCH <sub>3</sub>	C <sub>14</sub> H <sub>12</sub> O <sub>2</sub> S	90-92	68
3e	4-CH <sub>3</sub>	C <sub>6</sub> H <sub>5</sub>	C <sub>16</sub> H <sub>14</sub> O	108-10	72
3f	4-Cl	C <sub>6</sub> H <sub>5</sub>	C <sub>15</sub> H <sub>11</sub> ClO	101-03	67
3g	4-CH <sub>3</sub>	4-Cl	C <sub>16</sub> H <sub>13</sub> ClO	135-37	71
3h	4-OCH <sub>3</sub>	2-OH	C <sub>16</sub> H <sub>14</sub> O <sub>3</sub>	178-80	76
3i	4-CH <sub>3</sub>	2-OH	C <sub>16</sub> H <sub>14</sub> O <sub>2</sub>	164-66	73
3j	4-Cl	2-OH	C <sub>15</sub> H <sub>11</sub> ClO <sub>2</sub>	128-30	66

**General procedure for the synthesis of 1,3,5-trisubstituted pyrazolines (3a-j).** A solution of chalcones (**2a-j**) (0.01 mol) and guanicol hydrazide (**1**) (0.01 mol) in glacial acetic acid (25 ml) was refluxed for about 30-40 hrs. Excess of solvent was

removed under reduced pressure and the reaction mixture was poured into ice cold water. The product which was obtained was filtered, washed with water, dried and recrystallized from ethanol. The physical properties of compounds (**3a-j**) are given in table 2.

**Table-2. Physical properties of pyrazolines (3a-j).**

Compd.	Ar-CHO	Ar <sup>1</sup> -COCH <sub>3</sub>	Molecular formula	MP (°C)	Yield (%)
<b>3a</b>		4-Cl	C <sub>22</sub> H <sub>19</sub> ClN <sub>2</sub> O <sub>3</sub> S	76-78	62
<b>3b</b>		4-OH	C <sub>22</sub> H <sub>20</sub> N <sub>2</sub> O <sub>4</sub> S	86-88	58
<b>3c</b>		4-Br	C <sub>22</sub> H <sub>19</sub> BrN <sub>2</sub> O <sub>3</sub> S	100-02	62
<b>3d</b>		4-OCH <sub>3</sub>	C <sub>23</sub> H <sub>22</sub> N <sub>2</sub> O <sub>4</sub> S	92-94	66
<b>3e</b>	4-CH <sub>3</sub>	C <sub>6</sub> H <sub>5</sub>	C <sub>25</sub> H <sub>24</sub> N <sub>2</sub> O <sub>3</sub>	145-47	63
<b>3f</b>	4-Cl	C <sub>6</sub> H <sub>5</sub>	C <sub>24</sub> H <sub>21</sub> ClN <sub>2</sub> O <sub>3</sub>	164-66	65
<b>3g</b>	4-CH <sub>3</sub>	4-Cl	C <sub>22</sub> H <sub>17</sub> ClN <sub>2</sub> O <sub>3</sub>	151-53	67
<b>3h</b>	4-OCH <sub>3</sub>	2-OH	C <sub>25</sub> H <sub>24</sub> N <sub>2</sub> O <sub>5</sub>	122-24	61
<b>3i</b>	4-CH <sub>3</sub>	2-OH	C <sub>22</sub> H <sub>24</sub> N <sub>2</sub> O <sub>4</sub>	115-17	62
<b>3j</b>	4-Cl	2-OH	C <sub>24</sub> H <sub>21</sub> ClN <sub>2</sub> O <sub>4</sub>	132-34	63

**5-(4-chlorophenyl)-1-(3-methoxyphenoxy)-3-(thiophen-2-yl)-4,5-dihydro-1H pyrazole (3a):** IR (KBr)  $\nu$  (cm<sup>-1</sup>): 756 (Cl), 1563 (C=C), 1585 (C=N), 1683 (C=O), 2881 (OCH<sub>2</sub>), 3082 (C-H), <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  3.82 (s, OCH<sub>3</sub>, 3H), 3.84-3.93 (dd, 1H, H<sub>A</sub>), 4.60 (s, OCH<sub>2</sub>, 2H), 6.84-6.88 (dd, 2H, H<sub>B</sub>, H<sub>X</sub>), 7.02-8.37 (m, Ar-H, 11H). MS (m/z): 427 (M+1), 428 (M+2).

**4-(1-(3-methoxyphenoxy)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-5-yl phenol (3b):** IR (KBr)  $\nu$  (cm<sup>-1</sup>): 1566 (C=C), 1602 (C=N), 1636 (C=O), 2881 (OCH<sub>2</sub>), 3186 (OH). <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  3.70 (s, OCH<sub>3</sub>, 3H), 3.78-3.86 (dd, 1H, H<sub>A</sub>), 4.58 (s, OCH<sub>2</sub>, 2H), 5.09-5.15 (dd, 1H, H<sub>B</sub>), 6.49-6.53 (dd, 1H, H<sub>X</sub>), 6.73-8.01 (m, Ar-H, 1H), 9.50 (s, 1H, OH). MS (m/z): 409 (M+1).

**5-(4-bromophenyl)-1-(3-methoxyphenoxy)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole (3c):** IR (KBr)  $\nu$  (cm<sup>-1</sup>): 708 (Br), 1508 (C=C), 1586 (C=N), 1650 (C=O), 2881 (OCH<sub>2</sub>), 3103 (C-H). <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  2.33 (s, OCH<sub>3</sub>, 3H), 3.27-3.33 (dd, 1H, H<sub>A</sub>), 3.75-3.84 (dd, 1H, H<sub>B</sub>), 4.50 (s, OCH<sub>2</sub>, 2H), 5.83-5.87 (dd, 1H, H<sub>X</sub>), 6.90-7.70 (m, Ar-H, 11H). MS (m/z): 472 (M+1).

**1-(3-methoxyphenoxy)-5-(4-methoxyphenyl)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole (3d):** IR (KBr)  $\nu$  (cm<sup>-1</sup>): 1569 (C=C), 1603 (C=N), 1650 (C=O), 2882 (OCH<sub>2</sub>), 3011 (C-H). <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  3.33 (s, 2 X OCH<sub>3</sub>, 6H), 3.79-3.84 (dd, 1H, H<sub>A</sub>), 4.57 (s, OCH<sub>2</sub>, 2H), 5.83-5.85 (dd, 1H, H<sub>B</sub>), 6.58-6.66 (dd, 1H, H<sub>X</sub>), 6.89-7.82 (m, Ar-H, 11H).

**Antimicrobial activity.** The synthesized pyrazoline derivatives (**3a-j**) were screened for their antibacterial activity against two gram positive bacterial strains *Bacillus subtilis* and *Staphylococcus aureus* and two gram negative bacterial strains *Pseudomonas aeruginosa* and *Escherichia coli* as well as antifungal activity against *Aspergillus flavus* and *A. fumigatus* by using modified Kirby-Bauer disc diffusion method.<sup>19</sup> All the synthesized compounds were tested at a concentration of 100 µg/ml. The agar medium was incubated with different micro-organism cultures tested. The test compounds were dissolved in dimethylformamide (DMF) to prepare the stock solutions. To ensure that the solvent had no

effect on bacterial growth, a control test was performed with test medium supplemented with DMF at the same dilutions as used in the experiment. The plates of bacterial culture were incubated at 37°C for 18-24 hr and fungal cultures were incubated at 24°C for 24-48 hrs. The diameter of the zone of inhibition exhibited by the compounds was measured. For each treatment, three replicates were maintained. Ciprofloxacin and fluconazole were used as standard drugs for the comparison of antibacterial and antifungal activities respectively. The antimicrobial activities of the compounds (**3a-j**) are given in table 3.

**Table 3. Antimicrobial activity of 1,3,5-trisubstituted pyrazoline derivatives (3a-j).**

Compd.	Diameter of zone of inhibition in (mm) [mean± S.D (n=3)]					
	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>A. flavus</i>	<i>A. flumingatus</i>
<b>3a</b>	16 ± 4.83	9.33 ± 2.78	9.33 ± 2.78	18.66 ± 5.43	15.66 ± 4.67	14.66 ± 4.29
<b>3b</b>	7.66 ± 2.22	8 ± 2.3	5.33 ± 1.94	12.33 ± 3.57	7.66 ± 2.22	18.66 ± 5.43
<b>3c</b>	12.66 ± 3.69	13 ± 3.78	13.66 ± 4.01	10.66 ± 3.29	10 ± 3.21	18 ± 5.21
<b>3d</b>	8 ± 2.35	8.33 ± 2.42	17 ± 4.93	14.33 ± 4.17	19.66 ± 5.7	10 ± 3.03
<b>3e</b>	10 ± 3	8.33 ± 2.42	12 ± 3.55	22.33 ± 6.48	20.33 ± 5.91	15.33 ± 4.53
<b>3f</b>	13 ± 3.78	9.66 ± 2.88	11.33 ± 3.31	15.66 ± 4.67	13.33 ± 3.85	14 ± 4.06
<b>3g</b>	8.33 ± 2.51	6.33 ± 2.17	13.66 ± 3.95	15.33 ± 4.53	18.33 ± 5.34	10.33 ± 3.06
<b>3h</b>	10.33 ± 3.06	11.33 ± 3.31	9 ± 2.64	11.33 ± 3.31	20 ± 5.94	13.33 ± 3.91
<b>3i</b>	11.33 ± 3.28	12 ± 3.55	13.66 ± 4.06	15.33 ± 4.48	14 ± 4.12	13 ± 3.78
<b>3j</b>	11.33 ± 3.31	8.66 ± 2.56	11.33 ± 3.48	13 ± 3.78	13 ± 3.84	13.33 ± 3.91
Ciprofloxacin	23 ± 6.65	25 ± 7.23	22.33 ± 6.45	23 ± 6.68	--	--
Fluconazole	--	--	--	--	25 ± 7.23	24.33 ± 7.02
Control	--	--	--	--	--	--

The antibacterial activity results revealed that all the tested compounds showed weak to moderate activity against *B. subtilis* and *P. aeruginosa*. Some of the tested compounds **3a** and **3e** showed good activity against *S. aureus*. But most of the synthesized compounds displayed very weak activity against *E. coli*. None of the tested compounds was found to be potent when compared to the standard drug ciprofloxacin.

In the antifungal screening, the tested compounds **3d**, **3e**, **3g** and **3h** showed good activity against *A. flavus*. Compounds **3b** and **3c** displayed good activity

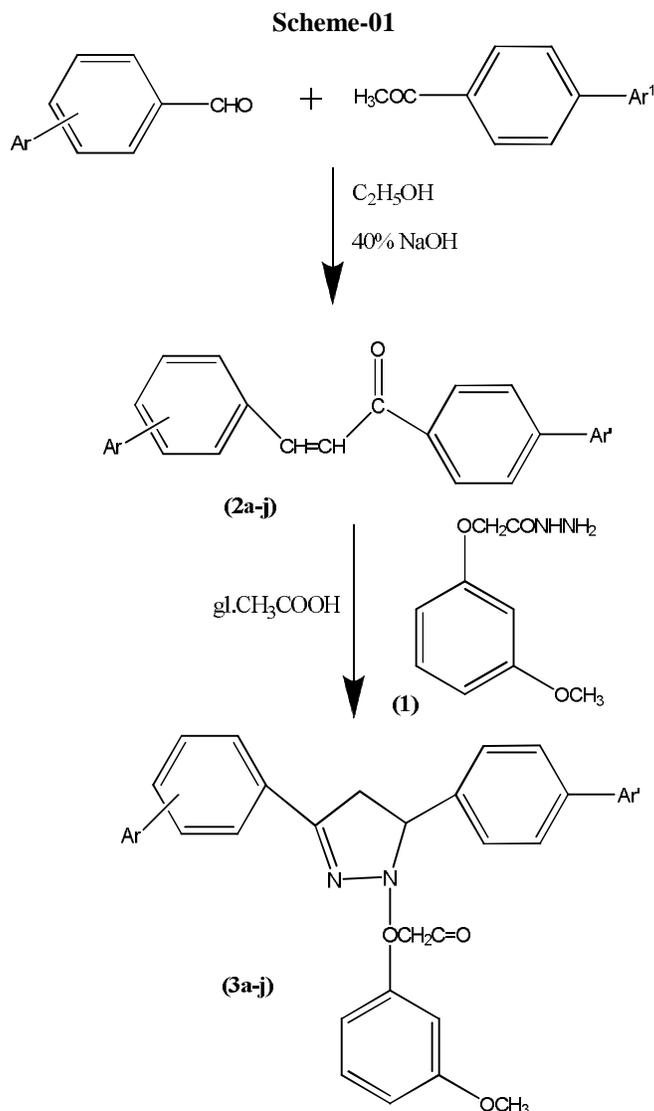
against *A. fumigatus*. The compounds with five membered heterocyclic moiety have displayed good activity against both the fungal organisms. But most of the tested compounds showed moderate activity against both the fungal organisms when compared with standard drug Fluconazole.

## RESULTS AND DISCUSSION

The objective of the present study was to synthesize and investigate the antibacterial and antifungal activities of a new series of pyrazoline derivatives. The reaction sequence is outlined in

Scheme 01. The newly synthesized compounds were characterized by various physicochemical parameters (mp, TLC) and also by spectroscopic methods (IR, NMR, mass). Characteristic peaks were observed in FT-IR, NMR and mass spectroscopy. The purity of the compounds was assessed by TLC. The key

intermediates chalcones<sup>15</sup> (**2a-j**) and guanicol hydrazide<sup>19</sup> (**1**) were prepared as per the reported procedure. Compounds (**3a-j**) were synthesized by reacting chalcones and guanicol hydrazide in glacial acetic acid medium. The compounds were obtained in good yields.



The IR spectra of compounds (**3a-j**) showed strong absorption bands at frequencies within 28812-2281 $\text{cm}^{-1}$  and 1683-1636  $\text{cm}^{-1}$  due to  $\text{OCH}_2$  and  $\text{C}=\text{O}$  stretching respectively. The absorption bands observed at 1603-1585  $\text{cm}^{-1}$  was due to  $\text{C}=\text{N}$  stretching. In  $^1\text{H-NMR}$  spectra, compounds (**3a-j**)

showed doublets of doublets at  $\delta$  3.27-6.88 corresponding to three protons of pyrazoline in  $\text{H}_A$ ,  $\text{H}_B$ ,  $\text{H}_X$  pattern. The methoxy protons was observed in the region at  $\delta$  2.33-3.82 and appeared as singlet, integrating for three protons. Aromatic protons resonated as multiplets at  $\delta$  6.73-8.37. Further

evidence for the formation of pyrazolines (**3a-j**) was obtained by recording their mass spectra. The mass spectra of the compounds (**3a-j**) showed a significantly stable molecular ion peak and they were consistent with the molecular formula.

## CONCLUSION

In summary, the paper describes the synthesis of pyrazoline derivatives and their antibacterial activity. The results indicated that most of the synthesized compounds have moderately good inhibitory potential for both bacterial and fungal strains. Among the synthesized pyrazolines, compounds with five membered heterocyclic substituents were found to be more potent in comparison to standard. The synthesized molecules might be helpful for the pharmaceutical industries in drug discovery program.

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