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# Synthesis, Characterization and Antimicrobial Studies of Some Substituted Pyrazolines from Aryloxy Acetyl Hydrazine

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A new series of 1,3,5-trisubstituted pyrazolines (**7a-h**) were synthesized by the condensation of various substituted chalcones (**6**) with aryloxy acetyl hydrazide (**3**) derived from eugenol (**1**). The assignment of the structure of all the newly synthesized compounds were based on IR, $^1$H NMR and Mass spectroscopic analysis. The final compounds synthesized were evaluated for antibacterial and antifungal activities.

**Key Words: Hydrazides, Chalcones, Pyrazolines, Heterocyclic compound, Antibacterial and Antifungal activity.**

## INTRODUCTION

The research work developed in the synthesis of a variety of heterocyclic compounds with potential biological activity may be used as dyes for textiles or other polymeric materials. The research and development projects carried out include the synthesis and/or reactivity studies of three, five, six and seven-membered heterocycles containing nitrogen, oxygen or sulphur and also 2, 3 and 4 fused heterocyclic rings.

Nitrogen heterocyclic compounds like pyrazolines have received considerable attention in recent years due to their biological and physiological activities. Several pyrazolines have shown promising results as chemotherapeutic agent.

2-Pyrazoline derivatives have been found to possess wide range of therapeutic activity such as antimicrobial, antiinflammatory, analgesic, anticarcinogenic, herbicidal[1], antidepressant, antiviral, antiproteolytic[2], antiarthritic[3], antiinflammatory, analgesic and COX-II inhibitory activities[4].

Eugenol (**1**) a phenolic compound is a major component of several essential oils like clove oil, cinnamon leaf oil which contains upto 90 % of eugenol. It is known for its antimicrobial[5] and antioxidant[6] properties.

## EXPERIMENTAL

Melting points were taken in open capillaries in liquid paraffin bath and are uncorrected. IR spectra was recorded in Shimadzu Perkin-Elmer 8201 PC IR Spectrometer using a thin film on potassium bromide pellets. The $^1$H NMR spectra were recorded on Bruker Avance II 400 NMR Spectrometer using $CDCl_3$. Chemical shift

values are reported as values in ppm relative to TMS ($\delta = 0$) as internal standard. The FAB mass spectra were recorded on Jeol SX-102/DA-6000 Mass spectrometer using argon/xenon (6Kv, 10 Ma) as the FAB gas.

In the present work, substituted pyrazolines (**7a-h**) have been synthesized by the condensation of aryloxy acetyl hydrazide (**3**) with various chalcones (**6**) in presence of glacial acetic acid. The synthesis consists of 3 steps, which are as follows:

**Step-I: Preparation of ethyl aryloxy acetate (2):** A mixture of eugenol (0.1 mol), chloro ethyl acetate (12.25 g, 0.1 mol) and anhydrous potassium carbonate (19.5 g, 0.15 mol) in dry acetone were refluxed on a water bath for 16 h at 50 ºC. The resultant reaction mixture was cooled and filtered. From the filtrate, excess of acetone was removed by distillation. The reaction mixture of filtrate was then poured on to the ice-cold water and stirred well. Organic layer was extracted with ether and further the ether layer was washed with water and dried over anhydrous sodium sulphate followed by the removal of etheral layer by drying on a water bath. The resultant liquid is collected to get the pure ethyl aryloxy acetate.

**Preparation of aryloxy acetyl hydrazide (3):** A mixture of ethyl aryloxy acetate (0.05 mol) and hydrazine hydrate (99 %, 3.525 g, 0.075 mol) in ethanol (100 mL) were refluxed on a water bath for 16 h. From the resultant mixture excess of ethanol was removed by distillation. On cooling, the resultant mixture of aryloxy acetyl hydrazide separates in the form of white needle like crystals and recrystallized from ethyl alcohol[7].

**Step-II: Synthesis of chalcones (6) [substituted 1,3-diphenyl propen-1-one] from substituted aldehydes (4) and substituted acetophenones (5):** Substituted aromatic aldehydes (0.01 mol) was treated with substituted acetophenones (0.01 mol) in presence of ethyl alcohol (25 mL) and 40 % NaOH (3 mL). The mixture was stirred for 24 h. The reaction mixture was poured into ice water and acidified with dil. HCl. The solid product was filtered and crystallized from ethanol[8].

**Step-III: Synthesis of pyrazolines (7a-h):** Chalcone (0.01 mol) and aryloxy acetyl hydrazide (0.02 mol) in 20 mL of glacial acetic acid was refluxed for a period of 10 h and cooled. Excess of solvent was removed under reduced pressure and the reaction mixture was poured into 250 mL of ice-cold water. The product obtained was filtered, washed with cold water and recrystallized from ethanol[9]. The physical characteristics of the synthesized substituted pyrazoline derivative are given in Table-1. The spectral data of some of the synthesized compounds (**7a-d**) are given below:

**7a:** IR (KBr, $\nu_{max}$, $cm^{-1}$): -NH (*str.*) 3012, -C=O (*str.*) 1641, C=N (*str.* in pyraroline) 1512, N-N (*str.*) 3244, OH (*str.*) 3390. $^1$H NMR ($\delta$ ppm) 6.70-8.00 (11H, m, Ar), 5.10 (2H, S, $OCH_2$), 9.20 (1H, s, OH), 2.43-2.54 (2H, m, $CH_2$ of pyrazoline), 4.65-4.66 (1H, m, CH of pyrazoline), 5.89-5.92 (3H, m, olefinic), 3.32-3.34 (2H, d, $CH_2$ of olefinic), 3.87 (3H, s, $OCH_3$), 2.05 (3H, s, $CH_3$). Mass m/z molecular peak-456, base peak-443.

TABLE-1
PHYSICAL DATA OF SUBSTITUTED PYRAZOLINE DERIVATIVES

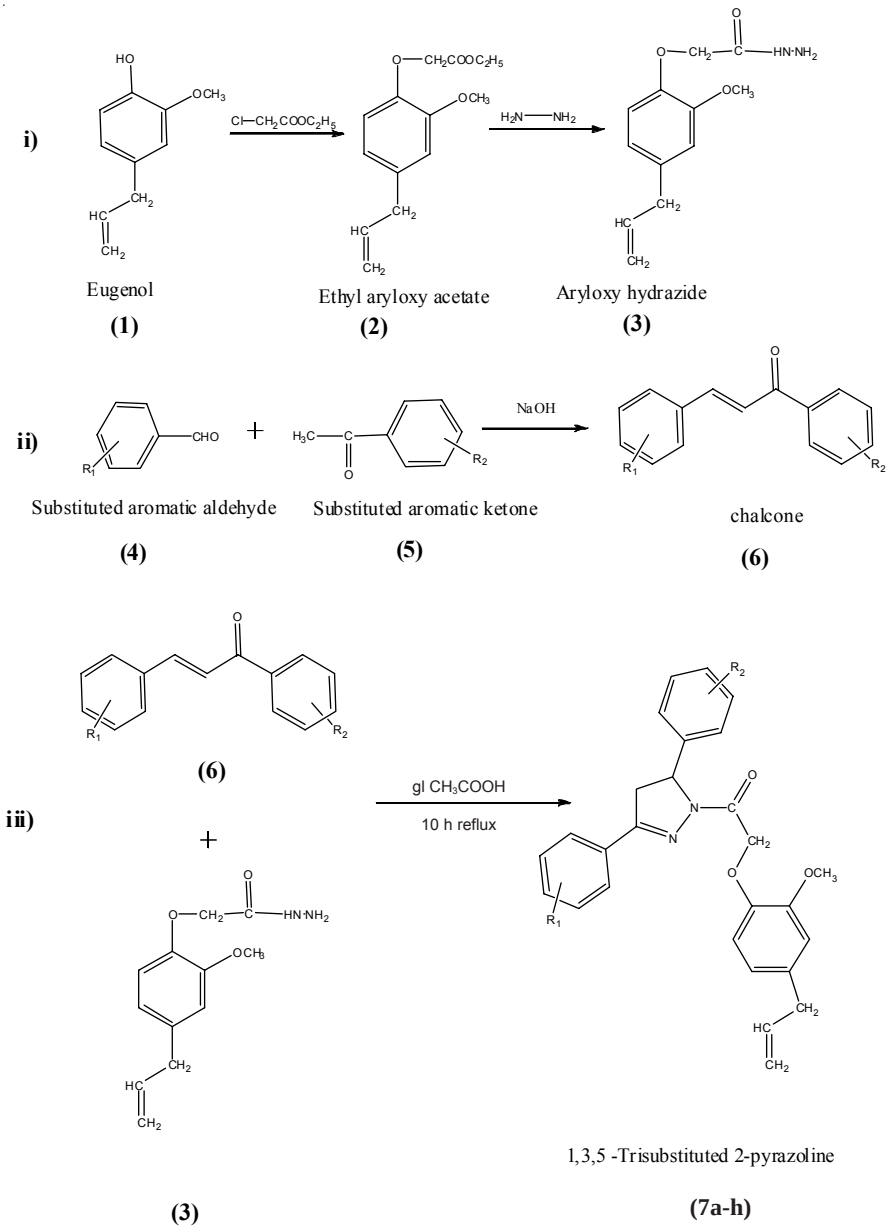
| Compd. | m.f. / (m.w.) | $R_1$ | $R_2$ | Colour | m.p. (ºC) | Yield (%) |
|---|---|---|---|---|---|---|
| **7a** | $C_{28}H_{28}N_2O_4$ (456.53) | -OH (*p*) | -$CH_3$(*p*) | Green powder | 121-123 | 67.43 |
| **7b** | $C_{27}H_{25}ClN_2O_4$ (476.95) | -Cl (*p*) | -OH (*p*) | Green | 115-119 | 62.35 |
| **7c** | $C_{28}H_{28}N_2O_5$ (472.53) | -OH (*p*) | -$OCH_3$ (*p*) | Yellow | 127-131 | 59.34 |
| **7d** | $C_{28}H_{27}ClN_2O_4$ (490.98) | -Cl (*p*) | -$OCH_3$ (*p*) | White flakes | 98-102 | 68.24 |
| **7e** | $C_{27}H_{25}N_3O_6$ (487.50) | -$NO_2$ (*m*) | -OH (*p*) | Black | 139-141 | 64.12 |
| **7f** | $C_{27}H_{25}ClN_2O_4$ (476.95) | -Cl (*p*) | -OH (*o*) | Brown | 124-127 | 62.33 |
| **7g** | $C_{28}H_{27}ClN_2O_3$ (474.98) | -Cl (*p*) | -$CH_3$(*p*) | Red | 118-120. | 65.32 |
| **7h** | $C_{28}H_{27}N_3O_5$ (485.53) | -$NO_2$ (*m*) | -$CH_3$(*p*) | Brown | 124-126 | 69.74 |

**7b:** IR (KBr, $\nu_{max}$, $cm^{-1}$): -NH (*str.*) 3076, -C=O (*str.*) 1644, C=N (*str.* in pyraroline) 1515, N-N (*str.*) 3273, C-Cl (*str.*) 761, OH (*str.*) 3416. $^1$H NMR (δ ppm) 6.72-8.01 (11H, m, Ar ), 5.11 (2H, S, $OCH_2$), 9.70 (1H, s, OH), 2.52-2.55 (2H, m, $CH_2$ of pyrazoline), 4.63-4.67 (1H, m, CH of pyrazoline), 5.89-5.97 (3H, m, olefinic), 3.00-3.04 (2H, d, $CH_2$ of olefinic), 3.35 (3H, s, $OCH_3$). Mass m/z (M+1) peak-477, base peak-95.

**7c:** IR (KBr, $\nu_{max}$, $cm^{-1}$): -NH (*str.*) 3005, -C=O (*str.*) 1645, C=N (*str.* in pyraroline) 1513, N-N (*str.*) 3184, OH (*str.*) 3414. $^1$H NMR (δ ppm) 6.64-8.02 (11H, m, Ar), 5.10 (2H, S, $OCH_2$), 9.70 (1H, s, OH), 2.51-2.55 (2H, m, $CH_2$ of pyrazoline), 4.62-4.66 (1H, m, CH of pyrazoline), 5.88-5.96 (3H, m, olefinic), 3.32-3.34 (2H, d, $CH_2$ of olefinic), 3.90 (3H, s, 2 × $OCH_3$). Mass m/z molecular peak-472, base peak-177.

**7d:** IR (KBr, $\nu_{max}$, $cm^{-1}$): -NH (*str.*) 3013, -C=O (*str.*) 1652, C=N (*str.* in pyraroline) 1515, N-N (*str.*) 3415, C-Cl (*str.*) 746. $^1$H NMR (δ ppm) 6.90-8.05 (11H, m, Ar ), 5.10 (2H, S, $OCH_2$), 2.52-2.56 (2H, m, $CH_2$ of pyrazoline), 4.62-4.66 (1H, m, CH of pyrazoline), 5.89-5.92 (3H, m, olefinic), 3.33-3.35 (2H, d, $CH_2$ of olefinic), 3.90 (3H, s, $OCH_3$).Mass m/z molecular peak-490, base peak-154.

**Antimicrobial activity studies:** The compounds **7a-h** were screened for their antibacterial activity against *B. subtilis, S aureus, E. coli, P. aeruginosa* and anti-fungal against. *Candida albicans* by cup plate method using DMF as a solvent at concentration of 50 µg. The activity was compared with known standard drugs gentamycin and griseofulvin, respectively at same concentration (50 µg)[10].




**Scheme-I**

## RESULTS AND DISCUSSION

All the synthesized pyrazoline derivatives have shown moderate to good anti-bacterial and antifungal activity in comparison to that of the standard drugs gentamycin and griseofulvin, respectively (Table-2).

TABLE-2
DATA OF ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF SUBSTITUTED PYRAZOLINE DERIVATIVES

| Compd. | Diameter of zone of inhibition (mm) | | | | |
|---|---|---|---|---|---|
| | *S. aureus* | *B. subtitis* | *E. coli* | *P. aeruginosa* | *C. albicans* |
| **7a** | – | – | 10 | 9 | 15 |
| **7b** | 16 | 17 | 15 | 15 | – |
| **7c** | 11 | 13 | 11 | 9 | 11 |
| **7d** | 12 | 9 | – | 8 | 14 |
| **7e** | 10 | 12 | 13 | 7 | 12 |
| **7f** | 15 | 17 | 16 | 13 | 9 |
| **7g** | 16 | 14 | 15 | 14 | 7 |
| **7h** | 9 | 10 | 7 | 11 | – |
| Gentamycin | 19 | 21 | 20 | 19 | – |
| Griseofulvin | – | – | – | – | 20 |

(–) Indicates no inhibition zone (no activity).

Compounds **7b**, **7f** and **7g** have shown significant antibacterial activity and the compounds **7c**, **7e** and **7h** have shown moderate activity when compared to the standard drug gentamycin. Compounds **7b** and **7d** have shown moderate antifungal activity when compared to the standard drug griseofulvin.

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