Synthesis and antibacterial activity of some Pyrazoline derivatives

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ABSTRACT

Pyrazoline derivatives, being used as potential medicinal agents, a series P₁-P₅ containing 3,5-diphenylpyrazoline and N-phenyl-3,5-diphenylpyrazoline derivatives were synthesized, structures were confirmed using melting point, IR, NMR and MS and evaluated for their antibacterial activity using disc diffusion method at concentration 2mg/ml. The reference used was amikacin and mostly, all synthesized compounds exhibited a significant antibacterial activity against Staphylococcus aureus (MTCC No. 96) as Gram positive bacteria, Staphylococcus epidermidis (MTCC No. 435) as Gram positive and Escherichia coli (MTCC No. 739) as Gram negative bacteria. It was observed that P₁ and P₂ possess good antibacterial potential against S. aureus, P₅ against S.epidermidis and P₁ and P₄ against E. coli.

Key words: Pyrazoline, Disc diffusion method, Amikacin.

INTRODUCTION

For combating bacterial infection wide variety of anti-bacterial agents has been synthesized¹.

Pyrazolines are important nitrogen-containing five-member heterocyclic compounds. Several Pyrazoline derivatives possess important pharmacological activities and therefore they are useful materials in drug research. Pyrazolines are used as antitumour², immunosuppressive³, antibacterial⁴ and antitubercular agents. Some of the pyrazoline derivatives are reported to possess antiinflammatory⁵, anticancer⁶, antidiabetic⁷ and antidepressant properties⁸. It also finds applications as dyestuffs, analytical reagents and agrochemicals⁹. Encouraged by its anti-bacterial activity, it was thought of interest to synthesize a new series of pyrazoline derivatives.

Aldol condensation between acetophenone and substituted benzenaldehyde yields chalcone derivatives, a condensation product. These chalcone derivatives were reacted with phenyl hydrazine and hydrazine hydrate to form N-phenyl-3,5-diphenylpyrazoline & 3,5-diphenylpyrazoline derivatives respectively. The characterization of various synthesized compounds was done by TLC, melting point, IR, NMR & MS.

EXPERIMENTAL

Materials

Materials used in synthesis of compounds P₁-P₅ includes, benzaldehyde, chloro benzaldehyde,
o-methoxy benzaldehyde, acetophenone, potassium hydroxide pellets, phenyl hydrazine, hydrazine hydrate, glacial acetic acid, sulphuric acid and ethanol.

Synthesis

General procedure for synthesis of chalcone [P₁ and P₅]

Trituration was done on adding potassium hydroxide pellets (0.094 mol) to the mixture of acetophenone (0.094 mol) and benzaldehyde (0.094 mol) at room temperature for 1 hr. The solid product 1,3-diphenyl-2-propen-1-one i.e. chalcone, so obtained was washed with water to remove excess of potassium hydroxide and then dried under UV light.

General procedure for synthesis of chalcone derivatives. [P₂, P₃, P₄]

[P₂] Trituration was done on adding potassium hydroxide pellets (0.072 mol) to the mixture of acetophenone (0.072 mol) and o-methoxy benzaldehyde (0.072 mol) at room temperature for 1 hr. The solid product 1-phenyl-3-(2-methoxy phenyl)-2-propen-1-one i.e. methoxy substituted chalcone so obtained was washed with water to remove excess of potassium hydroxide and then dried under UV light.

[P₃, P₄] Trituration was done on adding potassium hydroxide pellets (0.071 mol) to the mixture of acetophenone (0.071 mol) and o-chloro benzaldehyde (0.071 mol) at room temperature for 1 hr. The solid product 1-phenyl-3-(2-chloro phenyl)-2-propen-1-one i.e. chloro substituted chalcone so obtained was washed with water to remove excess of potassium hydroxide and then dried under UV light.

General procedure for synthesis of Pyrazoline derivatives from Chalcone and its derivatives [P₁, P₅]

To 1,3-diphenyl-2-propen-1-one i.e. chalcone (0.008 mol) in 20 ml of 1,4-dioxane, hydrazine hydrate (0.024 mol) and phenyl hydrazine (0.024 mol) were added for P₁ and P₅ respectively. To these mixtures 2-3 drops of sulphuric acid were added.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>X/Y</th>
<th>Molecular formula</th>
<th>m.p. (°C)</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>P₁</td>
<td>H</td>
<td>X</td>
<td>C₁₆H₁₄N₂</td>
<td>44-46</td>
<td>60</td>
</tr>
<tr>
<td>P₂</td>
<td>OCH₃</td>
<td>X</td>
<td>C₁₆H₁₆ON₂</td>
<td>52-54</td>
<td>30</td>
</tr>
<tr>
<td>P₃</td>
<td>Cl</td>
<td>Y</td>
<td>C₁₄H₁₃N₂Cl</td>
<td>129</td>
<td>53</td>
</tr>
<tr>
<td>P₄</td>
<td>Cl</td>
<td>X</td>
<td>C₁₄H₁₅N₂Cl</td>
<td>50-52</td>
<td>52</td>
</tr>
<tr>
<td>P₅</td>
<td>H</td>
<td>Y</td>
<td>C₁₃H₁₈N₂</td>
<td>72-74</td>
<td>45</td>
</tr>
</tbody>
</table>

Table 1: Experimental data of synthesized Pyrazoline compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>MIC (mg/ml)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S.aureus</td>
<td>E.coli</td>
</tr>
<tr>
<td>P₁</td>
<td>2</td>
<td>59</td>
</tr>
<tr>
<td>P₂</td>
<td>2</td>
<td>62</td>
</tr>
<tr>
<td>P₃</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>P₄</td>
<td>2</td>
<td>51</td>
</tr>
<tr>
<td>P₅</td>
<td>2</td>
<td>51</td>
</tr>
</tbody>
</table>

(⊥) Indicates bacteria are resistant to the compounds at concentration 2 mg/ml, MIC - minimum inhibitory concentration, i.e., lowest concentration to completely inhibit bacterial growth.
added and the contents were allowed to get reflux for 4h. 5ml glacial acetic acid was added to both the mixtures; again reflux was done for next 2h. On cooling to room temperature the contents were poured on crushed ice. As a result the solid products 3,5-diphenyl 4H-pyrazoline and 1,3,5-triphenyl 4H-pyrazoline were obtained which were recrystallized using ethanol.

\[ \text{[P}_2\text{]} \] To 1-phenyl-3-(2-methoxy phenyl)-2-propen-1-one (0.008mol) in 20ml of 1,4-dioxane, hydrazine hydrate (0.024mol) was added. To this mixture 2-3 drops of sulphuric acid was added and the contents were allowed to get reflux for 4hrs. 5ml glacial acetic acid was added to the mixture; again reflux was done for next 2hrs. On cooling to room temperature the contents were poured on crushed ice. As a result the solid product 3-phenyl-5-(2-methoxy phenyl) 4H-pyrazoline was obtained which was recrystallized using ethanol.

\[ \text{[P}_3\text{, P}_4\text{]} \] To 1-phenyl-3-(2-chloro phenyl)-2-propen-1-one (0.008mol) in 20ml of 1,4-dioxane, hydrazine hydrate (0.024mol) and phenyl hydrazine (0.024mol) were added for \( P_3 \) and \( P_4 \) respectively. To these mixtures 2-3 drops of sulphuric acid were added and the contents were allowed to get reflux for 4hrs. 5ml glacial acetic acid was added to both the mixtures; again reflux was done for next 2hrs. On cooling to room temperature the contents were poured on crushed ice. As a result the solid products 1,3-diphenyl-5-(2-chloro phenyl) 4H-pyrazoline and 3-phenyl-5-(2-chloro phenyl) 4H-pyrazoline were obtained which were recrystallized using ethanol.

**Antibacterial activity**

The newly synthesized pyrazoline compounds were screened for antibacterial activity against *Staphylococcus aureus* (MTCC No. 96), *Escherichia coli* (MTCC No. 739) and *Staphylococcus epidermidis* (MTCC No.435) by disc diffusion method\(^\text{10,11}\). Discs measuring 10.0 mm in diameter were punched from Whatman no.1 filter paper. Batches of 100 discs were dispensed to each
screw capped bottles and sterilized by dry heat at 50°C for 3hrs. Each disc containing full concentration (2mg/ml) were prepared using dimethylformamide (DMSO). The discs of each compounds was placed individually on nutrient agar medium seeded with fresh bacteria respectively using amikacin as the positive control. The nutrient agar plates were incubated at 37°C for 30min. before the discs were applied aseptically. The treated plates were incubated at 37°C for 48h. Minimum Inhibitory Concentrations (MIC) were noted and compared with positive control amikacin, the results of antibacterial studies are given:

The investigation of antibacterial screening data revealed that all the tested compounds showed moderate to good bacterial inhibition action.

CONCLUSION

We have synthesized a series of 3,5-diphenylpyrazoline and N-phenyl-3,5-diphenylpyrazoline derivatives. The synthesized compound P₁ and P₂ shows significant antibacterial activity against S. aureus, P₁ and P₄ against E. coli and P₅ against S. epidermidis. Also P₃ compound shows adequate activity against E. coli and S. epidermidis. Hence, it is concluded that there is ample scope for further study in developing these as good lead compounds.

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REFERENCES