SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME PYRAZOLINE DERIVATIVES BEARING AMIDE MOIETY

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ABSTRACT: In the present study, a series of pyrazoline derivatives were synthesized. The chemical structures of the compounds were elucidated by IR, 1H-NMR, 13C-NMR and FAB+MS spectral data and elemental analyses. The synthesized compounds were screened for their antimicrobial activities. All compounds exhibited the highest antibacterial activity against P. aeruginosa. All compounds except compounds 1-(chloroacetyl)-3-(2-furanyl)-5-(4-chlorophenyl)-2-pyrazoline and 1-(chloroacetyl)-3-(2-thienyl)-5-(3,4-methylenedioxyphenyl)-2-pyrazoline are more effective than ketoconazole against C. albicans, whereas compounds 1-(chloroacetyl)-3-(2-furanyl)-5-(4-chlorophenyl)-2-pyrazoline, 1-(chloroacetyl)-3-(2-thienyl)-5-(3,4-methylenedioxyphenyl)-2-pyrazoline and ketoconazole showed the same level of antifungal activity against C. albicans.

KEY WORDS: pyrazoline, amide, furan, thiophene, antimicrobial activity

INTRODUCTION

Microrganism resistance to antimicrobial agents still remains a major challenge in the medicinal chemistry, as many species of bacteria and fungi have developed resistance to currently available antimicrobial drugs. Therefore, there could be a major global healthcare problem in the clinical management of life-threatening infectious diseases caused by multi-drug resistant of Gram-positive pathogens like Staphylococcus, and Gram-negative pathogens like Escherichia, and Pseudomonas strains. In order to overcome this serious problem, the search for new antimicrobial agents have gained great importance (1-3).

Medicinal chemists have carried out considerable research for novel antimicrobial agents carrying haloacetamide moiety. Chloramphenicol and analogues, which are widely used antibiotics for the treatment of systemic bacterial infections, possess dichloroacetamide group. Dichloroacetamide group is one of the functional groups that determine antibacterial activity of chloramphenicol. Loss of this group results in significant loss of biological activity (4,5).

Bioisosteric replacement approach was used for designing the compounds. The bioisosteric replacement of benzene with a heteroaromatic ring resulted in analogues maintaining the same biological activity within a series of different pharmacological agents, lending great importance to ring-equivalent bioisosteres. Furan, and thiophene, which are structurally related to benzene, are widely used as ring equivalents in drug development (6-8). β-lactam antibiotics, which possess thiophene ring as the side chain, play a leading role in the treatment of systemic bacterial infections. Tioconazole is also an example of antifungal agents bearing thiophene moiety (4). Furthermore, nitrofuran derivatives represent an important class of antimicrobial agents in medicinal chemistry (9).

Pyrazolines are widely used and studied privileged pharmacophores in medicinal chemistry.
due to their synthetic and biological importance. Some studies have confirmed that pyrazoline derivatives possess antimicrobial activity (10-15).

In continuation of our previous work on the synthesis and antimicrobial evaluation of pyrazoline derivatives (11-15), herein we described the discovery of 2-pyrazoline derivatives bearing three important functional moieties, namely haloaceta-mide, furan and thiophene and focused on their potential antimicrobial effects.

**MATERIALS AND METHODS**

**Chemistry**

All reagents were used as purchased from commercial suppliers without further purification. Melting points were determined by using an Electrothermal 9100 digital melting point apparatus and were uncorrected (Electrothermal, Essex, UK). The compounds were checked for purity by TLC on silica gel apparatus and were uncorrected (Electrothermal, Essex, UK). Melting points were determined by using an Electrothermal 9100 digital melting point apparatus and were uncorrected (Electrothermal, Essex, UK). All reagents were used as purchased from commercial suppliers without further purification. Melting points were determined by using an Electrothermal 9100 digital melting point apparatus and were uncorrected (Electrothermal, Essex, UK).

**General procedure for the synthesis of the compounds**

1-(2-Furanyl/thienyl)-3-aryl-2-propen-1-ones (3a-h)

A mixture of 2-acetylfuran/thiophene (0.06 mol) (1), aromatic aldehyde (0.06 mol) (2), and 10% aqueous sodium hydroxide (10 mL) in ethanol (30 mL) was stirred at room temperature for about 5 h. The resulting solid was washed, dried, and crystallized from ethanol (16,17).

5-Aryl-3-(2-furanyl/thienyl)-2-pyrazolines (4a-h)

A solution of the appropriate furanyl/thienyl chalcone (0.03 mol) and hydrazine hydrate (0.06 mol) (3) in ethanol (30 mL) was stirred at room temperature for about 5 h. The resulting solid was filtered, the solvent was evaporated to dryness under reduced pressure, and the products were recrystallized from ethanol (16,17).

1-(Chloroacetyl)-3-(2-furanyl/thienyl)-5-aryl-2-pyrazolines (5a-h)

5-Aryl-3-(2-furanyl/thienyl)-2-pyrazolines (0.02 mol) and triethylamine (0.02 mol) were dissolved in dry acetone (30 mL) with constant stirring. Later, the mixture was cooled in an ice bath, and chloroacetyl chloride (0.02 mol) was added dropwise with stirring. The reaction mixture thus obtained was further agitated for 2 h at room temperature. The precipitate was filtered, the solvent was evaporated to dryness under reduced pressure, and the products were recrystallized from ethanol.

1-(Chloroacetyl)-3-(2-furanyl)-2-pyrazoline (5a)

IR (KBr) vmax (cm⁻¹): 1658 (C=O), 1564, 1506, 1446 (C≡N and C=C).

1H-NMR (500 MHz, DMSO-d₆): 3.82 (3H, s), 3.79 (1H, d, J = 18.03, 11.48 Hz), 4.60 (1H, d, J = 13.74 Hz), 4.70 (1H, d, J = 13.77 Hz), 5.00 (1H, d, J = 11.48, 4.52 Hz), 6.89 (2H, d, J = 8.52 Hz), 7.13 (2H, d, J = 8.51 Hz), 7.93 (1H, s).

13C-NMR (125 MHz, DMSO-d₆): 42.53 (CH₂), 59.69 (CH), 112.79 (CH), 115.46 (CH), 146.26 (CH), 146.45 (C), 146.62 (C), 146.93 (C), 158.60 (C), 163.28 (C).

For C₂₆H₂₅ClN₂O₂ calculated: 57.76 % C, 3.94 % H, 8.44 % N; found: 57.68 % C, 3.89 % H, 8.44 % N.

MS (FAB) [M+1⁺]: m/z 319
**RESULTS AND DISCUSSION**

Initially, the chalcones (1-(2-furanyl/thienyl)-3-aryl-2-propen-1-ones) (5a-h) were synthesized by literature methods as described (16,17) and treated with hydrazine hydrate (80%) to obtain 5-aryl-3-(2-furanyl/thienyl)-2-pyrazolines (4a-h) (Scheme 1). The desired 1-(chloroacetyl)-3-(2-furanyl/thienyl)-5-aryl-2-pyrazolines (5a-h) were synthesized via the nucleophilic acyl substitution reactions of 5-aryl-3-(2-furanyl/thienyl)-2-pyrazolines with chloroacetyl chloride in the presence of triethylamine. These reactions are summarized in Scheme 1 and some properties of the compounds are given in Table 2.

The structures of the compounds (5a-h) were confirmed by IR, 1H-NMR, 13C-NMR and FAB^+MS spectral data and Elemental analyses.
In the IR spectra of all compounds (5a-h), all derivatives have a strong, characteristic band in the region 1658-1683 cm\(^{-1}\) due to the amide C=O stretching vibration. The bands due to the C=C and C=N stretching vibrations are observed in the region 1610-1413 cm\(^{-1}\).

The \(^1\)H-NMR spectral data were also consistent with the assigned structures. In the 500 MHz \(^1\)H-NMR spectra of compounds, the CH\(_2\) protons of the pyrazoline ring resonated as a pair of doublets of doublets at \(\delta\) 3.04-3.22 ppm, 3.78-3.91 ppm. The CH proton appeared as doublet of doublets at \(\delta\) 5.48-5.58 ppm due to vicinal coupling with the two magnetically non-equivalent protons of the methylene group at position 4 of the pyrazoline ring (\(J_{AM} = 18.01-18.08\) Hz, \(J_{AX} = 4.51-4.74\) Hz, \(J_{MX} = 11.48-11.75\) Hz). The CH\(_2\) protons of acetyl (5a-h) are observed in the region 4.57-4.73 ppm as double doublets (\(J = 13.73-13.98\) Hz, \(J = 13.71-13.92\) Hz). This geminal coupling result from steric structure of the compounds. These geminal protons are observed as double doublet due to possible two different conformations since rigid protons were occurred (19,20). All the other aromatic and aliphatic protons were observed at expected regions.

In the \(^13\)C-NMR spectra of the compounds, the signal due to the carbonyl carbon appears at 163.28-163.78 ppm. \(^13\)C-NMR chemical shift values of the carbon atoms at 42.11-42.86 ppm (C-4), 59.32-60.27 ppm (C-5), and about 147.39-152.62 ppm (C-3) corroborate the 2-pyrazoline character deduced from the \(^1\)H-NMR data. The other aromatic and aliphatic carbons were observed at expected regions.

In the mass spectra of all compounds (5a-h), the M+1 peak is observed. All compounds gave satisfactory elemental analyses.

The antimicrobial activity of the compounds was studied with seven pathogenic microorganisms. Streptomycin and ketoconazole were used as reference drugs. The results are summarized in Table 2.

All compounds (5a-h) exhibited the highest antibacterial activity against *P. aeruginosa* with a MIC value of 250 μg/mL among the tested bacteria. The results clearly indicated that the func-

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**TABLE 1.** Some properties of the synthesized compounds (5a-h)

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>X</th>
<th>Yield (%)</th>
<th>M.p. (°C)</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
</tr>
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<tbody>
<tr>
<td>5a</td>
<td>CH(_3)</td>
<td>O</td>
<td>61</td>
<td>81</td>
<td>C(<em>{16})H(</em>{15})ClN(_2)O(_2)</td>
<td>302</td>
</tr>
<tr>
<td>5b</td>
<td>O-CH(_2)-O</td>
<td>O</td>
<td>67</td>
<td>104</td>
<td>C(<em>{16})H(</em>{13})ClN(_2)O(_4)</td>
<td>332</td>
</tr>
<tr>
<td>5c</td>
<td>OCH(_3)</td>
<td>O</td>
<td>63</td>
<td>150</td>
<td>C(<em>{16})H(</em>{15})ClN(_2)O(_3)</td>
<td>318</td>
</tr>
<tr>
<td>5d</td>
<td>Br</td>
<td>O</td>
<td>58</td>
<td>185</td>
<td>C(<em>{15})H(</em>{12})BrClN(_2)O(_2)</td>
<td>367</td>
</tr>
<tr>
<td>5e</td>
<td>Cl</td>
<td>O</td>
<td>70</td>
<td>125</td>
<td>C(<em>{15})H(</em>{12})ClN(_2)O(_2)</td>
<td>322</td>
</tr>
<tr>
<td>5f</td>
<td>F</td>
<td>O</td>
<td>62</td>
<td>149</td>
<td>C(<em>{15})H(</em>{12})ClF(_2)N(_2)O(_2)</td>
<td>306</td>
</tr>
<tr>
<td>5g</td>
<td>O-CH(_2)-O</td>
<td>S</td>
<td>70</td>
<td>137</td>
<td>C(<em>{16})H(</em>{13})ClN(_2)O(_3)S</td>
<td>348</td>
</tr>
<tr>
<td>5h</td>
<td>Br</td>
<td>S</td>
<td>60</td>
<td>146</td>
<td>C(<em>{15})H(</em>{12})BrClN(_2)O(_3)S</td>
<td>383</td>
</tr>
</tbody>
</table>

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**SCHEME 1.** The synthetic protocol of the compounds (5a-h)
All compounds except compounds 5e and 5g are more effective than ketoconazole against C. albicans, whereas compounds 5e, 5g and ketoconazole showed the same level of antifungal activity against C. albicans.

CONCLUSION

In conclusion, the synthesis of 2-pyrazoline derivatives was described and their in vitro antimicrobial effects of these compounds on various pathogenic bacteria and C. albicans were evaluated. The biological results indicate that P. aeruginosa and C. albicans are more susceptible to the synthesized compounds.

Considering all the results obtained from antimicrobial screen, in comparison with reference agents, it can be concluded that compound 5a is the most effective compound in the screen.

Based on eight compounds evaluated, it appears that 4-methyl substitution on the phenyl ring (5a) has made a good contribution to the antimicrobial activity in this series of pyrazoline-haloacetamide combination.

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REFERENCES


