A CONVENTIONAL SYNTHESIS AND CHARACTERIZATION OF SOME NOVEL PYRAZOLES AS CYTOTOXIC AGENTS

Dabas Rohit, Hareen K S, Yadav Mithlesh and Pathak Devender*
Department of Pharmaceutical Chemistry, Rajiv Academy for Pharmacy, Mathura, Delhi-Mathura Highway, Chhattikara - 281006, (U.P.), India.

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ABSTRACT
Present work deals with the synthesis, characterization and cytotoxic screening of novel substituted pyrazole derivatives. Different acetophenones (I) with various substituted aromatic aldehydes (II) were condensed into corresponding chalcones (1a-e), which upon bromination gave chalcone dibromides (2a-e). The treatment of 2a-e with hydrazine hydrate, phenylhydrazine and 2,4-dinitrophenylhydrazine in the presence of TEA (triethanolamine) afforded different di and tri-phenyl substituted novel pyrazoles (3a-d and 4a-h). All the newly synthesized compounds were characterized by UV, FTIR, 1H NMR, mass spectral analysis and elemental analysis. All the compounds were screened for anticancer activity against A549 cell line by SRB assay. Compounds 3d, 4b, 4d, 4f and 4h showed promising cytotoxic potential.

Keywords: Cytotoxicity; chalcone dibromides; pyrazole; SRB; anticancer; A549 cell line.

INTRODUCTION
The development and design of new synthetic approach is a challenge for the organic chemist. The pyrazole ring system is a useful structural moiety found in numerous biologically active compounds1,2. Therefore to meet the facile results of these tough challenges, pyrazole nucleus was being considered. Pyrazoles are well documented to possess antimicrobial3-7, anticancer8-11, anti-inflammatory12, anti-leishmanial13 etc. activities and have wide applications as pharmaceutical and agrochemical agents. There are some synthetic compounds with pyrazole nucleus used for anticancer activities. SAR studies revealed that the introduction of pyrazole nucleus between two aryl rings of chalcones14 played an integral role for the increase in cytotoxicity. In view of these observations and in continuation of our research to develop better and potent anticancer agents, it was contemplated to synthesize a series of novel compounds possessing pyrazole moiety.

Experimental
All the reagents were of commercial quality. Solvents were dried and purified by using standard techniques. Reactions were monitored by TLC. The purity of all the newly synthesized compounds was checked by TLC on silica gel G plates. Melting points were taken in open capillary tube and are uncorrected. The UV spectra were recorded on a SHIMADZU spec-1700 spectrophotometer, IR spectra on a SHIMADZU 8400S spectrophotometer, 1H NMR spectra on a Bruker DRX 300 MHz spectrometer in DMSO using TMS (Tetramethylsilane) as an internal standard and Mass spectrum on an MS-ESI (SHIMADZU-2010 AT, software class VP). Elemental analysis was carried out on Elemental Vario EL III Carlo Erba 1108.

Synthesis of chalcones (1a-e)
Different acetophenones (I) (0.045 mol) and various aromatic aldehydes (II) (0.043 mol) were added to a mixture of sodium hydroxide (2.25 g) in 20 ml of distilled water and rectified spirit (15 ml) and stirred for 3-7 hours at 10ºC. The reaction mixture was kept overnight at 5ºC. The crude product was filtered, washed with cold water, dried and recrystallized from absolute ethanol to obtain compounds 1a-e.

Synthesis of chalcone dibromides (2a-e)
Bromine (5 g, 1.6 ml, 0.032 mol) was dissolved in 7.5 ml of glacial acetic acid and this solution was slowly added to the mixture of 1a-e (0.03 mol) and glacial acetic acid (13 ml) with constant shaking. The reaction mixture was allowed to stand at room temperature for 30-45 minutes, poured into water, filtered, washed with cold water, dried and recrystallized from absolute ethanol to obtain compounds 2a-e.

Synthesis of Di-phenyl substituted pyrazoles (3a-d)
To a mixture of 2a-e (0.006 mol) and hydrazine hydrate (0.013 mol), 20 ml of triethanolamine (TEA) was added and heated for 15-30 minutes. The reaction mixture was cooled at room temperature, filtered and recrystallized from absolute ethanol to obtain compounds 3a-d (Scheme-1)
Derivatives synthesized (Table-1)

**5-(2-Chlorophenyl)-3-phenyl-1H-pyrazole (3a)**

- **UV**: \( \lambda_{\text{max}} ^{\text{DMSO}} \): 304 nm (log \( \varepsilon \) 4.17); IR \( \text{KBr} \) cm\(^{-1}\): 3338.10 (N-H, str.), 3057.10 (Ar C-H, str.), 1640.74 (C=C, str.), 1610.81 (C=N, str.), 1324.00 (C-N, str.), 1098.85 (C-Cl, str.), 1027.99 (C-C, str.); \(^1\)H NMR (DMSO-\( d_6 \)): \( \delta \) 6.610 (s, 1H, CH-pyrazole), 7.242–8.414 (m, 9H, Ar-H), 12.525 ppm (s, 1H, NH, D\(_2\)O exchangeable); MS-ESI: \( m/z \) 254.2925 (M); Calcd for C\(_{15}\)H\(_{11}\)ClN\(_2\): C, 70.73; H, 4.35; Cl, 13.92; N, 11.00; Found: C, 70.71; H, 4.34; Cl, 13.91; N, 10.97%.

**5-(4-Chlorophenyl)-3-phenyl-1H-pyrazole (3b)**

- **UV**: \( \lambda_{\text{max}} ^{\text{DMSO}} \): 306 nm (log \( \varepsilon \) 4.19); IR \( \text{KBr} \) cm\(^{-1}\): 3342.41 (N-H, str.), 3057.10 (Ar C-H, str.), 1640.74 (C=C, str.), 1610.81 (C=N, str.), 1324.00 (C-N, str.), 1098.85 (C-Cl, str.), 1027.99 (C-C, str.); \(^1\)H NMR (DMSO-\( d_6 \)): \( \delta \) 6.762 (s, 1H, CH-pyrazole), 7.199–8.221 (m, 9H, Ar-H), 12.545 ppm (s, 1H, NH, D\(_2\)O exchangeable); MS-ESI: \( m/z \) 254.4037 (M); Calcd for C\(_{15}\)H\(_{11}\)ClN\(_2\): C, 70.73; H, 4.35; Cl, 13.92; N, 11.00; Found: C, 70.63; H, 4.32; Cl, 13.91; N, 10.96%.

**5-(3-Chlorophenyl)-3-phenyl-1H-pyrazole (3c)**

- **UV**: \( \lambda_{\text{max}} ^{\text{DMSO}} \): 300 nm (log \( \varepsilon \) 4.24); IR \( \text{KBr} \) cm\(^{-1}\): 3303.83 (N-H, str.), 2979.82 (Ar C-H, str.), 1650.95 (C=C, str.), 1609.09 (C=N, str.), 1322.36 (C-N, str.), 1095.13 (C-Cl, str.), 1010.63 (C-C, str.); \(^1\)H NMR (DMSO-\( d_6 \)): \( \delta \) 6.972 (s, 1H, CH-pyrazole), 7.199–8.221 (m, 9H, Ar-H), 12.783 ppm (s, 1H, NH, D\(_2\)O exchangeable); MS-ESI: \( m/z \) 254.4199 (M); Calcd for C\(_{15}\)H\(_{11}\)ClN\(_2\): C, 70.73; H, 4.35; Cl, 13.92; N, 11.00; Found: C, 70.70; H, 4.34; Cl, 13.91; N, 10.97%.

**3-(4-Chlorophenyl)-5-(4-methoxyphenyl)-1H-pyrazole (3d)**

- **UV**: \( \lambda_{\text{max}} ^{\text{DMSO}} \): 292 nm (log \( \varepsilon \) 4.32); IR \( \text{KBr} \) cm\(^{-1}\): 3343.19 (N-H, str.), 3051.18 (Ar C-H, str.), 1651.23 (C=C, str.), 1614.02 (C-N, str.), 1325.99 (C-N, str.), 1276.89 (C-O, str.), 1151.42 (C-C, str.), 1096.28 (C-Cl, str.); \(^1\)H NMR (DMSO-\( d_6 \)): \( \delta \) 3.501 (s, 3H, -OCH\(_3\)), 7.032 (s, 1H, CH-pyrazole), 7.563–7.793 (m, 8H, Ar-H), 10.701 ppm (s, 1H, NH, D\(_2\)O exchangeable); MS-ESI: \( m/z \) 284.3961 (M); Calcd for C\(_{16}\)H\(_{13}\)ClN\(_2\)O: C, 67.49; H, 4.60; Cl, 12.45; N, 9.84; O, 5.62; Found: C, 67.43; H, 4.56; Cl, 12.41; N, 9.81; O, 5.60%.

**Table 1: Physical data of Di-phenyl substituted pyrazoles (3a-d)**

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<th>R(_1)</th>
<th>M.P. (°C)</th>
<th>Yield (%)</th>
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<td>H</td>
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<tr>
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<td>( \alpha )-Cl</td>
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<tr>
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<td>H</td>
<td>( m )-Cl</td>
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<td>3d</td>
<td>( \rho )-Cl</td>
<td>( \rho )-OCH(_3)</td>
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Table 2: Physical data of Tri-phenyl substituted pyrazoles 

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<th>X</th>
<th>M. P. (°C)</th>
<th>Yield (%)</th>
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<td>p-OCH₃</td>
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5-(4-Chlorophenyl)-1, 3-diphenyl-1H-pyrazole (4) 
UV λₘₐₓ (DMSO): 307.96 (Ar=C, C), 1647.59 (C=C, C), 1612.01 (C=N, C), 1301.58 (C-N, C), 1095.92 (C-Cl, C), 972.06 (C, C). 'H NMR (DMSO-d₆): δ 6.839 (s, 1H, CH-pyrazole), 7.189–8.161 ppm (m, 14H, Ar-H); MS-ESI: m/z 330.4107 (M⁺); Calcd for C₂₁H₁₅ClN₂: C, 76.24; H, 4.57; Cl, 10.72; N, 8.47; Found: C, 76.20; H, 4.57; Cl, 10.69; N, 8.43%.

1-(2, 4-Dinitrophenyl)-3, 5-diphenyl-1H-pyrazole (4) 
UV λₘₐₓ (DMSO): 305.29 (Ar=C, C), 1640.51 (C=C, C), 1581.73 (C=N, C), 1297.22 (C-N, C), 1098.14 (C-Cl, C), 999.40 (C, C). 'H NMR (DMSO-d₆): δ 6.686 (s, 1H, CH-pyrazole), 7.265–8.314 ppm (m, 13H, Ar-H); MS-ESI: m/z 420.3076 (M⁺); Calcd for C₂₃H₁₀ClN₂O₂: C, 79.94; H, 3.11; Cl, 8.43; O, 13.31; Obs: C, 79.97; H, 3.09; Cl, 8.40; O, 13.38.

5-(4-Chlorophenyl)-3-phenyl-1H-pyrazole (4) 
UV λₘₐₓ (DMSO): 3067.43 (Ar=C, C), 1645.15 (C=C, C), 1609.61 (C=N, C), 1348.15 (C-N, C), 1099.42 (C-Cl, C), 1020.27 (C, C). 'H NMR (DMSO-d₆): δ 6.680 (s, 1H, CH-pyrazole), 7.200–8.411 ppm (m, 14H, Ar-H); MS-ESI: m/z 330.3099 (M⁺); Calcd for C₂₁H₁₂ClN₂: C, 70.73; H, 4.35; Cl, 13.92; N, 11.00; Obs: C, 70.72; H, 4.34; Cl, 13.90; N, 10.99%.

1-(2, 4-Dinitrophenyl)-3, 5-diphenyl-1H-pyrazole (4) 
UV λₘₐₓ (DMSO): 306.12 (Ar=C, C), 2912.03 (Aliphatic C=C, C), 1642.95 (C=C, C), 1347.22 (C-N, C), 1234.08 (C-O, C), 1091.35 (C-Cl, C). 'H NMR (DMSO-d₆): δ 6.589 (s, 1H, CH-pyrazole), 7.499–8.264 ppm (m, 13H, Ar-H); MS-ESI: m/z 366.0841 (M⁺); Calcd for C₂₁H₁₄N₂O₂: C, 65.28; H, 3.65; N, 14.50; Obs: C, 65.25; H, 3.61; N, 14.48; Obs: C, 65.24; H, 3.57; N, 14.51; Obs: C, 65.23; H, 3.58; N, 14.50; Obs: C, 65.22; H, 3.59; N, 14.50; Obs: C, 65.21; H, 3.60; N, 14.50.

3-(4-Chlorophenyl)-5-(4-methoxyphenyl)-1-phenyl-1H-pyrazole (4) 
UV λₘₐₓ (DMSO): 306.03 (Ar=C, C), 2912.03 (Aliphatic C=C, C), 1642.95 (C=C, C), 1347.22 (C-N, C), 1234.08 (C-O, C), 1091.35 (C-Cl, C). 'H NMR (DMSO-d₆): δ 6.706 (s, 1H, CH-pyrazole), 7.200–8.411 ppm (m, 14H, Ar-H); MS-ESI: m/z 330.3099 (M⁺); Calcd for C₂₁H₁₂ClN₂O: C, 70.73; H, 4.35; Cl, 13.92; N, 11.00; Obs: C, 70.72; H, 4.34; Cl, 13.90; N, 10.99%.

Anticancer screening

The SRB assay possesses a colorimetric end point and is non-destructive and indefinitely stable. These practical advances make the SRB assay an appropriate and sensitive assay to measure drug-induced cytotoxicity. The results are reported in Table 3.

Principle

SRB (Kiton red) is a fluorescent dye. Under mild acidic conditions, SRB binds to protein basic amino acid residues in Trichloro acetic acid (TCA) fixed cells to provide a sensitive index of cellular protein content that is non-destructive and indefinitely stable. These practical advances make the SRB assay an appropriate and sensitive assay to measure drug-induced cytotoxicity. The results are reported in Table 3.
measured over a broad range of visible wavelength in either a spectrophotometer or a 96 well plate reader\textsuperscript{15}.

Table 3: Results for cytotoxicity by SRB assay in A549 cell line

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<th>S. No.</th>
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<th>CTC\textsubscript{50} (µg/ml)</th>
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<tr>
<td>2</td>
<td>3\textsubscript{b}</td>
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<tr>
<td>3</td>
<td>3\textsubscript{c}</td>
<td>148</td>
</tr>
<tr>
<td>4</td>
<td>3\textsubscript{d}</td>
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<tr>
<td>5</td>
<td>4\textsubscript{a}</td>
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</tr>
<tr>
<td>6</td>
<td>4\textsubscript{b}</td>
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<td>11</td>
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<tr>
<td>12</td>
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</table>

RESULTS AND DISCUSSION

The novel pyrazole derivatives were synthesized successfully in moderate to good yields. The newly synthesized compounds were identified on the basis of R\textsubscript{f} values; melting point range; solubility in different solvents; FTIR, \textsuperscript{1}H-NMR, mass spectral analysis and elemental analysis. All the newly synthesized pyrazole derivatives were screened for anticancer activity against A549 (Human lung adenocarcinoma epithelial) cell line by SRB (Sulforhodamine B) assay.

CONCLUSION

From the cytotoxicity screening data, it was concluded that the compounds possessing nitro and methoxy substitution viz. 3\textsubscript{d}, 4\textsubscript{a}, 4\textsubscript{e}, 4\textsubscript{g} and 4\textsubscript{h} exhibited highest degree of inhibition against A549 cell line and this fact warrants further investigation of these compounds as promising anticancer agents. The results are reported in Table 3.

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REFERENCES