Journal of Pharmaceutical Research Vol. 10, No. 1, January 2011 : 6-9.

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.

Received on : 03.12.2010 Revised : 11.01.2011 Accepted : 14.01.2011

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 **(1_{a-e})**, which upon bromination gave chalcone dibromides **(2_{a-e})**. The treatment of 2_{a-e} with hydrazine hydrate, phenylhydrazine and 2,4-dinitrophenylhydrazine in the presence of TEA (triethanolamine) afforded different di and tri-phenyl substituted novel pyrazoles **(3_{a-d} and 4_{a-h})**. All the newly synthesized compounds were characterized by UV, FTIR, ^{1}H NMR, mass spectral analysis and elemental analysis. All the compounds were screened for anticancer activity against *A549* cell line by SRB assay. Compounds 3_d, 4_b, 4_d, 4_f and 4_h 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 compounds[1,2]. Therefore to meet the facile results of these tough challenges, pyrazole nucleus was being considered. Pyrazoles are well documented to possess antimicrobial[3-7], anticancer[8-11], anti-inflammatory[12], anti-leishmanial[13]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 chalcones[14] 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, ^{1}H NMR spectra on a Brucker 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 (1_{a-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 distill 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 1_{a-e}.

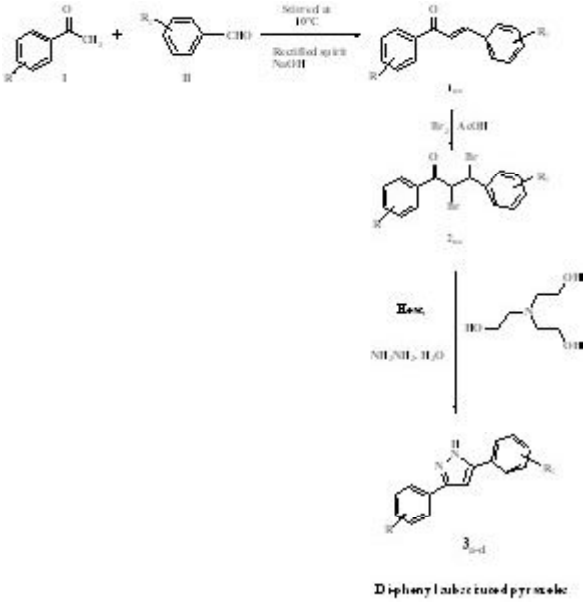
Synthesis of chalcone dibromides (2_{a-e})

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 1_{a-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 2_{a-e}.

Synthesis of Di-phenyl substituted pyrazoles (3_{a-d})

To a mixture of 2_{a-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 3_{a-d}. **(Scheme-1)**

***Correspondence :** dev_15@rediffmail.com



Scheme-1. Synthesis of Di-phenyl substituted pyrazoles (3_{a-d})

Derivatives synthesized (Table-1)

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

UV λ_{max} (DMSO): 304 nm (log ε 4.17); IR (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): δ 6.610 (s, 1H, CH-pyrazole), 7.242–8.414 (m, 9H, Ar-H), 12.525 ppm (s, 1H, NH, D_2O 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, 13.89; N, 10.98%.

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

Compound	R	R_1	M. P. (°C)	Yield (%)
3_a	H	*o*-Cl	185-186	75
3_b	H	*p*-Cl	197-198	72
3_c	H	*m*-Cl	188-189	69
3_d	*p*-Cl	*p*-OCH_3	185-186	78

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

UV λ_{max} (DMSO): 306 nm (log ε 4.19); IR (KBr) cm^{-1}: 3342.41 (N-H, str.), 3047.32 (Ar C-H, str.), 1626.08 (C=C, str.), 1604.08 (C=N, str.), 1314.08 (C-N, str.), 1151.42 (C-C, str.), 1093.28 (C-Cl, str.); ^{1}H NMR (DMSO-d_6): δ 6.762 (s, 1H, CH-pyrazole), 7.199–8.221 (m, 9H, Ar-H), 12.293 ppm (s, 1H, NH, D_2O 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, 70.68; H, 4.32; Cl, 13.90; N, 10.96%.

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

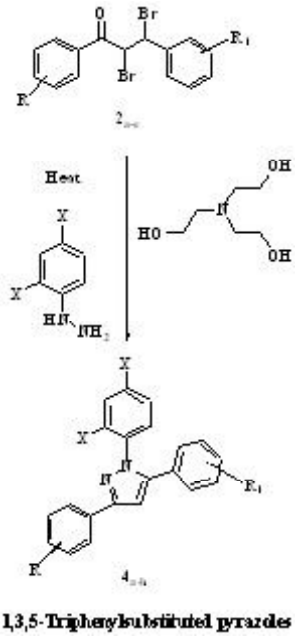
UV λ_{max} (DMSO): 300 nm (log ε 4.24); IR (KBr) cm^{-1}: 3303.83 (N-H, str.), 2979.82 (Ar C-H, str.), 1650.95 (C=C, str.), 1609.09 (C=N, str.), 1322.36 (C-N, str.), 1095.13 (C-Cl, str.), 1010.63 (C-C, str.); ^{1}H NMR (DMSO-d_6): δ 6.972 (s, 1H, CH-pyrazole), 7.589–8.414 (m, 9H, Ar-H), 12.783 ppm (s, 1H, NH, D_2O 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)-1*H*-pyrazole (3_d)

UV λ_{max} (DMSO): 292 nm (log ε 4.32); IR (KBr) cm^{-1}: 3343.19 (N-H, str.), 3051.18 (Ar C-H, str.), 2920.03 (Aliphatic 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): δ 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_2O exchangeable); MS-ESI: *m/z* 284.3961 $(M)^+$; Calcd for $C_{16}H_{13}ClN_2O$: 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%.

Synthesis of Tri-phenyl substituted pyrazoles (4_{a-h})

To a mixture of $\mathbf{2}_{a-e}$ (0.005 mol) and phenylhydrazine/ 2,4-dinitrophenylhydrazine (0.01 mol), 17 ml of TEA was added and heated for 15-30 minutes. The reaction mixture was cooled at room temperature, filtered and recrystallized from methanol to obtain compounds $\mathbf{4}_{a-h}$. **(Scheme-2)**



Scheme-2. Synthesis of Tri-phenyl substituted pyrazoles (4_{a-h})

Derivatives synthesized (Table-2)

1, 3, 5-Triphenyl-1*H*-pyrazole (4_a)

UV λ_{max} (DMSO): 294 nm (log ε 4.39); IR (KBr) cm^{-1}: 3029.54 (Ar C-H, str.), 1673.32 (C=C, str.), 1622.02 (C=N, str.), 1308.08 (C-N, str.), 1000.99 cm^{-1} (C-C, str.); ^1H NMR (DMSO-d_6): δ 6.536 (s, 1H, CH-pyrazole), 7.198–8.408 ppm (m, 15H, Ar-H); MS-ESI: *m/z* 296.1671 $(M)^+$; Calcd for $C_{21}H_{16}N_2$: C, 85.11; H, 5.44; N, 9.45; Found: C, 85.10; H, 5.42; N, 9.44%.

Table-2: *Physical data of Tri-phenyl substituted pyrazoles (4_{a-h})*

Compound	R	R_1	X	M.P. (°C)	Yield (%)
4_a	H	H	H	209-210	71
4_b	H	H	NO_2	190-191	67
4_c	H	o-Cl	H	194-195	72
4_d	H	o-Cl	NO_2	187-188	66
4_e	H	*p*-Cl	H	191-192	77
4_f	H	*p*-Cl	NO_2	184-185	68
4_g	H	*m*-Cl	H	197-198	73
4_h	*p*-Cl	*p*-OCH_3	H	192-193	70

1-(2, 4-Dinitrophenyl)-3, 5-diphenyl-1*H*-pyrazole (4_b)

UV λ_{max} (DMSO): 299 nm (log ε 4.41); IR (KBr) cm^{-1}: 3000.03 (Ar C-H, str.), 1638.52 (C=C, str.), 1604.51 (C=N, str.), 1504.12 (Ar NO_2, str.), 1346.22 (C-N, str.), 1065.63 (C-C, str.); ^1H NMR (DMSO-d_6): δ 6.489 (s, 1H, CH-pyrazole), 7.499–8.264 ppm (m, 13H, Ar-H); MS-ESI: *m/z* 386.0841 $(M)^+$; Calcd for $C_{21}H_{14}N_4O_4$: C, 65.28; H, 3.65; N, 14.50; O, 16.56; Found: C, 65.25; H, 3.61; N, 14.48; O, 16.53%.

5-(2-Chlorophenyl)-1, 3-diphenyl-1*H*-pyrazole (4_c)

UV λ_{max} (DMSO): 307 nm (log ε 4.40); IR (KBr) cm^{-1}: 3051.82 (Ar C-H, str.), 1651.51 (C=C, str.), 1619.51 (C=N, str.), 1325.87 (C-N, str.), 1151.35 (C-C, str.), 1081.99 (C-Cl, str.); ^1H NMR (DMSO-d_6): δ 6.516 (s, 1H, CH-pyrazole), 7.009–8.061 ppm (m, 14H, Ar-H); MS-ESI: *m/z* 330.2951 $(M)^+$; Calcd for $C_{21}H_{15}ClN_2$: C, 76.24; H, 4.57; Cl, 10.72; N, 8.47; Found: C, 76.22; H, 4.54; Cl, 10.70; N, 8.47%.

5-(2-Chlorophenyl)-1-(2, 4-dinitrophenyl)-3-phenyl-1*H*-pyrazole (4_d)

UV λ_{max} (DMSO): 298 nm (log ε 4.42); IR (KBr) cm^{-1}: 3043.82 (Ar C-H, str.), 1640.59 (C=C, str.), 1596.56 (C=N, str.), 1473.51 (Ar-NO_2, str.), 1316.94 (C-N, str.), 1153.35 (C-C, str.), 1080.06 (C-Cl, str.); ^1H NMR (DMSO-d_6): δ 6.803 (s, 1H, CH-pyrazole), 7.200–8.311 ppm (m, 12H, Ar-H); MS-ESI: *m/z* 420.2482 $(M)^+$, Calcd for $C_{21}H_{13}ClN_4O_4$: C, 59.94; H, 3.11; Cl, 8.43; N, 13.31; O, 15.21; Found: C, 59.91; H, 3.11; Cl, 8.41; N, 13.29 O, 15.19%.

5-(4-Chlorophenyl)-1, 3-diphenyl-1*H*-pyrazole (4_e)

UV λ_{max} (DMSO): 311 nm (log ε 4.43); IR (KBr) cm^{-1}: 3075.96 (Ar C-H, str.), 1647.59 (C=C, str.), 1612.01 (C=N, str.), 1301.58 (C-N, str.), 1095.92 (C-Cl, str.), 972.06 (C-C, str.); ^1H NMR (DMSO-d_6): 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 $C_{21}H_{15}ClN_2$: 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%.

5-(4-Chlorophenyl)-1-(2, 4-dinitrophenyl)-3-phenyl-1*H*-pyrazole (4_f)

UV λ_{max} (DMSO): 296 nm (log ε 4.52); IR (KBr) cm^{-1}: 2997.62 (Ar C-H, str.), 1645.51 (C=C, str.), 1618.73 (C=N, str.), 1528.59 (Ar-NO_2, str.), 1297.22 (C-N, str.), 1098.14 (C-Cl, str.), 999.40 (C-C, str.); ^1H NMR(DMSO-d_6): δ 6.686 (s, 1H, CH-pyrazole), 7.265–8.314 ppm (m, 12H, Ar-H); MS-ESI: *m/z* 420.3076 $(M)^+$; Calcd for $C_{21}H_{13}ClN_4O_4$: C, 59.94; H, 3.11; Cl, 8.43; N, 13.31; O, 15.21; Found: C, 59.89; H, 3.10; Cl, 8.40; N, 13.26, O, 15.19%.

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

UV λ_{max} (DMSO): 303 nm (log ε 4.36); IR (KBr) cm^{-1}: 3067.43 (Ar C-H, str.), 1645.15 (C=C, str.), 1609.61 (C=N, str.), 1348.15 (C-N, str.), 1099.42 (C-Cl, str.), 1020.27 (C-C, str.); ^1H NMR (DMSO-d_6): 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 $C_{21}H_{15}ClN_2$: C, 70.73; H, 4.35; Cl, 13.92; N, 11.00; Found: C, 70.72; H, 4.34; Cl, 13.90; N, 10.99%.

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

UV λ_{max} (DMSO): 297 nm (log ε 4.47); IR (KBr) cm^{-1}: 3063.12 (Ar C-H, str.), 2912.03 (Aliphatic C-H, str.), 1642.95 (C=C, str.), 1613.59 (C=N, str.), 1347.22 (C-N, str.), 1234.08 (C-O, str.), 1091.35 (C-Cl, str.), 1018.34 (C-C, str.); ^1H NMR (DMSO-d_6): δ 3.689 (s, 3H, -OCH_3), 6.586 (s, 1H, CH-pyrazole), 6.800–8.161 ppm (m, 13H, Ar-H); MS-ESI: *m/z* 360.4931 $(M)^+$; Calcd for $C_{22}H_{17}ClN_2O$: C, 73.23; H, 4.75; Cl, 9.83; N, 7.76; O, 4.43; Found: C, 73.21; H, 4.74; Cl, 9.81; N, 7.76; O, 4.40%.

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 linear over a cell density range of at least two orders of magnitude. Colour development in SRB assay is rapid, stable and visible. The developed colour can be

measured over a broad range of visible wavelength in either a spectrophotometer or a 96 well plate reader[15].

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

S. No.	Compound	CTC_{50} (µg/ml)
1	3_a	144
2	3_b	>200
3	3_c	148
4	3_d	123
5	4_a	172
6	4_b	125
7	4_c	138
8	4_d	105
9	4_e	>200
10	4_f	112
11	4_g	136
12	4_h	117

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_f values; melting point range; solubility in different solvents; FTIR, ^{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.* $\mathbf{3_d}$, $\mathbf{4_b}$, $\mathbf{4_d}$, $\mathbf{4_f}$ and $\mathbf{4_h}$ 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.

ACKNOWLEDGEMENT

The authors are thankful to I.I.T, New Delhi for conducting mass and ^{1}H NMR spectral studies. The authors also thank Dr. P. Vijayan, Professor and Head, Department of Pharmaceutical Biotechnology, JSS College of Pharmacy, Ootacamund for providing anticancer screening facilities. Thanks to Rajiv Academy for Pharmacy, Mathura for giving us the necessary facilities.

REFERENCES

1. Ahmed MSM, *et al*. Org Lett. 2005; 7: 4487.
2. Agrawal NN, *et al*. Indian J Chem. 2007; 46B: 532.
3. Goel S, *et al*. Indian J Het Chem. 2005; 15: 141.
4. Sharma KP, *et al*. Asian J Chem. 2007; 19: 4129.
5. Yadav AK, *et al*. Asian J Chem. 2007; 19: 3511.
6. Patankar JA, *et al*. Indian J Het Chem. 2005; 15: 125.
7. Thakare NR, *et al*. Asian J Chem. 2007; 19, 3633.
8. Sakai K, *et al*. Inorganica Chimica Acta. 2000; 297: 64.
9. Vyas R, *et al*. J Indian Chem Soc. 2008; 85: 1217.
10. Bhat BA, *et al*. Bioorg Med Chem Lett. 2005; 15: 3177.
11. Tan JH, *et al*. Eur J Med Chem. 2006; 41: 1041.
12. Bhaskar VH, *et al*. Indian J Het Chem. 2007; 17: 131.
13. Bernardino AM, *et al*. Eur J Med Chem. 2006; 41: 80.
14. Gomez L, *et al*. Bioorg Med Chem Lett. 2007; 17: 2723.
15. Philip S, *et al*. J. National Cancer Inst. 1990; 82: 1107.