SYNTHESIS, CHARACTERIZATION AND EVALUATION OF PYRZOLE DERIVATIVES FOR ANTIBACTERIAL ACTIVITY

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ABSTRACT

In the present work we have used the green and efficient synthesis for new series of bis- pyrazoles derivatives from 1-p-tollylpyrrolidine-2,5-dione under microwave irradiation. The 1-p-tolylpyrrolidine-2,5-dione was synthesized from succinic acid and p-toludine using thionyl chloride. The cyclic imides condensed with substituted aromatic aldehydes using solid support neutral Al_2O_3 in microwave. The resulting chalcone derivative underwent ring closer with phenyl hydrazine in presence of neutral Al_2O_3 under microwave irradiation afforded bis-pyrazole derivatives. All pyrazole derivatives were characterized by spectral and elemental analysis and these derivatives were screened for their antibacterial activity.

KEYWORDS: 1-p-tollyl pyrrolidin e -2,5-dione, phenyl hydrazine.

INTRODUCTION

Pyrazole is important class of nitrogen containing five member heterocyclic compound containing two nitrogen atoms in their ring structure make them biologically important and versatile heterocycle[1] which found to have power over Photoluminescence.[2] It has Hypertensive activity[3], Anticonvulsant activity[4], Some of its derivatives are act as anti- oxidant agent.[5] The different substituent's assemble on nitrogen atom of pyrazoles affords biologically important molecule which found to possess PGE2 inhibitory properties.[6] Some of the derivatives of pyrazole molecules are important in the field of medicine and these have Antimicrobial and antitubercular activities[7], Antimalarial and anti-leishmanial activity[8],

Antidepressant and anticonvulsant activity[9], Antihelminial[10], Antibacterial activities[11], Antitubercular agent[12], Antidepressant activities[13], and Anti-cancer activity.[14][15] The compound incorporated with pyrazole moiety is act as Anti-fungal agent.[16] By taking in to account all these incredible applications of pyrazole we have synthesized bis-N-phenyl substituted pyrazole from cyclic imides. The cyclic imides[17] are also biologically and chemically imperative molecule and It is used to synthesize bis-heterocyclic chalcone[18-19] the chalcone is an important synthon for synthesizing various heterocyclic compounds in this view we devise an environmental friendly method of synthesis of bis-N-phenyl substituted pyrazoline derivatives from 1-p-tolylpyrrolidine-2, 5-dione.

MATERIAL AND METHOD

Experimental

All chemicals used in the present work are of synthetic grade. The melting points were taken in to open capillaries and are uncorrected. The I.R spectra were recorded on FTIR shimazdu spectrophotometer using KBr disc method. The ^{1}H NMR spectra were recorded on Bruker mx-500 MHz in DMSO d_6. The chemical shift was recorded in δ unit relative to TMS as internal standard. All the compounds synthesized by using domestic microwave oven in hours. All reactions were executed in solid phase solvent free synthesis. The reaction was monitored by thin layer chromatography by using pre-coated silica gel aluminum plates and mixture of n-hexane: ethyl acetate 6:4 proportion was used as mobile phase. The identification of spots was done by visualizing plate in U.V chamber

General Procedure for Synthesis of Chalcone

The 0.1 mole of cyclic amide **1** taken in 100 ml borosilicate glass beaker then add 0.2 moles of substituted benzaldehydes **2a-e** in beaker in presence of 2 gm of neutral alumina then irradiated in microwave oven at 450 MHz power for 3 to 4 min thus yellow colored fused solid derivatives of chalcone **3a-e** are obtained and recrystalised it from ethyl alcohol.

(3Z,4Z)-3,4-bis(2-hydroxybenzylidene)-1-p-tolylpyrrolidine-2,5-dione(3a): M F: $C_{25}H_{19}NO_4$; M W: 397.42; Anal Cal.: C, 75.55; H, 4.82; N, 3.52; O, 16.10; Found: C, 75.15;
H, 4.22; N, 3.72; O, 16.30; FTIR (KBr, cm^{-1}): 1705 (C=O), 3368 (-OH), 2937(-CH_3),

1611(C=C); ^{1}H NMR (500 MHz, DMSO d_6, δ ppm): 2.40 (s, 1H, CH_3), 5.1(s, 1H, -OH),

7.31-7.17(m, 6H, Ar-H and =CH).

(3Z,4Z)-3,4-bis(3-nitro benzylidene)-1-p-tolylpyrrolidine-2,5-dione(3b): MF:$C_{25}H_{17}N_3O_6$; M W: 455.42; Anal Cal.: C, 65.93; H, 3.76; N, 9.23; O, 21.08; Found: C, 65.33; H, 3.21; N,
9.53; O, 21.28;FTIR (KBr, cm^{-1}): 2937(-CH_3), 1705 (C=O), 1611(C=C) 1345 (Ar-NO_2);^{1}H

NMR (500 MHz, DMSO d_6, δ ppm): 2.40 (s, 1H, CH_3), 8.47-6.67(m, 6H,Ar-H and =CH).

(3Z,4Z)-3,4-bis(2-chlorobenzylidene)-1-p-tolylpyrrolidine-2,5-dione(3c)

MF: $C_{25}H_{17}Cl_2NO_2$; M W: 434.31; Anal Cal.: C, 69.14; H, 3.95; Cl, 16.33; N, 3.23; O, 7.37;

Found: C, 69.34; H, 3.86; Cl, 16.43; N, 3.63; O, 7.57; FTIR(KBr, cm^{-1}): 1705 (C=O), 2937(-

CH_3), 713 (C-Cl), 1611(C=C); ^{1}H NMR (500 MHz, DMSO d_6, δ ppm): 2.40 (s, 1H, CH_3), 7.40-7.12(m, 6H,Ar-H and =CH).

(3Z,4Z)-3,4-bis(4-methoxybenzylidene)-1-p-tolylpyrrolidine-2,5-dione(3d): M F: $C_{27}H_{23}NO_4$ M W: 425.48; Anal Cal.: C, 76.22; H, 5.45; N, 3.29; O, 15.04; Found: C, 76.42;
H, 5.75; N, 3.59; O, 15.24; FTIR (KBr, cm^{-1}): 1705 (C=O)-, 2937(-CH_3), 1178 (C-O ether)

1611(C=C); ^{1}H NMR (500 MHz, DMSO d_6, δ ppm): 2.40 (s, 1H, CH_3), 3.7(s, 3H, -OCH_3),

8.22-6.43(m, 6H,Ar-H and =CH).

(3Z,4Z)-3,4-bis(4-methylbenzylidene)-1-p-tolylpyrrolidine-2,5-dione(3e): M. F: $C_{27}H_{23}NO_2$ M W: 393.48; Anal Cal.: C, 82.42; H, 5.89; N, 3.56; O, 8.13; Found: C, 82.62; H,
5.69; N, 3.76; O, 8.43; FTIR (KBr, cm^{-1}): 1705 (C=O), 2937(-CH_3), 1611(C=C); ^{1}H NMR

(500 MHz, DMSO d_6, δ ppm): 2.40 (s, 1H, CH_3), 8.22-6.43(m, 6H, Ar-H and =CH).

General Procedure for Synthesis of phenyl substituted pyrazoline

The 0.1 mole of chalcone 3a-e and 0.2 mole of phenyl hydrazine 4 taken in 100 ml borosilicate glass beaker in presence of 2 gm of neutral alumina then irradiated in microwave oven at 450 MHz power for 3 to 4 min thus brown colored fused solid derivatives of phenyl substituted pyrazoline 5a-e are obtained and recrystalised it from ethyl alcohol.

2,2'-(2,5-diphenyl-7-(p-tolyl)-3,3a,3b,4,5,7-hexahydro-2H-pyrrolo[2,3-c:5,4- c']dipyrazole-3,4-diyl)diphenol (5a): Brown solid, M. F: $C_{37}H_{31}N_5O_2$; M P (^{0}C): 80-85; Anal Cal.: C, 76.93; H, 5.41; N, 12.12; O, 5.54; Found: C, 76.33; H, 5.81; N, 12.42; O, 5.74;
FTIR (KBr, cm^{-1}): 1498.69 (C=N), 3464.15 (-OH), 3045.60 (-CH_3, Ar-CH_3), 1600.92 (Ar, C=C); ^{1}HNMR (500 MH_Z; DMSO d_6; δ ppm): 2.3 (s, 3H, CH_3), 2.5 (d, 1H, -CH pyrazole), 3.4 (d, 1H, -CH pyrazole), 7.6-6.7 (m, 11H, Ar-H), 10.2 (s, 1H, CH_3).

3,4-bis(3-nitrophenyl)-2,5-diphenyl-7-(p-tolyl)-3,3a,3b,4,5,7-hexahydro-2H-pyrrolo[2,3- c:5,4-c']dipyrazole (5b): Radish brown solid, M. F: $C_{37}H_{29}N_7O_4$; M P (^{0}C): 105-108; Anal Cal.: C, 69.91; H, 4.60; N, 15.42; O, 10.07; Found: C, 69.71; H, 4.80; N, 15.82; O, 10.27; FTIR (KBr, cm^{-1}): 1517.93 (C=N), 2931.80 (-CH_3, Ar-CH_3), 1394.54 (Ar-NO2);^{1}HNMR (500 MH_Z; DMSO d_6; δ ppm): 2.3 (s, 3H, CH_3), 2.5 (d, 1H, -CH pyrazole), 3.4 (d, 1H, -CH
pyrazole), 7.3-7.1 (m, 11H, Ar-H).

3,4-bis(2-chlorophenyl)-2,5-diphenyl-7-(p-tolyl)-3,3a,3b,4,5,7-hexahydro-2H- pyrrolo[2,3-c:5,4-c']dipyrazole (5c): Radish crystals; M.F: $C_{37}H_{29}Cl_2N_5$; M P (^{0}C): 98; Anal Cal.: C, 72.31; H, 4.76; Cl, 11.54; N,

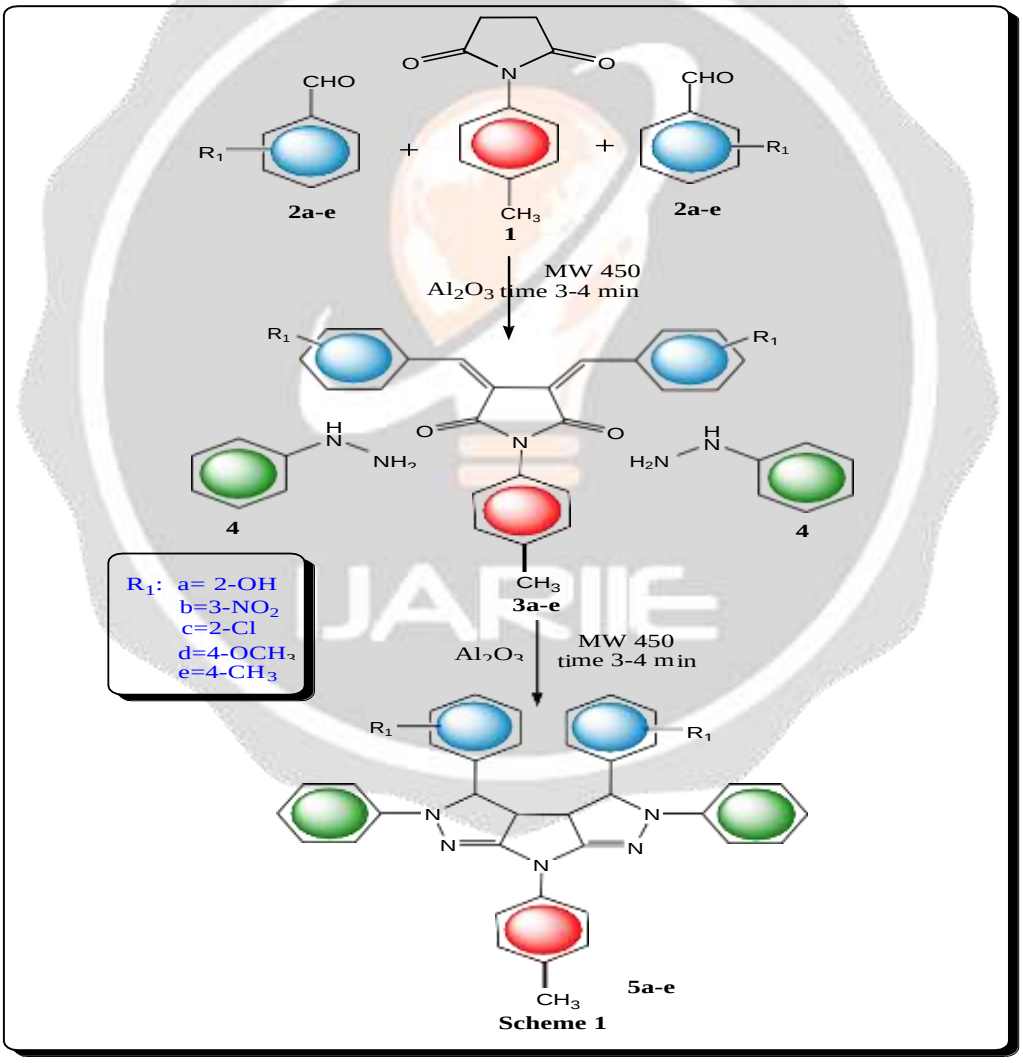
11.40; Found: C, 72.79; H, 4.36; Cl, 11.87; N, 11.83;
FTIR (KBr, cm^{-1}): 1498.69 (C=N), 2945.60 (-CH_3, Ar-CH_3), 758.12 (Ar-Cl), 1600.92 (Ar, C=C); ^{1}HNMR (500 MH_Z; DMSO d_6; δ ppm): 2.3 (s, 3H, CH_3), 2.5 (d, 1H, -CH pyrazole), 3.4 (d, 1H, -CH pyrazole), 7.8-7.1 (m, 11H, Ar-H).

3,4-bis(4-methoxyphenyl)-2,5-diphenyl-7-(p-tolyl)-3,3a,3b,4,5,7-hexahydro-2H- pyrrolo[2,3-c:5,4-c']dipyrazole (5d): Brown solid; M.F: $C_{39}H_{35}N_5O_2$; M P (^{0}C): 134; Anal Cal.: C, 77.33; H, 5.82; N, 11.56; O, 5.28; Found: C, 77.53; H, 5.68; N, 11.86; O, 5.78; FTIR
(KBr, cm^{-1}): 1517.93 (C=N), 2931.80 (-CH_3, Ar-CH_3), 1164.54 (O-CH3), ^{1}HNMR (500

MH_Z; DMSO d_6; δ ppm): 2.3 (s, 3H, CH_3), 3.8(s, 3H, -OCH_3), 2.5 (d, 1H, -CH pyrazole), 3.4 (d, 1H, -CH pyrazole), 7.8-7.1 (m, 11H, Ar-H).

3,4-bis(4-methylphenyl)-2,5-diphenyl-7-(p-tolyl)-3,3a,3b,4,5,7-hexahydro-2H- pyrrolo[2,3-c:5,4-c']dipyrazole (5e): Brown solid; M.F: $C_{39}H_{35}N_5$; M P (^{0}C): 90; Anal Cal.: C, 81.64; H, 6.15; N, 12.21; Found: C, 81.84; H, 6.85; N, 12.41; FTIR (KBr, cm^{-1}): 1554.63
(C=N), 3020.53 (CH_3, Ar-CH_3). ^{1}HNMR (500 MH_Z; DMSO d_6; δ ppm): 2.3 (s, 3H, CH_3), 2.5

(d, 1H, -CH pyrazole), 3.4 (d, 1H, -CH pyrazole), 7.8-7.1 (m, 9H, Ar-H).



Scheme 1

RESULT AND DISCUSSION

Chemistry

The chalcone **3a-e** required for synthesis of phenyl substituted pyrazoline is synthesized from cyclic imides **1** by reaction with substituted benzaldehydes **2a-e** in solid phase solvent free condition in presence of neutral alumina in microwave oven. The I.R band appears at 1705 cm^{-1} indicate the formation of α β unsaturated carbonyl compounds. The bis-heterocyclic chalcone on treatment with two moles of phenyl hydrazine **4** leading through cyclisation and furnished in to phenyl substituted pyrazole derivatives **5a-e**. The I.R band of 1705 cm^{-1} for carbonyl carbon is disappeared and new band of I.R stretching frequency is obtained at 1545 cm^{-1} is an indication for formation of pyrazoline ring system. The ^{1}H NMR signals appeared at 2.5 δ and 3.4 δ indicates the presence of -CH protons this is an evidence of cyclisation and formation of phenyl substituted pyrazoline which is shown in scheme **1**.

Microbial evaluation

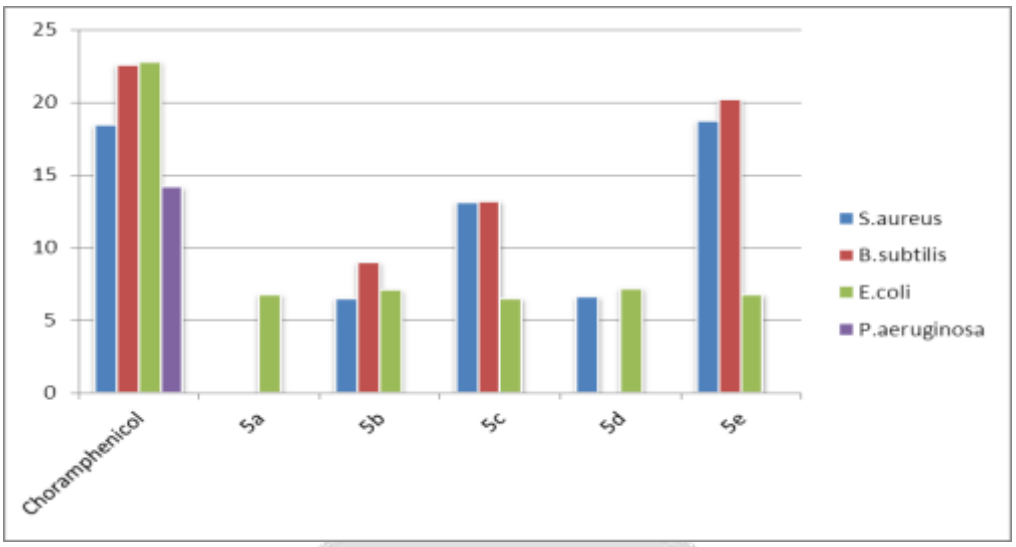
All synthesized phenyl substituted pyrazoline derivatives were evaluated for antibacterial activity for that purpose selective pathogenic bacteria strain of gram positive and gram negative bacteria were used. The DMSO was used as solvent to prepare aliquots of compounds **5a-e** and the final concentration 100µg per disc was adjusted. The disc diffusion method was used to evaluate antibacterial activity. For this, nutrient agar was employed as culture media. The results were obtained in the form of zone of inhibition and were noted after the period of incubation (at 37 ^{0}C for 24-28 hrs). The zone of inhibitions was measured in mm and the data is presented in table-**1.** Comparison of antibacterial activities of synthesized phenyl dipyrazole derivatives with standard antibiotic chloramphenicol is shown in graph 1.

Table 1: It shows antibacterial activity in form of zone of inhibition in mm.

Sr.no	Sample code	MIC100µg per disc			
		Gram +Ve		Gram -Ve	
		S.aureus	***B.subtilis***	***E.coli***	***P.aeruginosa***
1	5a	-	-	6.78	-
2	5b	6.51	-	7.07	-
3	5c	13.14	13.20	6.51	-
4	5d	6.61	-	7.20	-
5	5e	18.72	20.18	6.79	-
Std	Choramphenicol	18.43	22.56	22.80	14.19

Note: '-' means no zone of inhibition.

Fig. 1: Agar plates Shows zone of inhibition of compounds 5c and 5e at 100 µgm/ml concentrations.



Graph 1: Shows antibacterial activity in form of zone of inhibition in mm.

CONCLUSION

The above study concluded that synthesis of phenyl pyrazole derivatives in solid phase solvent free condition gave in high yield of products in very short period of time. The antibacterial evaluation of synthesized compounds concluded that 3,4-bis(2-chlorophenyl)- 2,5-diphenyl-7-(p-tolyl)-3,3a,3b,4,5,7-hexahydro-2H-pyrrolo[2,3-c:5,4-c']dipyrazole **(5c)** shown good antibacterial activities against *S.aureus* and *B.subtilis.* The compound 3,4-bis(4- methyl phenyl)-2,5-diphenyl-7-(p-tolyl)-3,3a,3b,4,5,7-hexahydro-2H-pyrrolo[2,3-c:5,4- c']dipyrazole **(5e)** showed promising antibacterial activities against *B.subtilis* and exhibited potent antibacterial activities against *S.aureus*.

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