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Research Article

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Synthesis and Antimicrobial activity Evaluation of some Novel Pyrazolines

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ABSTRACT

The pyrazoline nucleus is a ubiquitous feature of various therapeutically active compounds.They were reported to possess antimicrobial, antidiabetic, anticancer, antiHIV, antiamoebic, anthelmintic, anticonvulsant and tranquilizing activities. Therefore an attempt is made to synthesize novel pyrazolines from chalcones. All these synthesized compounds were characterized by means of their IR, ^{1}H NMR, ^{13}C NMR, and mass spectral data. These compounds were evaluated for antimicrobial activity by cup plate method.

Keywords: Pyrazolines, Chalcones, Synthesis, Antimicrobial activity, Cup plate method.

INTRODUCTION

Pyrazolines and chalcones were reported to possess various biological activities. In the present communication we report the synthesis of novel pyrazolines via chalcones. Hence, chalcones are important intermediates in the synthesis of various heterocyclic ring compounds like pyrazolines, pyrimidines, isoxazolines and thiazolines etc.. Therefore the present research work is viewed on the synthesis of Pyrazolines via chalcones by claisen-schmidt condensation using 3-acetylpyridine with either aromatic or heteroaromatic aldehydes (2_{a-j}) in the presence of alkali. The resulting chalcones [1-5] (3_{a-j}) after purification and characterization by physical and spectral methods have been successfully converted into novel substituted pyrazolines [6-17] (4_{a-j}) by reaction with phenyl hydrazine hydrochloride in absolute ethanol. The structures of the various synthesized compounds were assigned on the basis of elemental analyses, IR, ^{1}H NMR, ^{13}C NMR, and mass spectral data. These compounds were screened for their antimicrobial activity [18-20].

EXPERIMENTAL SECTION

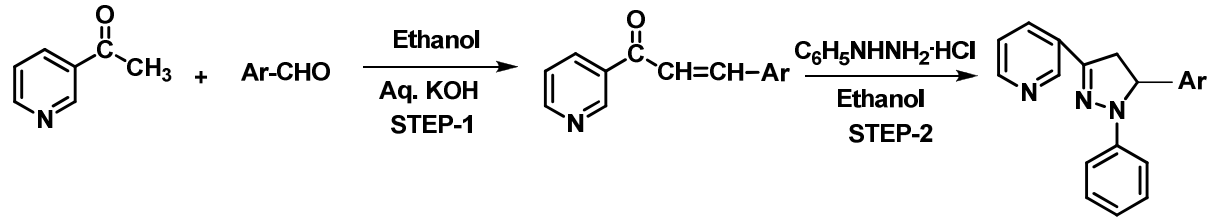
Melting points were determined on a capillary melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded in the indicated solvent on Bruker AMX 400 MHz spectrophotometer using TMS as an internal standard. Infrared spectra were recorded in KBr on Perkin-Elmer BXF1 spectrophotometer. Microanalyses were performed on carlo Ebra 1108 element analyzer and were within the ± 0.5% of the theoretical values. Column chromatography was performed on silica gel (Merck,100-200 mesh).

General procedure for the synthesis of pyrazolines via chalcones:
Step 1: Synthesis of chalcones:
Equimolar quantity (0.001mol) of 3-acetylpyridine and respective aldehydes were mixed and dissolved in minimum amount of alcohol. To this, 40 % aqueous potassium hydroxide solution (15 ml) was added slowly and mixed

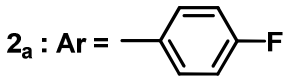
occasionally for 24 hrs, at room temperature. Completion of the reaction was identified by TLC using Silica gel-G. After completion of the reaction, the reaction mixture was poured into crushed ice, if necessary acidified with dil.HCl. The solid separated was filtered and dried. It was purified by column chromatography on silica gel (100-200 #, Merck), using ethylacetate and hexane mixture (1:1) as mobile phase.

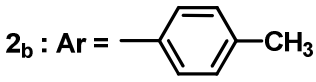
Step 2: Synthesis of pyrazolines :
Chalcone (0.001 mol) dissolved in absolute ethanol (20 ml) and phenyl hydrazine hydrochloride (0.001 mol) was added to it. After that the mixture was refluxed for 5-6 hr and the solvent was evaporated completely. The reaction mixture was poured into ice-cold water and the solid mass that separated out was filtered, dried and purified by column chromatography with ethyl acetate/hexane and recrystallized from chloroform.

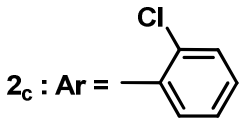
Reaction:

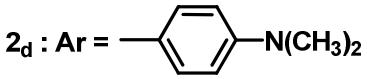


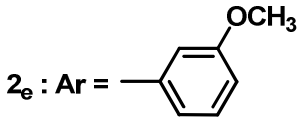
2 a-j **3 a-j** **4 a-j**

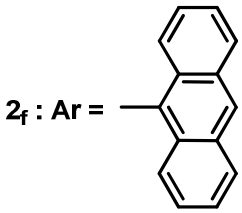


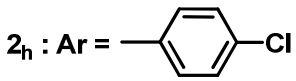


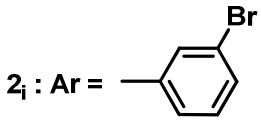


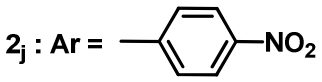












1-phenyl-3-(3'- pyridyl)-5-(4''-fluorophenyl)-2-pyrazoline (4_a):
Yield 85%; mp 142 ^oC; Relative molecular mass 317; IR (KBr) 1598 (C =N), 1323 (C-N), 1072 (C-F); ^{1}H-NMR 3.32 (1H, dd, H_A), 3.99 (1H, dd, H_B), 5.33 (1H, dd, Hx), 6.81 – 8.13 (13H, J_{AB} =17.72, J_{AX} =7.80, J_{BX} =10.8, Ar-H). Anal.calcd for $C_{20}H_{16}N_3F$: C, 79.94; H, 5.06; N, 13.29. Found: C, 75.92; H, 5.04; N, 13.26.

1-phenyl-3-(3'-pyridyl)-5-(4''-methylphenyl)-2-pyrazoline (4_b):
Yield 90%; mp 121 ^oC; Relative molecular mass 313; IR (KBr) 1565 (C =N), 1325 (C-N); ^{1}H-NMR 2.29 (3H, s, Ar-CH_3), 3.32 (1H, dd,H_A), 3.97 (1H, dd, H_B), 5.31 (1H, dd, H_X), 6.77 – 8.51 (13H, J_{AB} =17.6, J_{AX} =7.85, J_{BX} =10.8, Ar-H). Anal.calcd for $C_{21}H_{19}N_3$: C, 80.51; H, 6.07; N, 13.41. Found: C, 80.49; H, 6.06; N, 13.39.

1-phenyl-3-(3'-pyridyl)-5-(2''-chlorophenyl)-2-pyrazoline (4_c):
Yield 72%; mp 98 ^oC; Relative molecular mass 334; IR (KBr) 1592 (C=N), 1350 (C-N), 1040 (C-Cl); ^{1}H-NMR 3.25 (1H, dd, H_A), 4.09 (1H, dd, H_B), 5.71 (1H, dd, H_X), 7.19 – 8.51 (13H, J_{AB} =17.20, J_{AX} =7.30, J_{BX} =10.15, Ar-H). Anal.calcd for $C_{20}H_{16}ClN_3$: C, 71.85; H, 4.79; N, 12.58. Found: C, 71.89; H, 4.81; N, 12.60.

1-phenyl-3-(3'-pyridyl)-5-(4''-dimethylaminophenyl)-2-pyrazoline (4_d):
Yield 82%; mp 129 ^oC; Relative molecular mass 342; IR (KBr) 1590 (C=N), 1350 (C-N), 1181($N(CH_3)_2$); ^{1}H-NMR 2.89 (6H, s, N $(Me)_2$), 3.32 (1H, dd,H_A), 3.95 (1H, dd,H_B), 5.28 (1H, dd, H_X), 6.65 – 7.64 (13H, J_{AB} =17.20, J_{AX} =7.40, J_{BX} =10.15, Ar-H). Anal.calcd for $C_{22}H_{22}N_4$: C, 77.19; H, 6.43; N, 16.37. Found: C, 77.17; H, 6.44; N, 16.35.

1-phenyl-3-(3'-pyridyl)-5-(3''-methoxyphenyl)-2-pyrazoline (4_e):
Yield 68%; mp 61 ^oC; Relative molecular mass 329; IR (KBr) 1586 (C=N), 1329 (C-N); ^{1}H-NMR 3.74(3H, s, Ar-OCH_3), 3.34 (1H, dd, H_A), 4.00 (1H, dd, H_B), 5.31 (1H, dd, H_X), 6.76-8.53(13H, J_{AB} =17.10, J_{AX} =7.6, J_{BX} =9.8, Ar-H). Anal.calcd for C_{21} $H_{19}N_3O$: C, 76.59; H, 5.77; N, 12.76. Found: C, 76.62; H, 5.76; N, 12.73.

1-phenyl-3-(3'-pyridyl -5-(9''-anthracenyl)-2 -pyrazoline (4_f):
Yield 95%; mp 204 ^oC; Relative molecular mass 399; IR (KBr) 1590 (C=N), 1330 (C-N); ^{1}H-NMR 3.70 (1H, dd, H_A), 4.22 (1H, dd, H_B), 6.64 (1H, dd, H_X), 6.96-8.56 (18H, J_{AB} =17.10, J_{AX} =7.9, J_{BX} =10.32, Ar-H). Anal.calcd for $C_{28}H_{21}N_3$: C, 83.20; H, 5.26; N, 10.52. Found: C, 83.23; H, 5.27; N, 10.55.

1-phenyl-3-(3'-pyridyl)-5-(2''-thienyl)-2-pyrazoline (4_g):
Yield 93%; mp 194 ^0C; Relative molecular mass 305; IR (KBr) 1596 (C=N), 1342 (C-N), 647 (C-S); ^{1}H-NMR 3.49 (1H, dd, H_A), 3.97 (1H, dd, H_B), 5.60 (1H, dd, H_X), 6.82-8.53 (12H, J_{AB} =17.41, J_{AX} =7.89, J_{BX} =10.22, Ar-H). Anal.calcd for C_{18} $H_{15}S$ N_3: C, 70.81; H, 4.91; N, 13.77. Found: C, 70.83; H, 4.88; N, 13.73.

1-phenyl-3-(3'-pyridyl)-5- (4''-chlorophenyl)-2-pyrazoline (4_h):
Yield 93%; mp 85 ^0C; Relative molecular mass 334; IR (KBr) 1590 (C=N), 1328 (C-N); ^{1}H-NMR 3.32 (1H, dd, H_A), 3.99 (1H, dd,H_B), 5.33 (1H, dd, H_X), 6.81-8.53 (13H, J_{AB} =17.41, J_{AX} =7.89, J_{BX} =10.22, Ar-H). Anal.calcd for C_{20} H_{16} Cl N_3: C, 71.85; H, 4.79; N, 12.57. Found: C, 71.83; H, 4.77; N, 12.55.

1-phenyl-3-(3'-pyridyl)-5-(3''-bromophenyl)-2-pyrazoline (4_i):
Yield 93%; mp 124 ^0C; Relative molecular mass 378; IR (KBr) 1578 (C=N), 1320 (C-N); ^{1}H-NMR 3.39 (1H, dd, H_A), 4.10 (1H, dd,H_B), 5.42 (1H, dd, H_X), 7.19-8.52 (13H, J_{AB} =17.41, J_{AX} =7.89, J_{BX} =10.22, Ar-H). Anal.calcd for C_{20} H_{16} Br N_3: C, 63.49; H, 4.23; N, 11.11. Found: C, 63.45; H, 4.21; N, 11.13.

1-phenyl-3 -(3'-pyridyl)-5 -(4''-nitrophenyl)-2-pyrazoline (4_j):
Yield 83%; mp 203 ^0C; Relative molecular mass 344; IR (KBr) 1598 (C=N), 1540 (N=O, asymmetric), 1335 (N=O, symmetric), 1310 (C-N); ^{1}H–NMR 3.07 (1H, dd, H_A), 3.92 (1H, dd, H_B), 5.48 (1H, dd, H_X), 7.26-8.79 (13H, J_{AB} =17.41, J_{AX} =7.89, J_{BX} =10.22, Ar-H). Anal.calcd for C_{20} $H_{16}N_4$ O_2: C, 69.76; H, 4.65; N, 16.27. Found: C, 69.79; H, 4.62; N, 16.25.

Antimicrobial activity:
The antibacterial activity of synthesized pyrazolines was conducted against three Gram-positive bacteria viz., *Bacillus pumilis, Bacillus subtilis* and *Staphylococcus aureus* and two Gram-negative bacteria viz., *Escherichia coli, Proteus vulgaris* by using cup plate method. Preparation of nutrient broth, subculture, agar medium and peptone water was done as per standard procedure. Each test compound (5 mg) was dissolved in dimethylsulfoxide (5 ml) to give a concentration of 1000 µg/ml. All the compounds and the standard were tested at 50 µg (0.05 ml) and 100 µg (0.1 ml) dose levels and DMSO used as a control. Ampicillin as standard drug was also prepared at a concentration of 1000 µg/ml in sterilized distilled water.

All the compounds which were screened for antibacterial activity, also screened for their antifungal activity. The fungi employed for screening were *Aspergillus niger*, *Rhizopus oryzae* and *Candida albicans*. Fluconazole was employed as standard to compare the results. The test organisms were sub-cultured using potato-dextrose-agar (PDA) medium.

Each test compound (5mg) was dissolved in dimethylsulfoxide (5ml) to give a concentration of 1000 µg/ml. Fluconazole solution was also prepared at a concentration of 1000 µg/ml in sterilized distilled water. All the compounds and the standard were tested at 50 µg (0.05 ml) and 100 µg (0.1 ml) dose levels and DMSO used as a control.

RESULTS AND DISCUSSION

ANTIBACTERIAL ACTIVITY:

TABLE NO: 1

Compound No	*Zone of inhibition (in mm)*									
	Quantity in µg/ml									
	B. subtilis		***B. pumilis***		***S. aureus***		***E. coli***		***P. vulgaris***	
	50	**100**	**50**	**100**	**50**	**100**	**50**	**100**	**50**	**100**
4_a	12	18	11	17	13	18	15	19	13	15
4_b	09	10	09	11	06	08	10	12	11	13
4_c	10	11	09	12	12	13	09	11	10	12
4_d	10	12	09	13	10	14	09	11	11	14
4_e	09	11	09	10	10	12	09	11	11	13
4_f	07	08	09	10	06	08	09	10	08	09
4_g	09	10	10	11	09	11	10	12	09	11
4_h	10	14	12	18	11	14	12	16	11	18
4_i	09	10	11	13	08	10	09	10	09	11
4_j	10	13	09	12	11	15	10	18	12	19
Ampicillin	18	23	17	25	17	22	20	26	19	28

ANTIFUNGAL ACTIVITY:

TABLE NO: 2

Compound No	*Zone of inhibition (in mm)*					
	Quantity in µg/ml					
	A. niger		***C. albicans***		***R. oryzae***	
	50	**100**	**50**	**100**	**50**	**100**
4_a	11	14	12	20	16	19
4_b	10	13	09	12	11	15
4_c	10	12	14	16	12	13
4_d	10	13	09	12	11	15
4_e	08	10	08	10	10	11
4_f	10	11	09	12	12	13
4_g	07	08	09	10	06	08
4_h	10	14	11	15	12	13
4_i	07	08	09	10	06	08
4_j	09	12	10	14	11	13
Fluconazole	18	26	24	28	22	27

From the above results it is evident that all the synthesized compounds showed antibacterial and antifungal activities at both 50µg (0.05 ml) and 100µg (0.1 ml) dose levels but less than that of the ampicillin and fluconazole used as standards for antibacterial and antifungal activities respectively. Among the compounds tested, $4_{a,c,d,h}$ were found to be more potent antibacterial compounds and $4_{a,b,h,i,j}$ exhibited the highest antifungal activity. However, in particular pyrazoline containing fluoro (4_a) substitution at para position on phenyl ring enhanced both the antibacterial and antifungal activities.

The standard drugs used were Ampicillin and Fluconazole for antibacterial and antifungal activity respectively.

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