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MICROWAVE SYNTHESIS AND EVALUATION OF ANTIMICROBIAL ACTIVITY OF NOVEL 3, 5 DISUBSTITUTED 2-PYRAZOLINES

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Abstract:
2- Pyrazolines exhibiting various biological activities like antioxidant, antidepressant, anti-inflammatory, antimicrobial, antitumor and antitubercular drugs. This leads to synthesize 2-pyrazolines by the condensation of various substituted chalcones of 2-Acetyl pyridine and hydrazine hydrate in the presence of ethanol. The structure of synthesized molecules was confirmed on the basis of Physical data and Spectral studies. All 7 Compounds have been screened for antimicrobial activity. The results indicated that some of compounds show good antibacterial and antifungal activity.

Keywords: Pyrazolines, Chalcones, Microwave synthesis, Antimicrobial activity, Antifungal activity.

1. Introduction:

Heterocycles containing Nitrogen, Sulphur and Oxygen have been under investigation since from long time because of their important biological activities. Also, 2-pyrazolines have been reported to possess a variety of significant and diverse pharmacological activities such as antibacterial,[i-iv] antifungal,[v,vi] antiviral,[vii] antitubercular,[viii,ix] antidepressant,[x,xi] antiamoebic,[xii,xiii] anti-inflammatory,[xiv] anticonvulsant,[xv] analgesic,[xvi] anticancer[xvii] and antioxidant activities.

2-Pyrazolines seem to be the most frequently studied compounds. After pioneering work of Fischer and Knoevenagel in the late 19th century, the reaction of α, β unsaturated aldehydes and ketones to synthesize chalcones and then reaction with hydrazine become one of the most popular methods for the preparation of 2-pyrazolines. Pyrazolines have potential antimicrobial activity. As a result, numerous substituted 2-Pyrazoline have been synthesized. Pyridine derivatives are known to be associated with multiple biological activities. Therefore, both the pyrazoline and pyridine possess promising biological activities. In light of these finding, it was felt worthwhile to synthesize some new pyrazoline derivatives containing pyridine ring and evaluate for their antimicrobial activity.

So the current paper deals with the synthesis of novel 2-pyrazoline and screening of their antimicrobial activity.

2. Experimental

2.1 Materials and methods–

All the chemicals used in the synthesis were obtained from standard commercial sources. The melting point were determined by open capillaries, using Thiels tube and are uncorrected. Reaction were monitored by TLC using Silica gel-G (Merck grade)as the absorbent and the solvent systems are indicated at appropriate places. The 1HNMR spectra of the compounds were recorded on Bruker AMX 400 MHz spectrophotometer using TMS as an internal standard.

2.2 General Procedure for synthesis of Chalcone derivatives from 2-Acetyl Pyridine (Fig.1)

A mixture of 2-Acetyl Pyridine (0.01mol) and appropriate aldehydes (0.01mol) was dissolved in minimum quantity of alcohol. The reaction mixture was heated at about 60^0C and then 40 % NaOH added slowly with constant stirring about 45 minutes. Cool the reaction mixture and add dil. HCl, Filter the product and recrystallized by ethanol. Melting point is determined by open capillary method. The purity of each compound was checked by TLC using n-Hexane and Ethyl acetate (6:4).

2.2 General Procedure for Microwave synthesis of 3(pyridine-2-yl)-5-Phynyl-2-Pyrazoline derivatives(Fig.1)

A mixture of Chalcone (0.01mol) and Hydrazine hydrate (0.02 mol) in ethanol(20 ml) was irradiated under Microwave oven at 600 watt at time 3-4 minutes. Then pour the reaction mixture on crushed ice, the solid obtained. Melting Point of product was determined. The purity of compounds was checked by TLC using n-Hexane and Ethyl acetate (6:4).

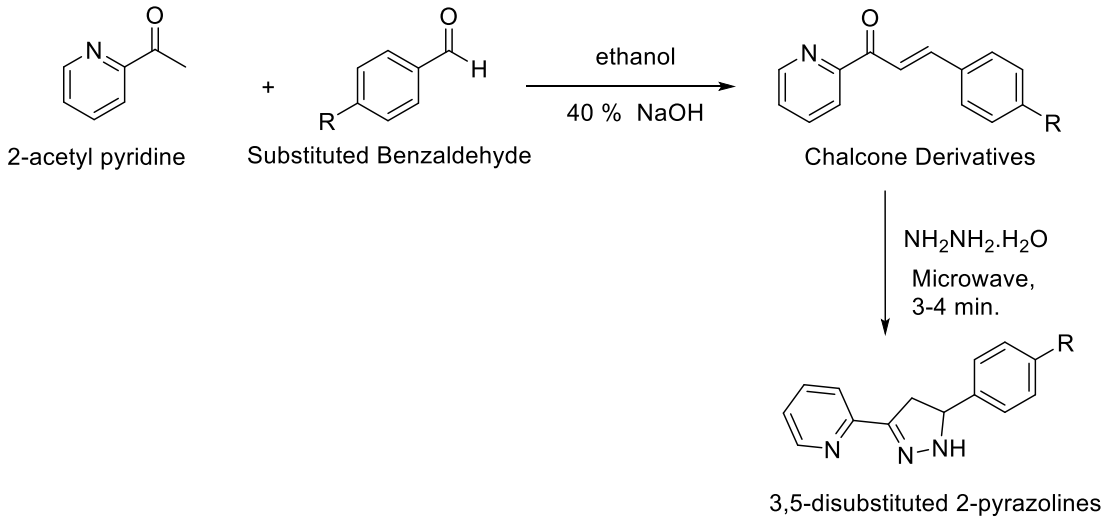

Fig. 1 Schematic synthesis of 3.5-disubstituted -2-pyrazolines

2.3 Detection Method:

All synthesized pyrazolines (2q-2x) were detected by their Physical data and Spectral analysis as shown below.

3(Pyridine-2-yl)-5-(4-Chloro phenyl)-2-Pyrazoline (2q)

Molecular Formula $C_{13}H_{11}N_2OCl$, Molecular weight 246.5, % Yield =85, M.P. = 135^0C

FT-IR (KBr disc): 3331 (Ar O–H); 3080, 3032 (Ar–H); 2956, 2926,2879 (C–H); 1645 (C,N); 1599, 1568, 1514, 1456 (ArC,C); 1203 (Ar C–OH),

1H-NMR: 3.29 (dd,1H,CH2(Pyraz)), 5.17 (dd,1H, CHPyraz), 6.44(d,1H, $CH_{pyridyl}$), 7.45-8.00 (dd,4H, Ar-H), 10,2(s,1H,N-H)

3(Pyridine-2-yl)-5-(4-Bromo phenyl)-2-Pyrazoline (2s)
Molecular Formula$C_{13}H_{11}N_2OBr$, Molecular weight 291g, % Yield =90 , M.P. = 170^0C
FT-IR (KBr disc):3320 (Pyrazoline N-H), 1593(ring C=N),1564(ring C=C), 1483(ring N-N), 1126(C-N), 1063(C-S-C), 803(C-Br)
^{1}H-NMR: 3.28 (dd,1H,CH2(Pyraz)), 5.16 (dd,1H, CHPyraz), 6.43(d,1H, $CH_{pyridyl}$) , 7.40-8.00 (dd,4H, Ar-H), 10.3(s,1H,N-H)

3(Pyridine-2-yl)-5-(4-Fluoro phenyl)-2-Pyrazoline (2t)
Molecular Formula$C_{13}H_{11}N_2OF$, Molecular weight 229, % Yield =80, M.P. = 200^0C
FT-IR (KBr disc): 3307 (Pyrazoline N-H),) 1590(ring C=N),1563(ring C=C), 1480(ring N-N), 1122(C-N), 1065(C-S-C), 1110(C-F)
^{1}H-NMR: 3.23 (dd,1H,CH2(Pyraz)), 5.15 (dd,1H, CHPyraz), 6.37(d,1H, $CH_{pyridyl}$) , 7.45-8.00 (dd,4H, Ar-H), 10,2(s,1H,N-H)

3(Pyridine-2-yl)-5-(4-methoxy phenyl)-2-Pyrazoline (2u)
Molecular Formula $C_{14}H_{14}O_2N_2$, Molecular weight 242, % Yield =140, M.P. = 140^0C
FT-IR (KBrdisc): 3315 (Pyrazoline N-H), 1595(ring C=N),1566(ring C=C), 1487(ring N-N), 1125(C-N), 1067(C-S-C), 1158(C-O)
^{1}H-NMR:3.29 (dd,1H,CH2(Pyraz)), 3.75(s,3H, OCH_3) 5.17 (dd,1H, CHPyraz), 6.30(d,1H, $CH_{pyridyl}$) , 7.45-8.00 (dd,4H, Ar-H), 10,2(s,1H,N-H),

3(Pyridine-2-yl)-5-(4-ethoxy phenyl)-2-Pyrazoline (2v)
Molecular Formula $C_{15}H_{16}O_2N_2$, Molecular weight 256, % Yield =78, M.P. = 160^0C
FT-IR (KBrdisc): 3315 (Pyrazoline N-H), 1595(ring C=N),1566(ring C=C), 1487(ring N-N), 1125(C-N), 1067(C-S-C), 1158(C-O)
^{1}H-NMR: 3.29 (dd,1H,CH2(Pyraz)), 3.75(s,3H, OCH_3) 5.17 (dd,1H, CHPyraz), 6.30(d,1H, $CH_{pyridyl}$) , 7.45-8.00 (dd,4H, Ar-H), 10,2(s,1H,N-H),

3(Pyridine-2-yl)-5-(4-Nitro phenyl)-2-Pyrazoline (2w)
Molecular Formula C13H11N3O3, Molecular weight 257, % Yield =90, M.P. = 240^0C
FT-IR (KBrdisc): 3331 (Ar O–H); 3080, 3032 (Ar–H); 2956, 2926,2879 (C–H); 1645 (C,N); 1599, 1568, 1514, 1456 (ArC,C); 1203 (Ar C–OH),
1H-NMR:
2.80–2.74 (dd, 1H,CHHa), 3.40–3.29 (dd, 1H, CHbH), 3.78 (s, 3H, OCH3), 4.73(t, 1H, CHAr), 6.75 (d, 1H, NH), 7.23–6.95 (m, 7H, Ar), 9.2(s, 1H, ArOH).

3(Pyridine-2-yl)-5-(4-methyl phenyl)-2-Pyrazoline (2x)
Molecular Formula $C_{14}H_{14}N_2O$, Molecular weight 226, % Yield =79 , M.P. = 210^0C
FT-IR (KBr disc): 3312 (Pyrazoline N-H), 1594(ring C=N),1543(ring C=C), 1480(ring N-N), 1122(C-N), 1065(C-S-C), 1150(C-O)
^{1}H-NMR: 3.29 (dd,1H,CH2(Pyraz)), 3.75(s,3H, OCH_3) 5.18 (dd,1H, CHPyraz), 6.40(d,1H, $CH_{pyridyl}$) , 7.45-8.00 (dd,4H, Ar-H),

2.4 Evaluation of Antimicrobial Activity –

Faecalis, E. Coli, Pseudomonas Fluorescenes. The agar medium and peptone water was done as per the standard procedure. Each test compound (5mg) was dissolved in 5 ml of DMSO. Cup Plate Method using Muller-Hinton agar medium was employed to study the preliminary antibacterial activity of compound (2q-2x) against Staphylococcus aureus, S. Faecalis, E. Coli, Pseudomonas Fluorescenes. OFLOXACIN (20mg) was employed as a reference standard drug to compare the results. The pH of all the solutions and control was maintained at 2-3 by using Conc. HCl, because the compounds were not diffused through agar medium at below 3. All the compounds were tested at a Conc. of 0.05ml. DMSO as control did not show any inhibition.
Same Cup plate method using PDA (Potato-Dextrose-Agar) medium was employed to study the preliminary antifungal activity of compound (2q-2x) against Trichophyton rubrum and

Candida albicans. Preparation of nutrient broth, subculture, base layer medium and PDA medium was done as per standard procedure. Each test compound (5mg) was dissolved in 5ml of DMSO. OFLAXACIN (20mg) was employed as reference standard to compare the results. The pH of all the test solutions and control was maintained at 2-3 by using Conc. HCl, because the compounds were not diffused through Agar medium at pH below 3. All the compounds were tested at a Conc. of 0.05ml. DMSO as control did not show any inhibition.
The cups of 7mm diameter were made by scooping out medium with a sterilized cork borer in a petridish, which was streaked with the organisms. The solution of each test compound, control and reference standards were added separately in the cups and petridishes were subsequently incubated at 37^0C for 24 hours for antibacterial activity and kept aside at room temperature for 48 hr for antifungal activity. Zone of inhibition produced by each compound was measured in mm and the results are presented in Table 1 for antibacterial activity and Table 2 for antifungal activity.

Table 1: Antibacterial Activity of 2-Pyrazoline (2q-2x)

Sr. No.	Compound	Zone of inhibition in mm			
		S. aureus	S. faecalis	E. coli	P. fluorescenes
1	2q	20	16	23	18
2	2s	--	--	--	--
3	2t	14	--	18	--
4	2u	20	13	--	14
5	2v	18	12	--	13
6	2w	--	--	--	--
7	2x	--	--	--	--
8	Control DMSO	--	--	--	--
9	Reference OFlOXACIN	20	10	10	12

Table 2: Antifungal Activity of 2-Pyrazoline (2q-2x)

Sr. No.	Compound	Zone of inhibition in mm	
		Trichophyton rubrum	Candida albicans
1	2q	20	14
2	2s	--	12
3	2t	--	12
4	2u	--	13
5	2v	--	12
6	2w	--	--
7	2x	--	--
8	Control DMSO	10 mm	8
9	Reference OFlOXACIN	14 mm	--

2.5 Result and Discussion –

The results of antibacterial activity of novel 2-Pyrazoline derivatives (2q-2X) indicated that some compounds have some degree of inhibitory activity on all the bacteria when

compared with reference std. OFLOXACIN. Among all the compounds tested , compound 2q,2u,2t produced maximum inhibition zones.

The antifungal data of 2-Pyrazoline derivatives (2q-2x) revealed that some compounds have some degree of inhibitory activity on all fungi when compared with reference standard OFLOXACIN. Among all the compounds tested compound 2q, 2s, 2t, 2u produced maximum inhibitory zones. The results clearly revealed the contribution of electron withdrawing groups and electron releasing groups on the aromatic ring enhancing the antibacterial and antifungal activity.

2.6 Conclusion -

We reported the synthesis and evaluation of antimicrobial activity of 3,5-disubstituted-2-pyrazoline analogs. The results were almost comparable to standard Ofloxacin. The findings are highly significant. It is proposed to use of electron withdrawing groups in the 3,5 – disubstituted-2-pyrazoline analogs if aimed to be having a potential antimicrobial agent.

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