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SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME NEW PYRAZOLINES

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ABSTRACT
Pyrazoline is dihydropyrazole, a five member heterocyclic compound containing two nitrogen atoms in adjacent positions and possessing only one double bond. The compound of six different Pyrazoline derivatives were synthesized by cyclisation of substituted chalcone derivatives in the presence of 2, 4 dintro phenyl hydrazine hydrate. All synthesized compounds were characterized by spectral analysis. All synthesized compounds screened for their antimicrobial activities.

KEYWORDS: Phenothiazines 2,4 dinitro phenyl hydrazine hydrate, anti microbial activity

INTRODUCTION

Pyrazole is a π-excessive five membered heterocyclic system and possess N-hetero aromatic character, contains two nitrogen atoms. Pyrazole is a colourless solid, melting point 70^0 C and crystallizes in long needles. Pyrazoles exhibit aromatic character with properties resembling both pyrrole and pyridine. Pyrazolines are important nitrogen-containing five membered heterocyclic compounds. 1-pyrazoline, 2-pyrazoline and 3-pyrazoline are the three partially reduced forms of pyrazole structure with the different positions of the double bonds. 2-pyrazoline (1) exhibits monoimino character and hence more stable than the rest eventhough all the three types have been synthesized.

Experimental

General procedure for Synthesis of pyrazolines

Chalcone (0.001 mol) was dissolved in methanol/ethanol (20 ml) and hydrazine hydrate (0.1 ml) was added to it. To this mixture triethylamine (0.3 ml) was added drop wise at room temperature. 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 recrystallised from chloroform. Physical characterization and spectral data of the synthesized compounds were given in table 1 and 2.

Table 1: Physical characterization data of the synthesized compound.

Molecular formula	$C_{21}H_{17}N_3S$
Molecular weight	343.44
Melting point (^{0}C)	163.4
Yield (%)	90
R_f	0.64 (20% EtOAc/Hexane)

Table 2: Spectral data of the synthesized compound.

IR (KBr)	
Type Of Vibration	Wave Number (cm^{-1})
C=N	1550
N-H	3327
C-N	1331

Antibacterial activity

The antibacterial activity was tested by *cup-plate method*. The following organisms were used. Test organisms:
Gram positive bacteria: *Staphylococcus aureus and Bacillus pumilis*
Gram negative bacteria:*Pseudomonas aeruginosa*

Experimental Procedure

The test organisms were subcultured using nutrient agar medium. The tubes containing sterilized medium were inoculated with the respective bacterial strain. After incubation at 37^0C ±1^0C for 18 hours, they were stored in a refrigerator. Nutrient agar (Hi-media) was dissolved and distributed in 25 ml quantities in 100 ml conical flasks and were sterilized in an autoclave at 121^oc(151lbs/sq.in) for 20 minutes. The medium was inoculatd at one percent level using 18 hrs old cultures of

the test organism mentioned above aseptically into sterile petri dishes and allowed to set at room temperature for about 30 minutes. In a size of 4 inches petridishes, twelve cups of 8mm diameter at equal distance were made in each plate. In each plate, one cup was used for control i.e. Dimethyl sulfoxide (DMSO), other for standard chloramphenicol with 100 µg/ml and other ten cups with concentrations of test compound i.e. 50 µl solutions.

The plates thus prepared were left for 90 minutes in refrigerator for diffusion. After incubation for 24 hrs at $37^{o}c \pm 1^{o}c$, the plates were examined for inhibition zones. The experiments were performed in triplicate and the average diameters of the zone of inhibition measured were recorded. There is no zone of inhibition for control. The results are presented in **Table 3.**

Table 3: Antibacterial activity of 2-Pyrazolines.

Compound Code	Zone Of Inhibition (in mm)		
	S.aureus	***B.pumilis***	***P.aeruginosa***
	(50µl)	**(50µl)**	**(50µl)**
Standard(Chloramphenicol)	35	48	40
P1	-	10	-
P2	-	13	-
P3	-	12	-
P4	-	13	-
P5	-	15	-
P6	-	15	-

COCLUSION

Theory and detailed experimental work involved in the evaluation of antimicrobial studies were described. From the results it was observed that 2-Pyrazolines showed significant antibacterial activity.

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