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Synthesis of certain Pyrazoline 5-One Derivatives of 6-Methyl Nicotinicacid and Evaluation of their Antimicrobial activities

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ABSTRACT

Pyrazolines are known to possess a wide variety of biological activities. Due to the wide range of biological activities that Pyrazolines posses, it was our aim to prepare pyrazolin-5-one derivatives containing substituted Nicotinic acid and to explore, their therapeutic advantage. Among the six newly synthesized pyrazoline-5-one derivatives MPZ-1 showed promising antimicrobial activity even at a concentration 250mcg/disc. and MPZ-2 showed moderate antimicrobial activity at a concentration 500mcg/disc.

Keywords: *Pyrazoline-5- one derivatives, substituted Nicotinic acid, Antibacterial, antifungal.*

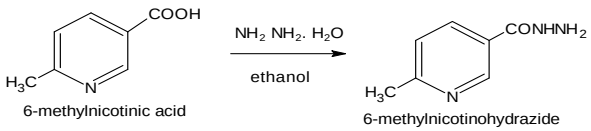
INTRODUCTION

Pyrazolines are known to possess a wide variety of biological activities. Due to the wide range of biological activities that Pyrazolines posses, it was our aim to prepare pyrazolin-5-one derivatives and to explore, their therapeutic advantage, when incorporated with other aromatic system in a molecular framework. The investigation that was carried out during the course of work was divided into 3 parts, Synthesis of pyrazolin-5-one derivatives by condensing unsaturated ester of diazotized anilines with substituted nicotinohydrazide, Antimicrobial screening of the synthesized pyrazolin-5-one derivatives.

MATERIALS AND METHODS

Preparation of 6-Methylnicotinohydrazide: [1]

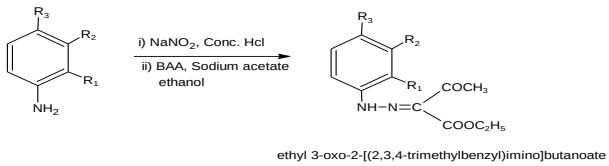
A mixture of 6-methyl nicotinic acid (0.01mol)and hydrazine hydrate (0.01mol) was placed in a round bottom flask fitted with a reflux condenser and the mixture was heated gently under reflux for 15 minutes. Then sufficient quantity of ethanol was added to give a clear solution. The mixture was refluxed for a further 2-3 hours. And then the mixture was concentrated to half its volume by distillation. The crystals of 6-Methylnicotinohydrazide are filtered and recrystalized from an aqueous ethanolic solution. The purity of the compound was established by single spot on TLC. Reaction given in the **Scheme-1**.



Scheme 1: Preparation of 6-Methyl Nicotinohydrazide

Diazotization: [2, 3]

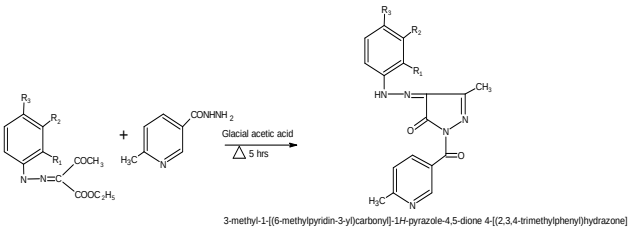
Substituted aromatic amines (0.01mol) were dissolved in a mixture of 40 ml of hydrochloric acid (8ml) and water (6ml) then cooled to 0°c in ice water. And a cold aqueous solution of sodium nitrite (0.03mol)was added. The diazotization salt solution was filtered directly in to the cold solution of ethylacetoacetate (0.01mol) and sodium acetate (0.122mol) in 50 ml of ethanol. The resultant solid was filterd, washed with water then recrystallized from ethanol. The purity of the compound was established by single spot on TLC. Rection given in the **Scheme-2**.



Scheme 2: Diazotization

Cyclization: [4]

A mixture of diazotizedand estrified aromatic substituted amines (0.002mol) and the 6-methyl nicotinohydrazide (0.002mol)in glacial acetic acid were refluxed for 5 hours, cooled and then allowed to stand overnight. The product was precipitated by addition of cold water. The resultant solid separated by filtration was dried and recrystallized from ethanol. The purity of the compound was established by a single spot on a TLC plate. Reactions given in the **Scheme-3**.



Scheme 3: Cyclization

Anti Microbial Screening: [5, 6]

Screening for Antibacterial Activity:

Kirby-Bauer method:

Mueller Hinton agar plates were prepared aseptically .The plates were dried at 37°C before inoculation. The organisms were inoculated in the plates prepared. It was allowed to dry at room temperature, with the lid closed. The sterile disc containing test drugs, standard and blank were placed on the previously inoculated surface of the Mueller Hinton agar plate and it was kept in the refrigerator for one hour to facilitate uniform diffusion of the drug. Plates were prepared in triplicate and they were then incubated for 18-24 hrs at37°C. Observations were made for zone of inhibition around the discs containing the drugs and compared with that of standard. Results were tabulated in the **Table No. 1 & 2**.

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Table No. 1: Antibacterial Activity against Gram Positive Organisms

S. No.	Compound Code*	Diameter of Zone of Inhibition in mm			
		Staphylococcus aureus		*Bacillus subtilis*	
		500µg/disc	250µg/disc	500µg/disc	250µg/disc
1	MPZ -1	20	17	18	16
2	MPZ -2	14	-	13	-
3	MPZ -3	-	-	-	-
4	MPZ -4	-	-	-	-
5	MPZ -5	-	-	-	-
6	MPZ -6	-	-	-	-
	DMF (Blank)	-	-	-	-
	Standard (Ciprofloxacin) 10 µg/disc	24	24	24	24

Table No. 2: Antibacterial Activity against Gram Negative Organisms

S. No.	Compound Code*	Diameter of Zone of Inhibition in mm			
		Escherichia coli		*Pseudomonas aeruginosa*	
		500µg/disc	250µg/disc	500µg/disc	250µg/disc
1	MPZ-1	19	14	14	12
2	MPZ -2	14	-	14	-
3	MPZ -3	-	-	-	-
4	MPZ -4	-	-	-	-
5	MPZ -5	-	-	-	-
6	MPZ -6	-	-	-	-
	DMF (Blank)	-	-	-	-
	Standard (Ciprofloxacin) 10 µg/disc	24		24	

Antifungal Screening:

Sabouraud dextrose agar plates were prepared aseptically to get a thickness of 5-6mm. The organisms Candida albicans and Aspergillus niger was inoculated in the respective plates prepared earlier by dipping sterile swab in the inoculum, removing the excess of inoculum by pressing and rotating the swab firmly against the sides of the culture tube above the level of the liquid and finally streaking the swab all over the surface of the medium three times, rotating the plates through the 60°C after each application. Finally the swab was pressed around the edges of the agar surface. The inoculum was left to dry at room temperature with lid closed. Sterile discs containing the test, standard and blank were placed in the petridish aseptically. 10mcl/disc of the different drug concentrations were used. The Petri dishes were incubated at 25°C for 24-48 hours. Observations were made for the zone of inhibition around the discs containing the drugs and compared with that of Fluconazole. All the compounds were tested for antifungal activity. . Results were given in the **Table No. 3**.

Table No. 3: Antifungal Activity

S. No.	Compound Code*	Diameter of Zone of Inhibition in mm			
		Candida albicans		*Aspergillus Niger*	
		500 g/disc	250 g/disc	500 g/disc	250 g/disc
1	MPZ-1	16	14	16	14
2	MPZ-2	14	-	14	-
3	MPZ-3	-	-	-	-
4	MPZ-4	-	-	-	-
5	MPZ-5	-	-	-	-
6	MPZ6	-	-	-	-
	DMF (Blank)	-	-		-
	Standard (Fluconazole) 10 mcg/disc	18	18	18	18

RESULTS AND DISCUSSION

Six new pyrazolin-5-one derivatives were prepared by condensing unsaturated ester of diazotized anilines with 6-methyl nicotinohydrazide was carried out and structure of the newly synthesized compounds were confirmed by by their elemental analysis, Infrared spectra (IR), Nuclear magnetic resonance (NMR) and Mass spectra.Among the derivatives synthesized MPZ-1showed promising antibacterial activity against gram positive microorganisms namely *staphylococcus aureus* and *bacillus subtilis,* gram negative organisms *Eschrichia coli* and *pseudomonas aeruginosa* and antifungal activity against *candida albicans* and *aspergillus Niger* even at 250mcg/disc. MPZ -2 showed moderate antimicrobial activity at a concentration 500mcg/disc.

CONCLUSION

Among the six newly synthesized compounds MPZ -1 showed promising antimicrobial activity. MPZ -2 showed moderate antimicrobial activity at a concentration 500mcg/disc.

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