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RESEARCH ARTICLE

SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL SCREENING OF NOVEL PYRAZOLONE DERIVATIVES

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ABSTRACT

Pyrazolones, versatile nitrogen containing heterocyclic compounds displaying broad spectrum of biological and pharmacological activities. The main objective of the present study was to explore newer molecules with potent biological activity like antimicrobial activity. 5-methyl-2-(pyridin-4-ylcarbonyl)-2,4-dihydro-3*H*-pyrazol-3-one were synthesized by the cyclization reaction of pyridine-4-carbohydrazide and ethyl acetoacetate in the presence of ethanol, which on treatment with different amines and formaldehyde to give mannich bases. The structure of newly synthesised compounds were characterised by IR, ^{1}H NMR and MASS spectral analysis. Compounds were screened for antimicrobial activity against strains of bacteria such as Staphylococcus aureus, Escherichia coli, Psuedomonus aurenginosa and fungal strains of Pencillium chrysogenum and Aspergillus niger. All the compounds exhibited good antimicrobial activity.

KEYWORDS: 5-methyl-2-(pyridin-4-ylcarbonyl)-2,4-dihydro-3*H*-pyrazol-3-one,Mannich bases, cyclization, Antimicrobial activity,

INTRODUCTION:

Medicinal chemistry is the application of chemical research techniques to the synthesis of pharmaceuticals [I, II]. Heterocyclic compounds are considered as the most promising molecules for the design of new drugs [III]. Cycloaddition reaction is an efficient synthetic tool for constructing biologically potent five membered heterocyclic compounds [IV]. Pyrazolone compounds refer to the class of compounds characterized by 5- membered ring structure composed of three carbon and two nitrogen atoms in the adjacent position, out of which one is basic nitrogen and other is neutral nitrogen [V]. The aromatic nature these compounds arises from the four electrons and the unshared pair of electrons on the NH nitrogen. Pyrazolone compounds also have complex formation property [VI]. They can coordinate to metal ions via carbonyl oxygen atom and may be considered as good oxygen donor ligands [VII]. Pyrazolone derivatives shows different pharmacological activities like antimicrobial, anti-inflammatory,analgesic,antidepressent, anticonvulsant, antidiabetic, antihyperlipidemic, antiviral, antitubercular, antioxidant, anticancer activities etc[VIII]. Mannich bases of pyrazolone also have been reported as potential biological agents [IX]. This works focused on the antimicrobial screening of mannich bases of pyrazolone, which could furnish better therapeutic results.

MATERIALS AND METHODS:

All the chemicals and reagents used in the research work were of analytical grade or synthetic grade. Melting point were determined by melting point apparatus (KHERA) and TLC plates were prepared by using silica gel G. Spots were visualised by exposure to iodine vapour or UV light. IR spectra of the synthesized compounds were recorded using FTIR in the range of 3500-500cm^{-1} on a Perkin Elmer FT-IR Spectrophotometer. ^{1}H NMR spectra were measured with a Bruker Spectrophotometer [500 MHz] in DMSO using TMS as an internal standard. Mass spectra were obtained with LC-MSD Trap- SL 2010 A-Shimadzu.

Experimental section

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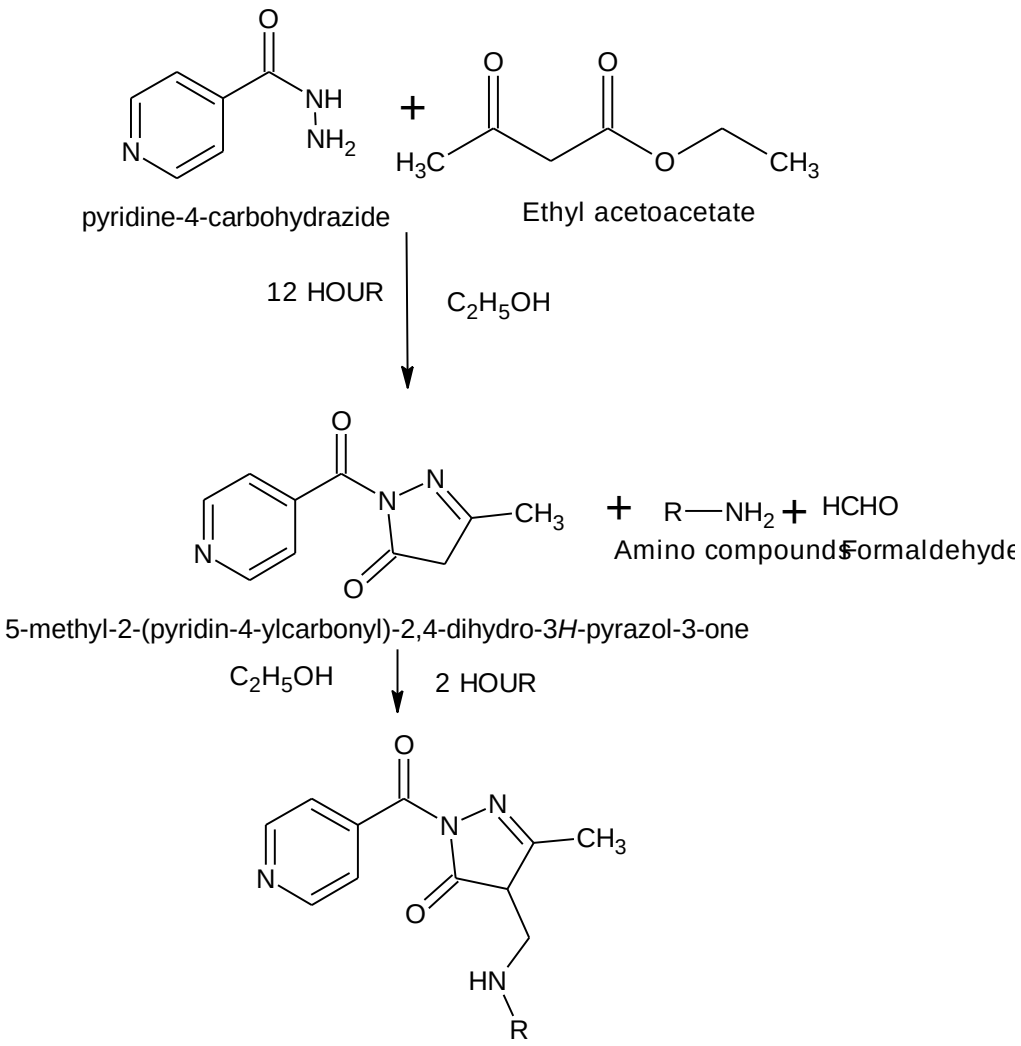
SCHEME OF SYNTHESIS



Mannich bases of Pyrazolone

SYNTHETIC PROCEDURE:

STEP 1: Synthesis of 5-methyl-2-(pyridin-4-ylcarbonyl)-2, 4-dihydro-3*H*-pyrazol-3-one (PZI)

A mixture of pyridine-4-carbohydrazide (isoniazid) 1.37g (0.01 mol) and ethyl acetoaceate 1.30g (0.01 mol) were taken in absolute ethanol (30 ml) and reflux for 12 hours. After completion of reaction, excess of solvents was distilled off and the resulted residue was poured on crushed ice to obtain the light yellow/cream needle shaped crystals. Solid precipitate was filtered and recrystallized using ethanol.

STEP 2: Synthesis of Pyrazolone derivatives

Synthesis of 4-{[(4-acetylphenyl) amino] methyl}-5-methyl-2-(pyridin-4-ylcarbonyl)-2, 4- dihydro-3H-pyrazol-3-one (PZI 1)

Placed 0.01 mol of 4-Amino acetophenone, 5ml formaldehyde and 0.01 mol of compound 1 in a 250 ml round bottom flask attached to a reflux condenser. Introduced 25 ml of 95% ethanol to which 0.5 ml of concentrated hydrochloric acid had been added, and refluxed the mixture on a water bath for 2 hours; the reaction mixture ultimately almost clear and homogenous. Filtered the yellowish solution into a conical flask and while still warm, add 20 ml of acetone. Allowed to cool to room temperature and left in a refrigerator overnight. Filtered the crystal and washed with 10 ml of acetone and dried for 6 hours at 40-50^{0}c. Recrystallized the crude product by using ethanol.

IRpeaks(cm$^{-1)}$-3430.55(N-Hstr),2971(C-Hstr),1660(C=O),1549.56(C=N),1494(C=C), 1302.38(C-), 687(C-H bending).^{1}H NMR(ppm)- 7.6-8.6(4H

Pyridine),6.6(2H Ar),4.4(1H CH),4.5(1H NH),2.5(1H CH_3).m/z 350.10(M^+)

Synthesis of 2-({[3-Methyl-5-oxo-1-(pyridin-4-ylcarbonyl)-4, 5-dihydro-1H-pyrazol-4-yl] methyl} amino) benzoic acid (PZI 2)

Placed 0.01 mol of anthranillic acid, 5ml formaldehyde and 0.01 mol of compound 1 in a 250 ml round bottom flask attached to a reflux condenser. Introduced 25 ml of 95% ethanol to which 0.5 ml of concentrated hydrochloric acid had been added, and refluxed the mixture on a water bath for 2 hours; the reaction mixture ultimately almost clear and homogenous. Filtered the yellowish solution into a conical flask and while still warm, add 20 ml of acetone. Allowed to cool to room temperature and left in a refrigerator overnight. Filtered the crystal and washed with 10 ml of acetone and dried for 6 hours at 40-50^{0}c. Recrystallized the crude product by using ethanol.

IRpeaks($cm^{-1)}$-360.89(N-Hstr),2921.31(ArHstr),1582(C=C),1660.50(C=O),1266(C-Cstr), 1423(Ar C-C str),682(C-H bending).^{1}H NMR(ppm)- 8-8.5(2H Pyridine),8.2(1H NH),6.5-7.5(4H (m) Ar),4.2(2H CH_2),3.5 (1H (s) CH).m/z 350(M^+)

Synthesis of 4-({[3-Methyl-5-oxo-1-(pyridin-4-ylcarbonyl)-4, 5-dihydro-1H-pyrazol-4-yl] methyl} amino) benzoic acid (PZI 3)

Placed 0.01 mol of para amino benzoic acid, 5ml formaldehyde and 0.01 mol of compound1 in a 250 ml round bottom flask attached to a reflux condenser. Introduced 25 ml of 95% ethanol to which 0.5 ml of concentrated hydrochloric acid had been added, and refluxed the mixture on a water bath for 2 hours; the reaction mixture ultimately almost clear and homogenous. Filtered the yellowish solution into a conical flask and while still warm, add 20 ml of acetone. Allowed to cool to room temperature and left in a refrigerator overnight. Filtered the crystal and washed with 10 ml of acetone and dried for 6 hours at 40-50^{0}c. Recrystallized the crude product by using ethanol.

IR peaks($cm^{-1)}$- 3430.83(N-H str),3199.58(Ar-CH str),1535.62(C=C),1677.74(C=O),1180(C-N str),667(C-H bending).^{1}HNMR(ppm)-7.5-8.5(4HPyridine),6.5-7.5(2HAr),4.5-5(1H NH),4.4(2H CH_2),3.4 (1H (s) CH).m/z 352(M^+)

Synthesis of 4-({[3-Methyl-5-oxo-1-(pyridin-4-ylcarbonyl)-4, 5-dihydro-1H-pyrazol-4-yl] methyl} amino) benzenesulfonamide (PZI4)

Placed 0.01 mol of sulphanilamide, 5ml formaldehyde and 0.01 mol of compound 1 in a 250 ml round bottom flask attached to a reflux condenser. Introduced 25 ml of 95% ethanol to which 0.5 ml of concentrated hydrochloric acid had been added, and refluxed the mixture on a water bath for 2 hours; the reaction mixture ultimately almost clear and homogenous. Filtered the yellowish solution into a conical flask and while still warm, add 20 ml of acetone. Allowed to cool to room temperature and left in a refrigerator overnight. Filtered the crystal and washed with 10 ml of acetone and dried for 6 hours at 40-50^{0}c. Recrystallized the crude product by using ethanol.

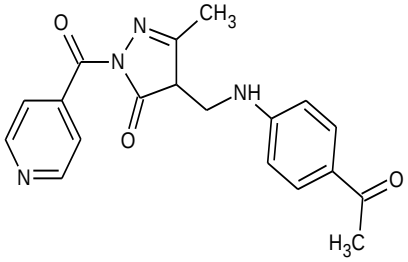
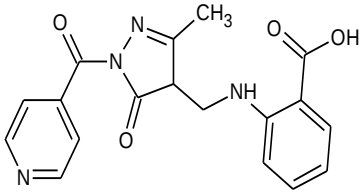
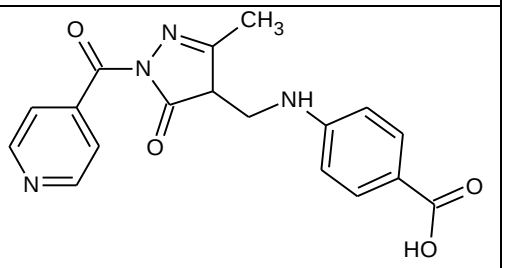
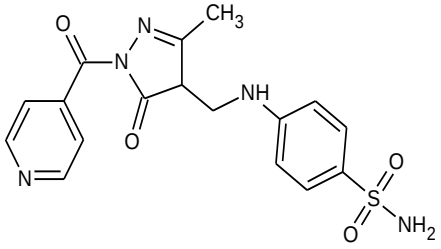
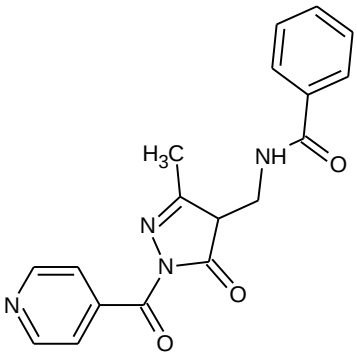
IRpeaks($cm^{-1)}$-3426.07(N-Hstr),2970.70(C-Hstr),1698.67(C=O),1597.42(C=N), 3267.62(Amide),678.19(C-S).^{1}H-NMR(ppm)-7-8(4H Pyridine),6.5-7(4H Ar),5-5.5(1H NH),4.3(2H NH_2),4.4(2H CH_2),3.7(1H (s) CH).m/z 387(M^+)

Synthesis of N-{[3-Methyl-5-oxo-1-(pyridin-4-ylcarbonyl)-4, 5-dihydro-1H-pyrazol-4-yl] methyl} benzamide (PZI 5)

Placed 0.01 mol of benzamide, 5ml formaldehyde and 0.01 mol of compound 1 in a 250 ml round bottom flask attached to a reflux condenser. Introduced 25 ml of 95% ethanol to which 0.5 ml of concentrated hydrochloric acid had been added, and refluxed the mixture on a water bath for 2 hours; the reaction mixture ultimately almost clear and homogenous. Filtered the yellowish solution into a conical flask and while still warm, add 20 ml of acetone. Allowed to cool to room temperature and left in a refrigerator overnight. Filtered the crystal and washed with 10 ml of acetone and dried for 6 hours at 40-50^{0}c. Recrystallized the crude product by using ethanol.

IRpeaks ($cm^{-1)}$-3431.07(N-Hstr), 3198.27(Amide), 2966.43(C-H), 1671.31(C=O), 1416.11(C-C), 1299(C-N), 684(C-H bending). ^{1}H NMR (ppm) - 8-9(2H Pyridine) 7.5-8 (4H Ar), 4.6 (1H NH), 3.4(1H CH). M/z-336($M^{+)}$

Table I: List of derivatives

Compounds	Structure
PZI 1	
PZI 2	
PZI 3	
PZI 4	
PZI 5	

ANTIMICROBIAL ACTIVITY

The synthesised compounds were subjected to antimicrobial activity. Antimicrobial activities were observed for all compounds using strains of bacteria such as Staphylococcus aureus, **(**ATCC 25923), Escherichia coli (ATCC 25922), Psuedomonus aurenginosa (ATCC 27853) and fungal strains of Pencillium chrysogenum (NCIM 3102) and Aspergillus niger(NCIM 596). The antimicrobial

activities of the synthesized compounds were studied by cup plate method. Using a sterile cork borer of about 5mm diameters, wells were made in each petridishes. Standard, control and test were marked on the bottom of the petridishes to identify each cup. Using sterile syringe injected 0.1 ml of standard, control and test into the cups. After injection the petridishes were kept at room temperature for 24 hours for uniform diffusion of the agent to occur in seeded agar medium. The petridishes incubated at 37±0.5^{0}C for 24 hours. To extent the diameter of inhibition after 24 hours was measured as the zone of inhibition in millimeters as compared with standard drug. Ciprofloxacin (100 µg/ml) for bacteria and Ketoconazole (100 µg/ml) for fungi were used as standard. The zone of inhibition was measured in mm to estimate the potency of the test compounds [X].

RESULT AND DISCUSSION:

The preliminary characterization of synthesised compounds [Table II]. The synthesized compounds were characterized through Elemental analysis, IR, ^{1}H NMR and MASS spectra. Compound PZI 1(100 µg/ml) showed maximum activity against these bacterial strains and compounds PZI 2, PZI 4 (100 µg/ml) showed moderate activity against these bacterial strains. The results of antifungal screening revealed that compound PZI 1 (100 µg/ml) showed better activity and compounds PZI 2, PZ1 4(100 µg/ml) showed moderate activity against Aspergillus nigris and Pencillium chrysogenum using ketaconazole (100 µg/ml) as standard[Fig. I, II]. The investigation of antimicrobial screening data revealed that all the tested compounds shown good antimicrobial activity [Table III].

Table II: Preliminary characterization of synthesized compounds

Compounds	Molecular formula	Molecular weight	Melting point	Percentage yield	R_f value
PZI 1	$C_{19}H_{18}N_4O_3$	350.37122	137^{o}C	68	0.66
PZI 2	$C_{18}H_{16}N_4O_4$	352.34404	155^{o}C	74	0.65
PZI 3	$C_{18}H_{16}N_4O_4$	352.34404	134^{o}C	76	0.62
PZI4	$C_{17}H_{17}N_5O_4S$	387.41298	160^{o}C	75	0.67
PZI 5	$C_{18}H_{16}N_4O_3$	336.34464	136^{o}C	69	0.64

Table III: Antimicrobial activity of Pyrazolone derivatives (zone of inhibition in mm)

Compounds	S.aureus	E.coli	P.aeruriginosa	A. niger	P.chrysogenum
PZI 1	20	20	25	21	19
PZI 2	18	16	18	16	14
PZI 3	14	14	15	09	06
PZI4	16	18	19	15	13
PZI5	12	13	12	08	06
Ciprofloxacin	22	21	26	0	0
Ketoconazole	0	0	0	20	18

Standard =ciprofloxacin (100µg/ml) for bacteria; Ketaconazole (100µg/ml) for fungi

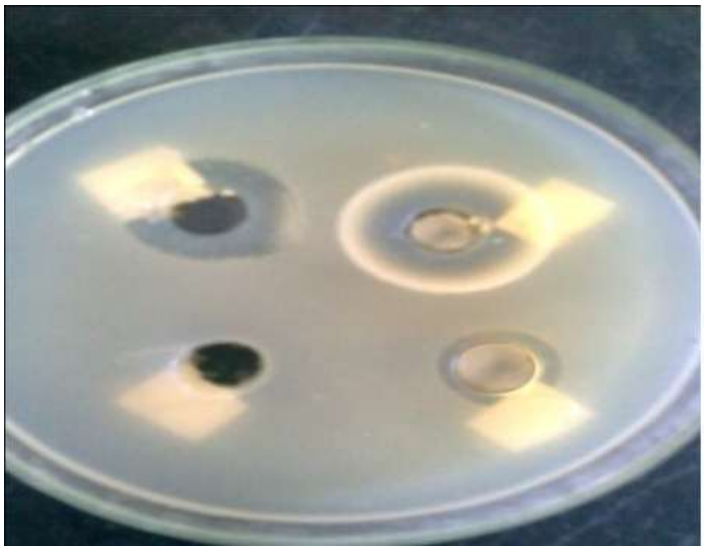



Figure I: **Antibacterial activity against S. aureus Fig.II: Antifungal activity against A. nigris**

CONCLUSION:

The synthesised compounds were subjected to antimicrobial activity. Concentration of 100 µg/ml was screened for antimicrobial activity. Among the synthesized compounds PZI showed good antibacterial and antifungal activity. The order of antimicrobial activity is PZI1>PZI2 > PZI4> PZI > 3 > PZI4.

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