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SYNTHESIS, CHARACTERISATION AND BIOLOGICAL EVALUATION OF SOME PYRAZOLONE DERIVATIVES

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ABSTRACT:
The synthetic part involves the formation of N'-({1-[(4substituted phenyl)carbonyl]-3-methyl-5-oxo-4,5-dihydro-1Hpyrazol-4-yl}methyl)pyridinecarbohydrazide from the substituted acid hydrazide. The structures of the final compounds were established on the basis of IR, 1H-NMR and MASS spectral data. Drug likeness was determined by Molinspiration software program. Parameters related to drug likeness of the derivatives were established on the basis of Lipinski's rule of 5. The molecular properties were calculated from suitable computational tools. All of the derivatives showed a zero violations of the rule of 5 which indicates good bioavailability. The positive bioactivity score of the derivative were also in agreement with their probability of drug likeness. The entire synthetic derivative was evaluated for their antimicrobial studies. Most of the derivatives were showed good activity towards gram positive bacteria and less activity towards gram negative bacteria. Some of the derivatives showed moderate activity against tested fungi.

Keywords: Pyrazolone, Molinspiration, Druglikeness, Anti Microbial studies.

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INTRODUCTION

The main purpose of the present study is to synthesize different derivatives with pyrazolone as basic heterocyclic nucleus condensed with isonicotinic acid hydrazide and screening for their biological activity. Pyrazolone is a five membered lactam ring and is a derivative of pyrazole that has additional keto group. Pyrazolones are important class of heterocyclic compounds that occur in many drugs and is a non-steroidal anti-inflammatory agent used in the treatment of arthritis and other musculoskeletal and joint disorders.

Pyrazolones are biologically important group of compounds having different activities like antibacterial, antifungal, anti-inflammatory, antidiabetic, analgesic, antipyretic, immunosuppressive agents, hypoglycemic, antiviral, antineoplastic activity and other biological activities. This literature has encouraged dealing on pyrazolones. The wide range of biological activities exhibited by pyridine and pyrazolone, it was our aim is to prepare derivatives of incorporated pyridine with pyrazolone ring system in a molecular frame work and to explore the therapeutic advantage of this combination. All the synthesized compounds were characterized by recrystallization, TLC, Melting point, UV, IR, 1HNMR analysis, and Mass fragmentation pattern. All the synthesized structures showed satisfactory result.

Drug likenesses were determined by mol inspiration software program. All of the derivatives showed a zero violations of the rule of 5 which indicates good bioavailability. The entire synthetic derivative was evaluated for their antimicrobial studies. Most of the derivatives were showed good activity towards gram positive bacteria and less activity towards gram negative bacteria. Some of the derivatives showed moderate activity against tested fungi[1,2].

MATERIALS AND METHOD

All the chemicals were of synthetic grade and are procured from S. D. Fine Chemicals Ltd., Jiangsu Huani International Trade Pvt. Ltd., Sisco Research Laboratory Pvt. Ltd., Finar Chemicals Ltd. and Nice Chemicals Pvt. Lt. Melting points were determined by using melting

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point apparatus MP-DS TID 2000 V. Scientific and were uncorrected. Reactions were monitored by thin layer chromatography (TLC) on pre coated silica gel G plates using iodine vapours as visualizing agents.IR spectra were recorded on JASCO FT/IR-140 spectrophotometer .PMR spectra were recorded using BRUCKER FT-NMR-400MHz, IIRBS.

CALCULATION OF MOLECULAR PHYSICOCHEMICAL PROPERTIES:[3-5]

The physiochemical properties involve determination of drug-like property of the synthesized compounds. It is based on Lipinski‟s rule of five and can be determined by using molinspiration cheminformatics software. All the synthesized compounds showed zero violation of Lipinski‟s rule of five, which indicates good bioactivity and bioavailability. The Rule of Lipinski's Rule of Five states that in general, an orally active drug has not more than one violation of the following criteria.

- Not more than 5 hydrogen bond donors (nitrogen or oxygen atoms with one or more hydrogen atoms).
- Not more than 10 hydrogen bond acceptors (nitrogen or oxygen atoms)
- A molecular weight under 500 g/ mol.
- A partition coefficient log P less than 5.
- Not more than 15 rotatable bonds.

Physico-chemical Properties and biological activities discussed in Table no.1 and 2 respectively.

Table 1: Physico-Chemical Properties of compound

COMP	Log P	TPSA	MW	No of hydrogen bond acceptor	No of hydrogen bond donor	Violation	No of rotatable bond	Molar volume
INH I	1.182	103.76	385.81	8	2	0	5	321.98
INH II	0.463	149.58	396.36	10	2	1	6	331.77
INH III	0.561	112.995	381.39	9	2	0	6	333.99
INH IV	0.157	133.22	397.39	10	3	0	6	342.00
INH V	0.439	149.58	396.36	10	2	1	6	337.77

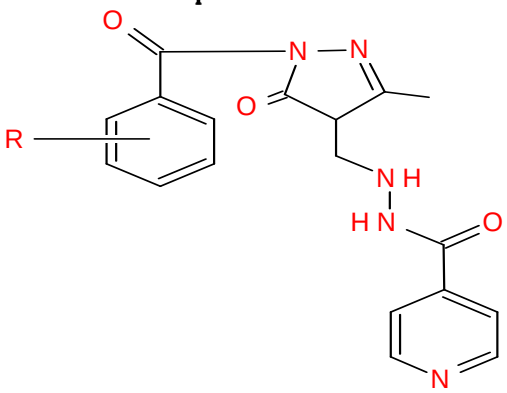
Table 2: Biological Activites

Comp	GPCR	Ion Channel	Kinase Inhibitor	Nuclear Receptor Ligand	Protease Inhibitor	Enzyme Inhibitor
INH I	-0.20	-0.52	-0.20	-0.68	-0.43	-0.29
INH II	-0.33	-0.53	-0.30	-0.71	-0.49	-0.34
INH III	-0.23	-0.56	-0.21	-0.65	-0.43	-0.29
INH IV	-0.21	-0.52	-0.17	-0.59	-0.49	-0.29
INH V	-0.33	-0.54	-0.30	-0.72	-0.50	-0.36SS

Selection of Lead & Lead Optimization

The selection of lead was done by screening various nucleus such as benzthiazole, imidazole, indole, pyridazine, pyrimidine, naphthyridine, pyrazolone etc by using molinspiration software. Molinspiration is a cheminformatic software tool which gives the molecular properties of any chemical structure(for e.g. Log P, polar surface area, number of hydrogen bond donors and acceptor), as well as prediction of bioactivity score for the most important drug targets (GPCR Ligands, Kinase receptors, ion channel modulators, Nuclear receptors) and possible molecular toxicity. Among the various nuclei investigated pyrazolone moiety was found to have good bioactivity score.

Structure of compound



Lead optimization was made by screening different heterocyclic ring systems incorporated with acid hydrazide. pyrazolone showed significant bioactive score when incorporated with acid hydrazide. These choosen compounds were synthesized by simple scheme starting from Benzoic acid derivatives. Due to wide range of biological activities exhibited by pyrazolone an attempt was made to investigate the anti oxidant, antifungal and anti TB, anti inflammatory activities of above designed compounds.

Experimental[6-9]

STEP 1: A weighed quantity of Benzoic acid (substituted) 22.8gm (0.1mole) was dissolved in 50ml of ethanol in a RBF. Add 10ml of Con.H_2SO_4 to the reacting mixture and reflux for 1 ½ hr. The reaction mixture becomes concentrated by distillation. A crushed ice piece was added to the RBF and the solid mass becomes precipitate. It is filtered and dried.

STEP 2: Hydrazinolysis of benzoate (0.01 mol) with 10 ml of 99% hydrazine hydrate carried out with ester in presence of absolute ethanol (50ml) for 5-6 hr. After cooling and removing excess of solvent by distillation, the solid obtained was filtered, dried and recrystallised from ethanol.

STEP 3: A mixture of 0.01 mol of hydrazide and 0.1 mol (13ml) of ethyl acetoacetate were heated on water bath for 2hr with stirring from time to time with a glass rod. The resultant heavy reddish syrup was allowed to cool to room temperature. It was washed thoroughly with ether to remove coloured impurities. The solid

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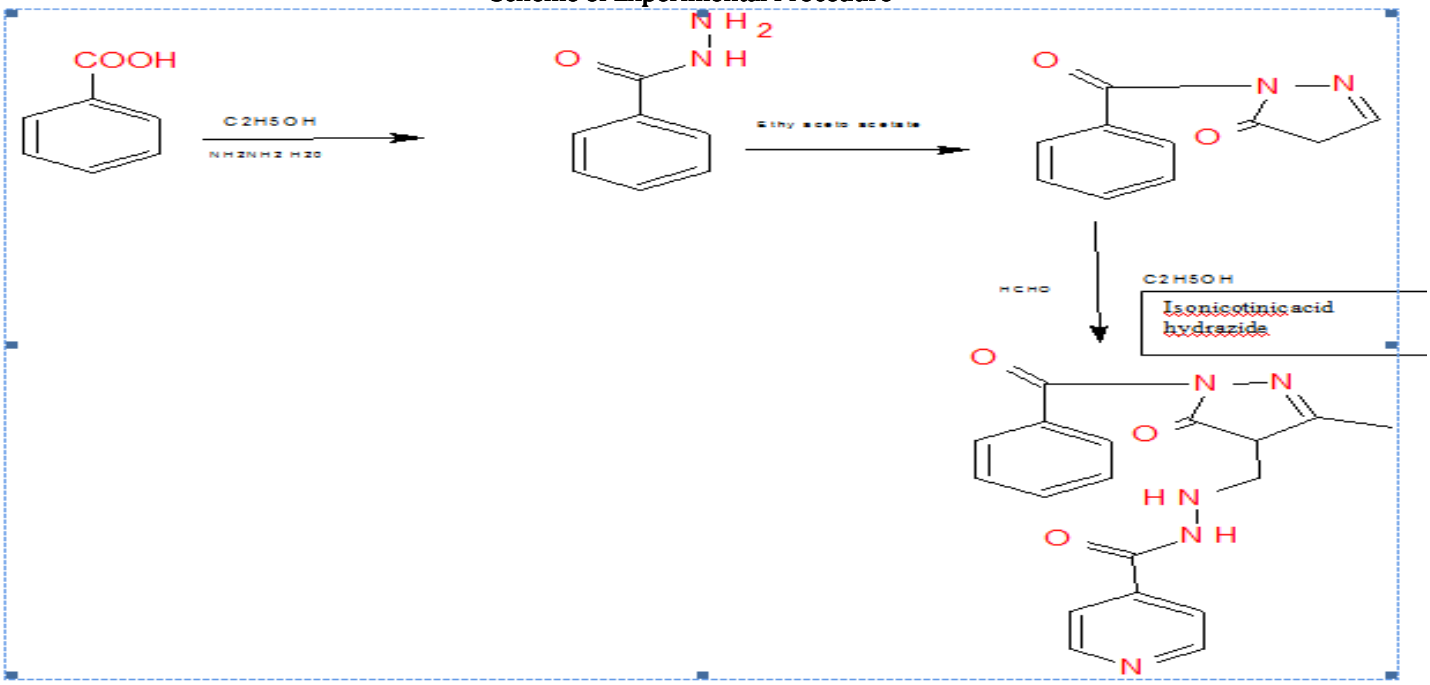
separated out was filtered, dried and purified by recrystallization from ethanol .

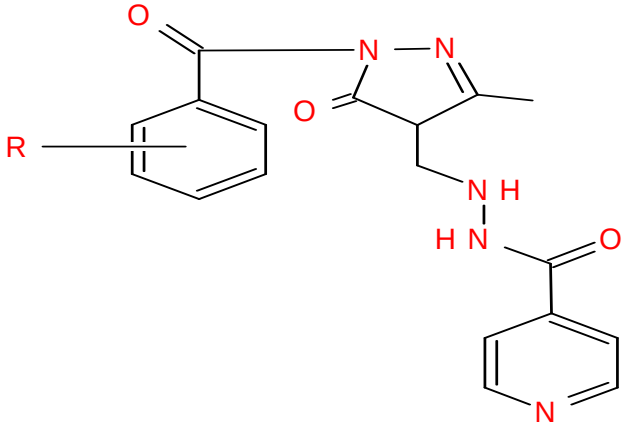
STEP 4: A mixture of 0.005mol (1.09gm) of 3-methyl pyrazol-5 one, 5ml of formaldehyde and 0.05mol (0.68gm) of isonicotinic acid hydrazide was refluxed with 25ml of 95% ethanol for 2hrs. The resultant mixture was concentrated. The resultant solid mass was dried and purified by recrystallization from ethanol.

Scheme of Experimental Procedure

Scheme of Experimental Procedure



Physical Characterization of newly Synthesised Compounds



Spectral Studies[10-11]

Compound INH I:

IR (KBr,cm-1): 3150(CH(str) Aromatic) 1681.93(C=O(str)amide) 1591(C=N(str) 761.88(C-Cl(str)) 3500(N-N(pyrrole)) 1425.4(C-H def (CH3)) 1062.78(C-N(str))
1485(C-H (def) (-CH2-)1307 (C-C (str))

Compound INH II:

IR (KBr,cm-1): 3342.64(CH(str) Aromatic) 1670.35(C=O(str)amide 1604.77(C=N(str)) 1521.84(Ar-NO2(N=Ostr))
3413 (N-N (pyrrole)) 1062.78 (C-N(str)) 1440 (C-H (def) (-CH2-))
860.25(C-C (str).

HNMR (DMSO-d6/TMS): (3H CH3)8.1, (8H C-H aryl2) 8.1- 8.3, (2H CH2) 4.19,(1H NH) 10.18

Compound INH V:

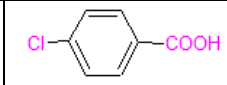
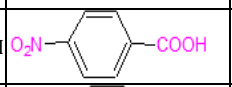
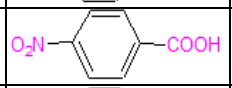
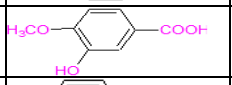
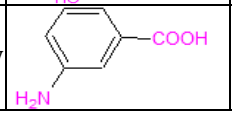
IR (KBr,cm-1): 3219.19(CH(str) Aromatic) 1668.43(C=O(str)amide) 1604.77(C=N(str)) 3400 (Ar-NH2(C-Nstr)) 3327(N-N(pyrrole)) 1014.56 (C-N(str)) 1441.89(C-H (def) (-CH2-)) 840.96(C-C (str))

Biological Activity[12-13]

Antibacterial Activity :

Sample Used	: Pyrazolone derivatives
Standard Used	: Gentamycin (10mcg/well)
Vehicle Used	: Dimethyl sulphoxide (DMSO)
Method	:Cylindrical plate method
Organisms Used	: *Bacillus subtilis* NCIM 2079 & *Escherichia coli* NCIM2118

Table 3:

Compound	R	Molecular Formula	Molecular Weight	Melting Point (°C)	Rf value
INH I		$C_{18}H_{16}ClN_5O_3$	385.80	162	0.71
INH II		$C_{18}H_{16}N_6O_5$	396.35	130	0.32
INH III		$C_{19}H_{19}N_5O_4$	381.38	172	0.62
INH IV		$C_{19}H_{19}N_5O_5$	397.38	256	0.47
INH V		$C_{18}H_{18}N_6O_3$	366.3	84	0.35

Screening of Test Compounds For Antibacterial Activity Against Gram Positive& Gram Negative Organism

Table 4: Zone of Inhibition (mm)

Comp. code	ZONE OF INHIBITION (mm)	
	Bacillus subtilis(100µg)	**E.Coli**(100µg)
INH I	13	12
INH II	10	9
INH III	8	8
INH IV	10	9
INH V	8	8
Gentamycin	18	16
DMSO	-	12

(-) indicates no activity.

Antifungal Activity

Sample Used : Pyrazolone derivatives
Standard Used : Ketoconazole
Vehicle Used : Dimethyl sulphoxide (DMSO)
Method : Cylindrical plate method
Organisms Used : *Candida albicans* NCIM 3100 & *Saccharomyces cervisea*

Screening of Test Compounds for Antifungal Activity against Gram Positive & Gram Negative Organism

Table 5:

Comp. Code	ZONE OF INHIBITION (mm)	
	Candida albicans (100µg)	Saccharomyces (100µg)
INH I	-	-
INH II	8	
INH III	9	11
INH IV	-	-
INH V	12	8
Ketoconazole	14	15
DMSO	-	-

(-) indicates no activity.

RESULT AND DISCUSSION

DRUGLIKENESS:

- Parameters related to drug likeness of the derivatives were established on the basis of Lipinski's rule of 5.
- All of the derivatives showed a zero violations of the rule of 5 which indicates good bioavailability.
- The positive bioactivity score (Table 2) of the derivative were also in agreement with their probability of drug likeness.

Synthesis:

- All the synthesized compounds were characterized by physical and spectral characterization.

Antibacterial Activity

Antimicrobial Activity

Bacilus subtilis

Antibacterial activities of synthesized compounds were evaluated against Bacilis subtilis and the zone of inhibition was measured as the parameters of the activity. Gentamycin, the standard drug showed a zone of inhibition of 18 mm in concentration of 100 µg ml. Out of five synthesized compounds INH I, INH II and INH IV posses high degree of antibacterial activity. INH III and INH V showed moderate antibacterial activity.

Escherichia coli

Antibacterial activities of synthesized compounds were evaluated against E.coli and the zone of inhibition was measured as the parameters of the activity. Gentamycin, the standard drug showed a zone of inhibition of 16 mm in concentration of 100 µg ml Out of five synthesized compounds INH I posses high degree of antibacterial activity and INH II, INH III, INH IV and INH V showed moderate antibacterial activity.(Fig.1)

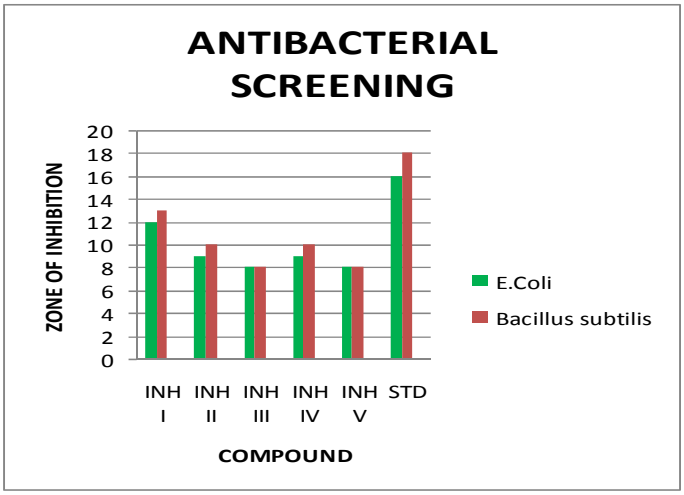


Fig.1 Gram (+) and Gram (-) antibacterial activity of Synthesized comp.

Antifungal Activity

Candida albicans

Antifungal activities of synthesized compounds were evaluated against E.coli and the zone of inhibition was measured as the parameters of the activity.

Ketoconazole, the standard drug showed a zone of inhibition of 14 mm in concentration of 100 µg ml. Out of five synthesized compounds INH V posses high degree of antifungal activity and INH III showed moderate antifungal activity.

Saccharomyces cervisea

Antifungal activities of synthesized compounds were evaluated against E.coli and the zone of inhibition was measured as the parameters of the activity. ketoconazole, the standard drug showed a zone of inhibition of 14 mm in concentration of 100 µg ml. Out of five synthesized compounds INH III posses high degree of antifungal activity and INH II and INH V showed moderate antifungal activity.

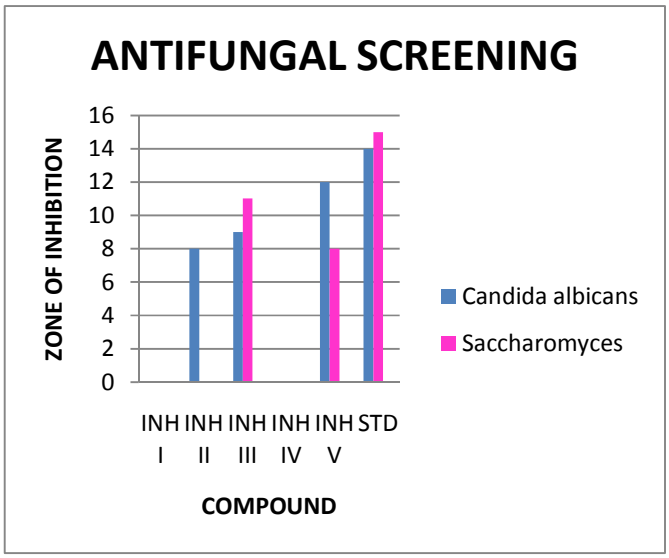


Fig.1 Gram (+) and Gram (-) antifungal activity of Synthesized comp.

CONCLUSION

The present study has been taken up to investigate possible Biological activity of pyrazolone derivatives. Some pyrazolone derivatives were reported to possess some degree of biological activity. A total of 5 derivatives were prepared and the structure of these compounds has been confirmed by the IR, NMR, and also supported by physical data. All the compounds were screened for anti bacterial and anti fungal using Gentamycin and Ketoconazole as the standard. The synthesized derivatives were characterized and identified on the basis of physical and spectral data. The derivatives were tested for biological activity using cylindrical plate method. Among all the pyrazolone derivatives compounds INH I, INH II and INH IV posses high degree of antibacterial activity against ***Bacilus subtilis*** compared to other derivatives. INH I posses high degree of antibacterial activity and INH II, INH III, and INH V showed moderate antibacterial activity against E.coli. INH V posses high degree of antifungal activity and INH III showed moderate antifungal activity against ***Candida albicans.*** INH III posses high degree of antifungal activity and INH II and INH V showed moderate antifungal activity against ***Saccharomyces cervisea.***

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