Research Article

Synthesis and Antimicrobial Activities of New Indolyl-Pyrazoline Derivatives

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ABSTRACT

The purpose of research was to synthesize a series of new indolyl-pyrazoline from indole-3-carbaldehyde.The reaction of 1-H-Indole-3-carbaldehyde and substituted acetophenone in presence of methanolic sodium hydroxide gives the (2*E*)-3-(1*H*-indol-3-yl)-1-phenylprop-2-en-1-one in 70-80% yield. These corresponding chalcones are condensed with thiosemicarazide to gives 5-(1*H*-indol-3-yl)-3-phenyl-4,5-dihydro-1*H*-pyrazole-1-carbothioamide. Structure of the synthesized compounds was confirmed by means of their IR spectral data and elemental analysis Melting points were determined by using melting point apparatus MP-DS TID 2000 V. scientific and were uncorrected .Reactions were monitored by TLC on pre coated silica gel G plates using iodine vapours as visualizing agents. Drug likeness properties were computed by calculating Lipinski's rule of five for the lead molecule. All the compounds showed zero violation of Lipinski's rule of five, which indicates good bioactivity and bioavailability.

The antimicrobial testing of the synthesized compounds were evaluated. Out of five synthesized compounds IPC IV & IPC V posses high degree of antibacterial activity against ***Bacilussubtilis and*** compounds IPC II and IPC V posses high degree of antibacterial activity against ***Escherichia coli.*** The compounds IPC IV & IPC V posses high degree of antifungal activity against ***Aspergilus Niger.***

Keywords: Drug likeness properties, Pyrazoline Antimicrobial studies.

INTRODUCTION

The main purpose of the present study is to synthesize different derivatives with pyrazolIne and screening for their biological activity. PyrazolIne is a five membered ring and is a derivative of pyrazole that has additional keto group. Pyrazoline are important class of heterocyclic compounds that occur in many drugs and is a nonsteroidal anti-inflammatory agent used in the treatment of arthritis and other musculoskeletal and joint disorders.

The azoles are five membered-cyclic systems, which in addition to carbon and nitrogen also contain at least one other heteroatom. Thus oxazole contain an oxygen atom; thiazole a sulphur atom; and pyrazole a nitrogen atom.The parent substance of these compounds, pyrazole (2), is a pyrrole (1) in which a methine group has been replaced by nitrogen.

(1) (2)

The nomenclature of the pyrazole group is based on that suggested byknorr for pyn'ole derivatives. The dihydropyrazoles are termed pyrazolines (3) and the completely reduced tetrahydro derivatives, pyrazolidines (4)

(3) (4)

Pyrazolines 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 pyrazolines. The wide range of biological activities exhibited by pyridine and pyrazolone, it was our aim is to prepare derivatives of incorporated indole with pyrazoline ring system in a molecular frame work and to explore the therapeutic advantage of this combination.

THERAPEUTIC IMPORTANCE

From the literature survey, it was revealed that 2-pyrazolines are better therapeutic agents. They possess valuable bioactivities like figure: 1

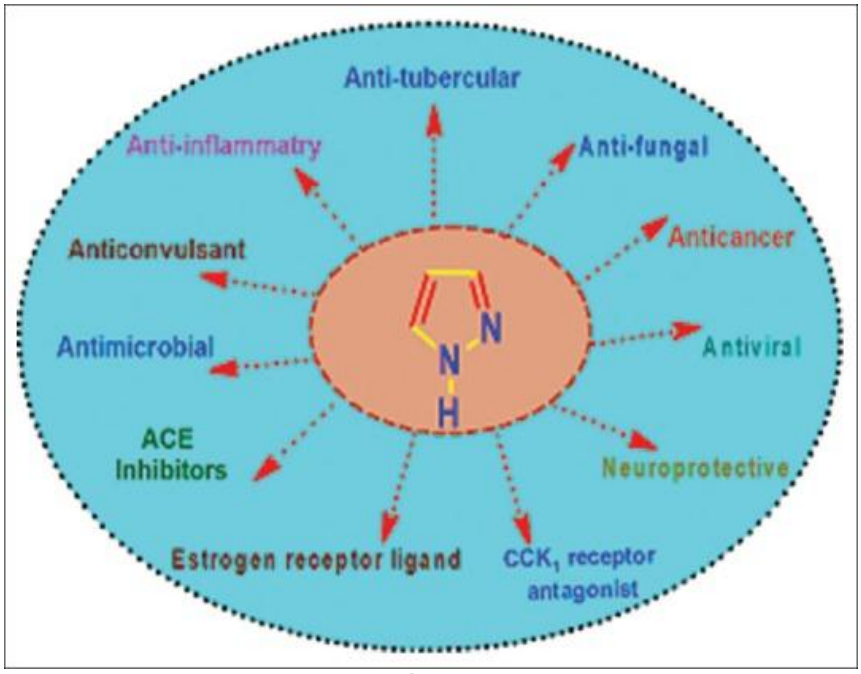


Fig. 1:

Structure of the synthesized compounds was confirmed by means of their IR spectral data and elemental analysis Melting points were determined by using melting point apparatus MP-DS TID 2000 V. scientific and were uncorrected .Reactions were monitored by TLC on pre coated silica gel G plates using iodine vapours as visualizing agents

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.

MATERIALS AND METHODS

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 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-NMR400MHz, IIRBS.

DRUGLIKENESS PROPERTIES

Drug likeness is a qualitative concept used in drug design for how "druglike" a substance is with respect to factors like bioavailability. It is estimated from the molecular structure before the substance is even synthesized and tested. A druglike molecule has properties such as:

- ✓ **Solubility** in both water and fat, as an orally administered drug needs to pass through the intestinal lining after it is consumed, be carried in aqueous blood and penetrate the lipid-based cell membrane to reach the inside of a cell. A model compound for the lipophilic cellular membrane is 1-octanol (a lipophilic hydrocarbon), so the logarithm of the octanol/water partition coefficient, known as LogP, is used to predict the solubility of a potential oral drug. This coefficient can be experimentally measured or predicted computationally, in which case it is sometimes called "cLogP".
- ✓ Potency at the target of interest. High potency (high value of pIC_{50}) is a desirable attribute in drug candidates, as it reduces the risk of non-specific, off-target pharmacology at a given concentration. When associated with low clearance, high potency also allows for low total dose, which lowers the risk of idiosyncratic drug reactions.
- ✓ Several scoring methods can be used to express druglikeness as a function of potency and physicochemical properties, for example ligand efficiency and lipophilic efficiency.
- ✓ Since the drug is transported in aqueous media like blood and intracellular fluid, it has to be sufficiently water-soluble in the absolute sense (i.e. must have a minimum chemical solubility in order to be effective). Solubility in water can be estimated from the number of hydrogen bond donors vs. alkyl sidechains in the molecule. Low water solubility translates to slow absorption and action. Too many hydrogen bond donors, on the other hand, lead to low fat solubility, so that the drug cannot penetrate the cell membrane to reach the inside of the cell.

Molecular weight

The smaller the better, because diffusion is directly affected. Eighty percent of traded drugs have molecular weights under 450 daltons; they belong to the group of small molecules

- ✓ Substructures that have known chemical or pharmacological properties. For example, alkylnitro compounds tend to be irritants, and Michael acceptors, such as enones, are alkylating agents and thus potentially mutagenic and carcinogenic.

A traditional method to evaluate druglikeness is to check compliance of **Lipinski's Rule of Five,** which covers the numbers of hydrophilic groups, molecular weight and hydrophobicity.

Based on one definition, a drug-like molecule has a logarithm of partition coefficient (log P) between -0.4 and 5.6, molecular weight 160-480 g/mol, molar refractivity of 40-130, which is related to the volume and molecular weight of the molecule and has 20-70 atoms.

Also, other factors such as substructures with known toxic, mutagenic or teratogenic properties affect the usefulness of a designed molecule. In fact, several poisons have a good druglikeness. Natural toxins are used in pharmacological research to find out their mechanism of action, and if it could be exploited for beneficial purposes.

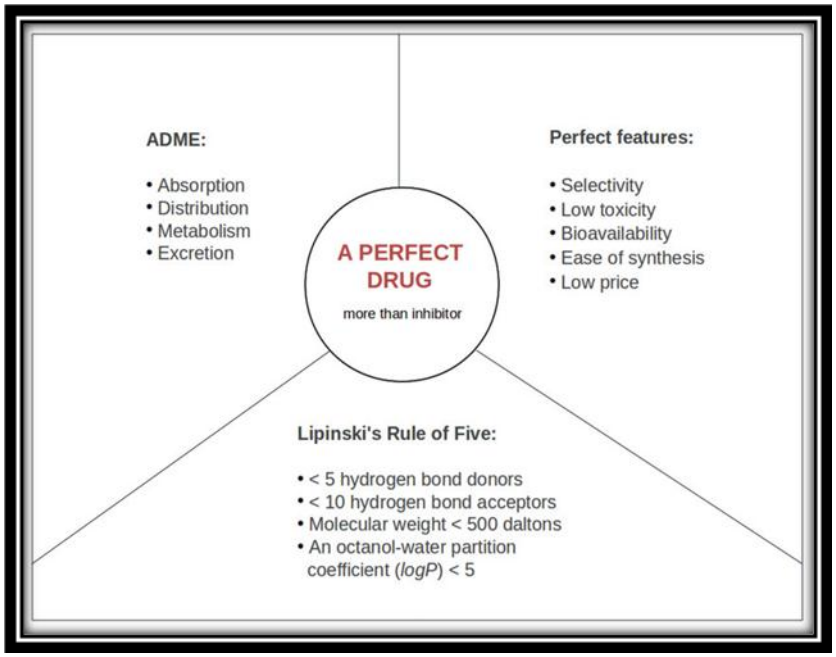


Fig. 2:

CALCULATION OF MOLECULAR PHYSICOCHEMICAL PROPERTIES

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.

MOLECULAR PROPERTIES AND BIOACTIVE SCORE OF COMPOUNDS

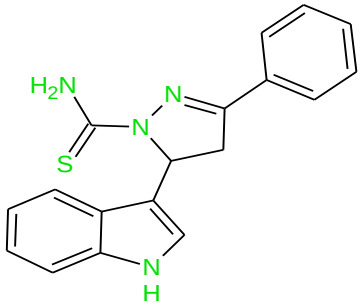
Table 1:

COMP	LogP	TPSA	MW	No of Hydrogen bond acceptor	No of Hydrogen bond donor	Violation	No of Rotatable bond	Molar volume
IPC I	3.47	50.44	238.4	3	1	0	1	208.01
IPC II	4.12	50.44	272.62	3	1	0	1	221.5
IPC III	4.15	50.44	272.69	3	1	0	1	221.55
IPC IV	2.99	70.67	254.24	4	2	0	1	216.03
IPC V	3.43	96.26	283.24	6	1	0	2	231.35

Table 2:

Comp	GPCR	Ion channel	Kinase inhibitor	Nuclear receptor	Protease inhibitor	Enzyme inhibitor
IPC I	-0.17	-0.06	0.06	0.15	-0.34	0.21
IPC II	-0.12	-0.07	0.09	0.20	-0.35	0.19
IPC III	-0.11	-0.06	0.08	0.17	-0.33	0.18
IPCIV	-0.88	-0.03	0.14	0.26	-0.26	0.25
IPC V	-0.21	-0.12	-0.01	0.13	-0.34	0.07

Structure of compound



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 pyrazoline moiety was found to have good bioactivity score.

Experimental studies

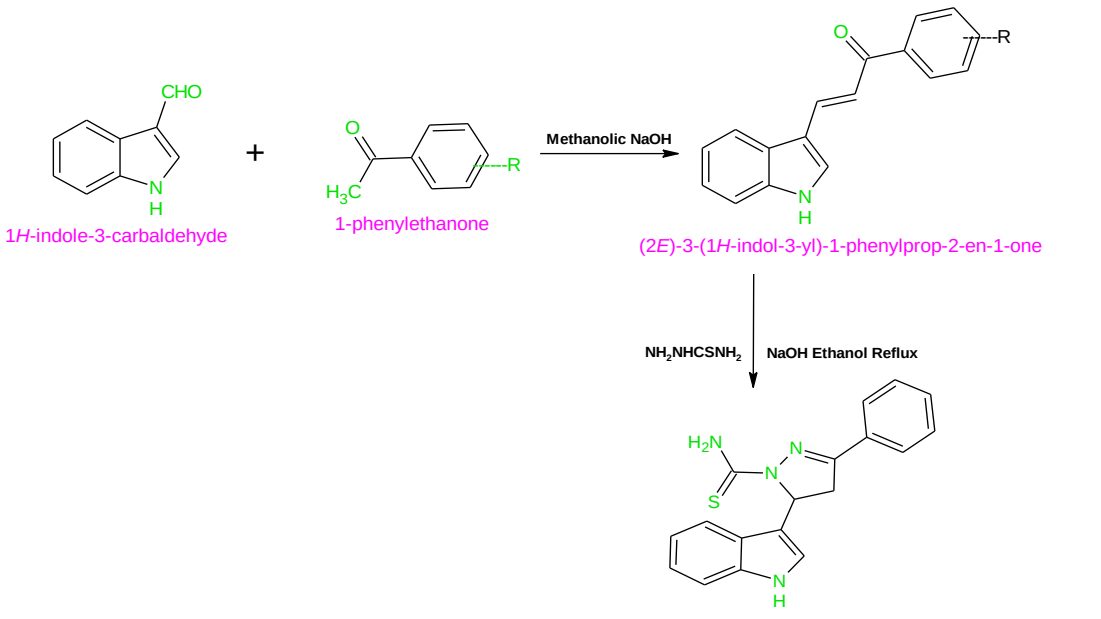
Step 1: Synthesis of chalcones-(2*E*)-3-(1*H*-indol-3-yl)-1-phenylprop-2-en-1-one

To a solution of indole-3- carbaldehyde (0.01 mol)substituted Ketones (0.01 mol) in ethanol (25ml), a solution of NaOH (6 ml 40%) was added. Then the reaction mixture was stirred at a reaction time for a period of 24 hours and acidified with Conc.HCl.The product obtained was filtered, washed with water and recrystallized from ethanol.

Step 2: Synthesis of 5-(1*H*-indol-3-yl)-3-phenyl-4,5-dihydro-1*H*-pyrazole-1-carbothioamide

A mixture of appropriate indolylchalcone (0.01mol),thiosemicarbazide (0.015mol) and NaOH (0.02mol) in dryethanol was refluxed for 16 hrs. The progress of the reactionwas monitored by TLC. The excess of solvent was removedunder reduced pressure and the reaction mixture was pouredinto ice cold water. The product obtained was filtered, washedwith water and recrystallised with ethanol

SCHEME



R= H, P-OH , P-OCH3 , P- NH2 , P-Cl

Physical Characterization of newly Synthesized Compounds

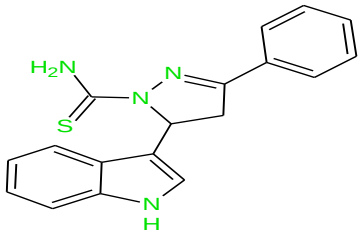
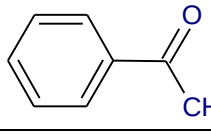
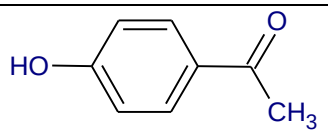
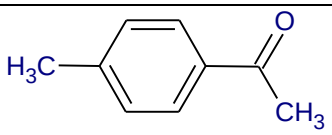
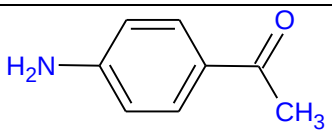
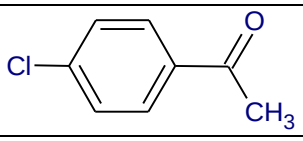


Table 3:

Compound	R	Molecular Formula	Molecular Weight	Melting Point (^{0}C)	Rf value	Elemental analysis calculated(found)%
IPC1		$C_{18}H_{16}N_4S$	320.41	210	0.62	C(67.47%) H(5.03%) N(17.49%) S(10.01%)
IPC2		$C_{18}H_{16}N_4OS$	336.41	180	0.68	C(64.26%) H(4.79%) N(16.65%) O(4.76%) S(9.53%)
IPC3		$C_{19}H_{18}N_4S$	334.43	220	0.74	C(68.23%) H(5.42%) N(16.75%) S(9.59%)
IPC4		$C_{18}H_{17}N_5S$	335.42	225	0.72	C(64.45%) H(5.11%) N(20.88%) S(9.56%)
IPC5		$C_{18}H_{15}ClN_4S$	354.85	246	0.78	C(60.92%) H(4.26%) Cl(9.99%) N(15.79%) S(9.04%)

Spectral Studies

Spectral studies of compound IPC- I

IR (KBr,cm-1): CH(str) Aromatic-3039.81, NH (Indole ring)- 3448.72, C=N(str)- 1581.63, C- N(str)- 1120.64, C=S (str)- 696.30, NH Amide-1614

Spectral studies of compound IPC- II

IR (KBr,cm-1): CH(str) Aromatic-3232.7, NH (Indole ring)- 3448, C=N(str)- 1550.77, C- N(str)- 1182.36, C=S (str)- 653.87, NH Amide-1612.49, C-OH-3311.78

Spectral studies of compound IPC- IV

IR (KBr,cm-1): CH(str) Aromatic-3230.77, NH (Indole ring)- 3446.79, C=N(str)- 1548.84, C- N(str)- 1118.71, C=S (str)- 653.87, NH Amide-1616.35, Ar-NH2 -3320.25

Biological Activity

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 2063&E.Coli*NCIM 2064

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

Table 4: Zone of Inhibition (mm)

Compound	Zone of Inhibition(mm)	
	E coli (100µg)	Bacillus subtilis (100µg)
IPC-I	6	5
IPC-II	7	6
IPC-III	5	6
IPC-IV	6	7
IPC-V	7	7
Gentamycin	10	12
DMSO	-	-

(-) indicates no activity.

Antifungal Activity
Sample Used: Pyrazolone derivatives
Standard Used: Fluconazole
Vehicle Used: Dimethyl sulphoxide (DMSO)
Method: Cylindrical plate method
Organisms Used: **Aspergillusniger *NCIM 3063***

Screening of Test Compounds for Antifungal Activity against Gram Positive & Gram Negative Organism
Table 5:

Compound	Zone of Inhibition(mm)
	Aspergillus niger (100 µg)
IPC-1	06
IPC-2	07
IPC-3	06
IPC-4	08
IPC-5	09
KETOCONAZOLE	11
DMSO	-

(-) indicates no activity

RESULT AND DISCUSSION
In the present study, a series of new Indolyl-pyrazoline derivatives were synthesized. The structures of the synthesized compounds were established on the basis of elemental and spectral (IR) studies. Finally, the drug likeness and bioactivity were predicted using Molinspiration software. The results revealed that all new compounds show good drug likeness score and bioactivity score.
Various stage involved in the present work were summarized below.

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.

EXPERIMENTAL WORK
Selected leads were synthesized
- ✓ During the present work totally, five new compounds were synthesized. This involves two steps.
- ✓ **Step 1:** This step involves the formation of chalcone from Indole-3carboxaldehydeand various acetophenone.
- ✓ **Step 2:**In this step various chalcone were treated with thiosemicarbazide which resulted in the formation of indolyl pyrazoline derivatives.

CHARECTERIZATION OF SYNTHESISED COMPOUNDS

The synthesized compounds are characterized by various methods such as melting point, infra-red spectroscopy and its reports were in complete agreement with their chemical structure. The purity of the compounds were further established by chromatographic methods (TLC).

ANTI-MICROBIAL ACTIVITY

Antibacterial activity (g+ve)

Bacilus subtilis

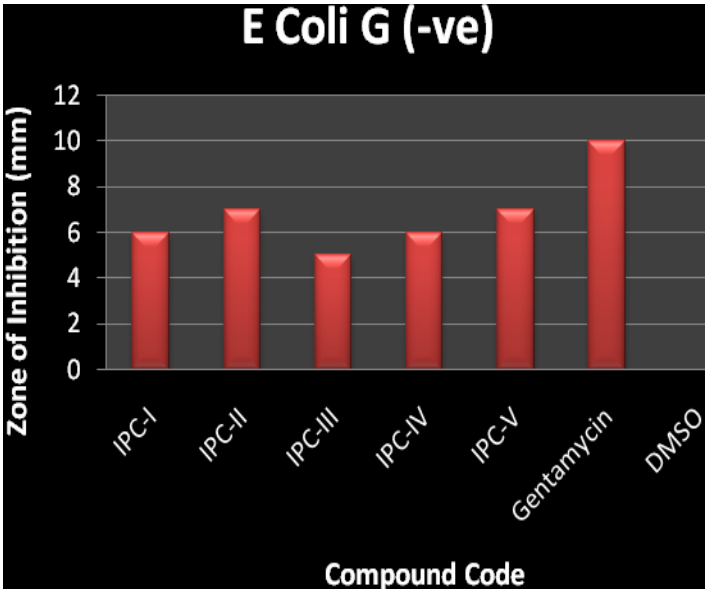
Antibacterial activities of synthesized compounds were evaluated against Bacilis subtilis and the zone of inhibition was measured as the parameters of the activity. Gentamycin, the standard drug showed a zone of inhibition of 12 mm in concentration of 100 µg/ ml. Out of five synthesized compounds IPC IV & IPC V posses high degree of antibacterial activity. IPCI, IPC II and IPC III showed moderate antibacterial activity.

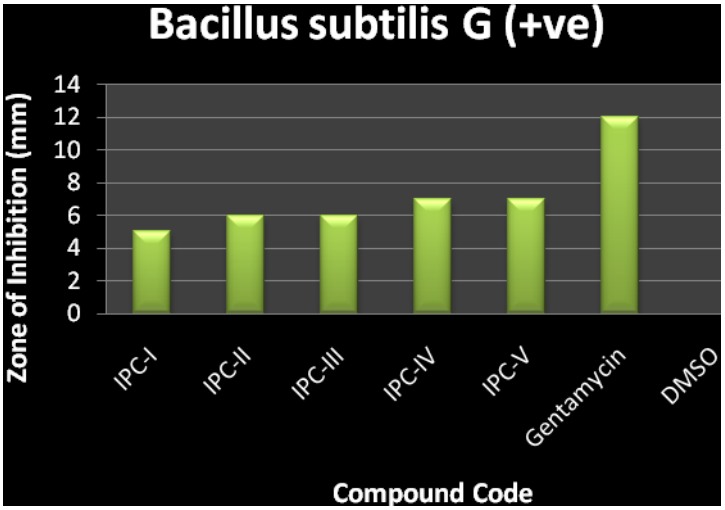
Antibacterial activity (g-ve)

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 10 mm in concentration of 100 µg/ ml. Out of five synthesized compounds IPC II and IPC V posses high degree of antibacterial activity and IPCI, IPC III and IPC IV showed moderate antibacterial activity.

GRAPHICAL REPRESENTATION OF ANTIBACTERIAL SCREENING ACTIVITY



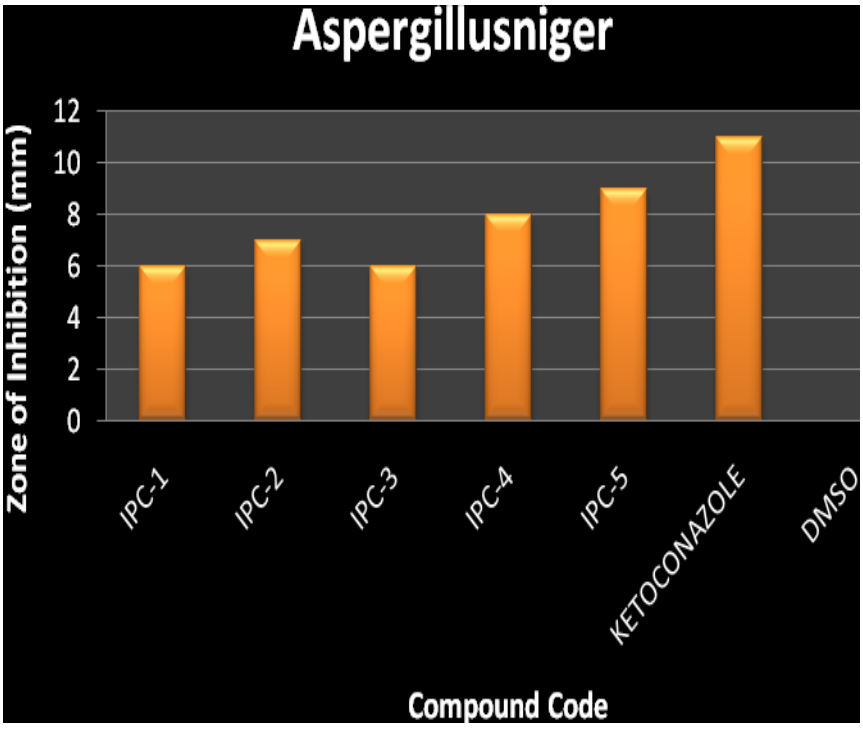


Antifungal activty (g+ve)

Aspergilus Niger

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 11 mm in concentration of 100 µg/ml. Out of five synthesized compounds IPC IV & IPC V posses high degree of antifungal activity. IPC I, IPC II and IPC III showed moderate antifungal activity.

GRAPHICAL REPRESENTATION OF ANTIFUNGAL SCREENING ACTIVITY



CONCLUSION

A series of pyrazoline were synthesized, characterized by analytical and spectral study. The derivatives were tested for biological activity using cylindrical plate method. Among all the pyrazoline derivatives compounds IPC IV & IPC V posses high degree of antibacterial activity against **Bacilus subtilis** compared to other derivatives. IPC II and IPC V posses high degree of antibacterial activity against **E.coli.** INH V posses high degree of antifungal activity and IPC IV & IPC V posses high degree of antifungal activity against ***aspergilus Niger.***

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