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RESEARCH ARTICLE

SYNTHESIS, SPECTRAL CHARACTERISATION AND ANTIMICROBIAL STUDIES OF PYRAZOLE DERIVATIVES

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ABSTRACT

Novel 1-(4-substitutedphenyl)-3-(4-substitutedphenyl)-prop-2-en-1-oneswere synthesized from a Claisen-Schmidt reaction of4-substituted benzaldehyde with several acetophenone derivatives. Subsequently, the cyclocondensation reaction of chalcones with hydrazine gives5-(4-substitutedphenyl)-3-(4-substituted phenyl)-1H-pyrazole derivatives. Several of these compounds were screened for the ability as antibacterial and antifungal activity. Growth of *S. aureus* (gram positive), *E.Coli* (gram negative) bacteria and *Candida albicans*, *Aspergillus niger* are inhibit by the action on synthesized compounds.

Key words: *Chalcone, Pyrazole, Antibacterial, Antifungal, FTIR, ^{1}H NMR,^{13}C NMR.*

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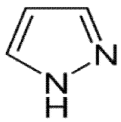
INTRODUCTION

Pyrazoles are five member ring heterocyclic compounds, have some structural features with two nitrogen atoms in adjacent position. Among the two nitrogen atoms; one is basic and the other is neutral in nature. These are aromatic molecules due to their planar conjugated ring structures with six delocalized π-electrons. The aromatic nature arises from the four π electrons and the unshaired pair of electrons on the –NH nitrogen (Ajay Kumar *et al.,* 2013).



Comparison of Basicity AmongPyrazole, Imidazole and Pyridine

Basicity of compound is based on electron pair availability an atoms. Among of these heterocyclic compounds containing a nitrogen atom as a hetro atom in a ring; there is basicity depends upon the ability to donate the lone pair of nitrogen atom. Both imidazole, and pyrazole containing two nitrogen atom in their ring. But pyrazole form a dimer so, its lone pairs are busy in intramolecular hydrogen bonding. This type of dimer structure is not possible in imidazole. So It's lone pairs are available to donate electrons.

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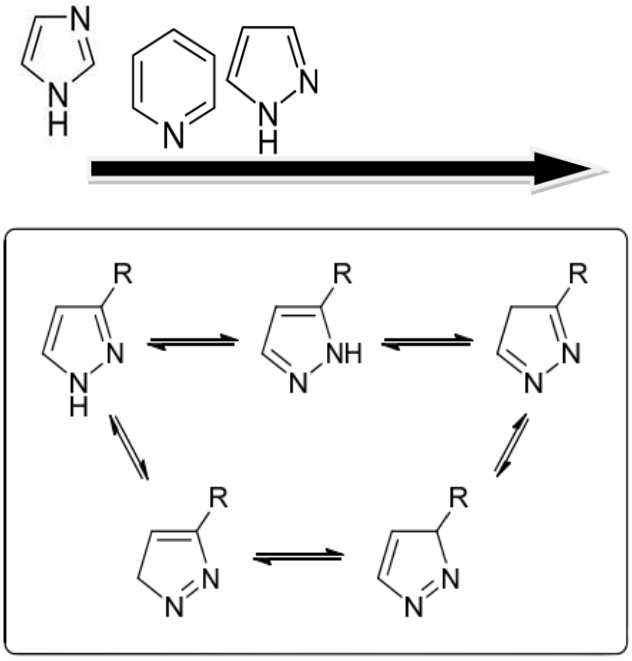
Soimadazole more basic than pyrazoles. But in comparison with pyridine the more basic heterocyclic compound is imidazole because the presence of additional N, which is more electronegative the C, makes the whole ring more electron rich, which means more basic. So, we can say that imidazole is most basic among of these three heterocyclic compounds .but in comparison with pyridine and pyrazole, there is pyrazole is less basic than pyridine because in pyrazole lone pair of electrons on N is involved in resonance whereas in pyridine this is not possible. SoPyrazole is least basic or weak base.

Increasing order of basicity

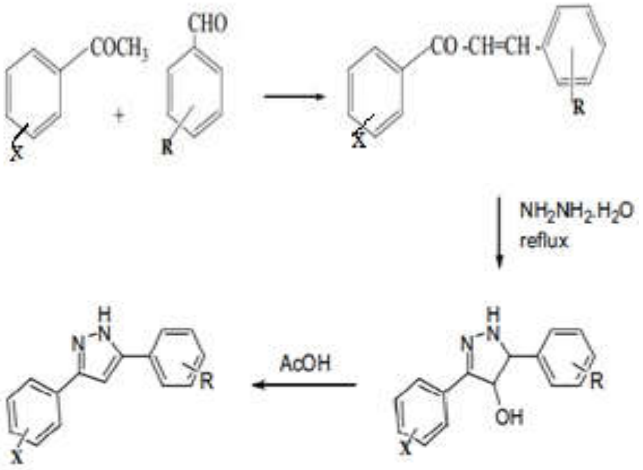
For pyrazoles in which two carbon neighbouring the nitrogen atom in ring have different substituent, Five tautomeric structures are possible are given as following:

Pyrazole is synthesised from chalcones which were obtained by condensation of acetophenone with aromatic aldehydes derivatives in presence of suitable condensing agent. Chalcones have been used as intermediate for the preparations of compounds having therapeutic value. They undergo a various chemical reactions and are found useful in synthesis of variety of pyrazole derivatives (Mohamed, 2012; Kalirajan, 2009). Pyrazole derivatives are the subject of many research studies due to their widespread potential biological activities such as antimicrobial (Pimerova, 2001), antiviral (Janus, 1999), antitumor (Park, 1995; Bouabdallah *et al.,* 2006), antidepressant (Bailey, 1986), insecticides (Chu, 1986) and fungicides (Pratap Kumar Patra, 2014).



In the view of the varied biological and pharmacological applications, we have planned to synthesize some pyrazole derivatives from various chalcones and test their antibacterial activity and antifungal properties.

Scheme 1



EXPERIMENTAL

Material and Instruments

All chemicals and reagents were obtained analytical grade and used without further purification. The IR spectra (in KBr pellets) were recorded on a Perkin-Elimer 400 FTIR spectrometer (Germany).[13]C NMRspectra were recorded in Bruker Avance II 400 NMR (Fallanden), NMR spectrometer using TMS as an internal standard. The compounds were analyzed for elemental analysis and the percentages of elements were found to be very near that of the calculated values by using software Accelrys Draw 4.1. Physical data of the compounds are recorded in Table-II.

Synthesis of chalcone (I)

Benzaldehyde derivative (0.01 mol) and acetophenone (0.01 mol) were dissolved in ethanol (25 mL) Sodium hydroxide solution 10% (25 mL) was added slowly and the mixture stirred for 9 hrs then it was poured into 400 mL of water with adding few drops of HCl and left overnight in Refrigerator. The precipitate obtained was filtered, washed and recrystallized from ethanol.

Synthesis of Pyrazole derivatives (II)

A mixture of chalcone (0.02 mol) and hydazine hydrates (0.02 mol) in glacial acetic acid or ethanol (25 mL) was refluxed for 9-11 hrs, and then the reaction mixture was poured into ice water (50 mL). The precipitate obtained was filtered, washed and recrystallized from methanol or ethanol.

Spectral analysis of synthesized Compounds

5-(4-bromophenyl)-3-(4-nitrophenyl)-1H-pyrazole

In the **[13]C** NMR spectrum of compound pyrazole derivative a confirmed pattern was observed peak signals. The characteristics signal corresponding to sp^3 carbon(C-NH) inpyrazole ring was observed at δ 47.48 ppm. The characteristics signal obtained by vinyl carbon(C=C and C=N) in pyrazole ring was characteristics at 124.7 and 130.17 ppm. The characteristics downfield shift due to aromatic ring attached to the pyrazole ring was observed in range of 120-175 ppm.

Table 1.

Sr.No.	code	R	X	Name of compound
1	P1	Br	NO_2	5-(4-bromophenyl)-3-(4-nitrophenyl)-1Hpyrazole
2	P2	Cl	OCH_3	5-(4-chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazole
3	P3	Cl	-	3-(4-chlorophenyl)-5-phenyl-1H-pyrazole

Table 2. Physical Parameters and Elemental Analysis of Synthesized Compounds

Cmpd No.	Molecular Formula	Mol.wt.	Solubility	Elemental Analysis (Calculated)					
				%C	%H	%O	%N	%Cl	%Br
I	$C_{15}H_{10}BrN_3O_2$	342.995	$CDCl_3$	52.25	2.93	9.30	12.21	-	23.22
II	$C_{16}H_{13}ClN_2O$	284.74	$CDCl_3$	67.49	4.60	5.62	9.84	12.45	-
III	$C_{15}H_{11}ClN_2$	254.061	$CDCl_3$	70.73	4.35	-	11.00	13.92	-

5-(4-chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazole;

The characteristics band at 3023 cm^{-1} attributing to Ar-H stretching vibrations. Banddue to C=C stretching vibrations was observed in the range of 1513-1614 cm-1. The band observed at 2939 cm^{-1} was characteristic of C-H stretching vibrations. The band due to stretching vibration of C=N group was observed at 1658 cm^{-1}. A band at 3300 cm^{-1} was observed due to N-H stretching vibrations. The band due to stretching vibration of N=CH group was observed at 1614 cm-1. The band due to streching vibration of O-CH_3 group was observed at 1423 cm^{-1}. The band due to streching vibration of C-Cl group was observed at 825 cm^{-1}. In the ^{1}HNMR spectrum of compound pyrazole derivative a confirmed pattern was observed peak signals. The characteristics signal corresponding to H at nitrogen atom in pyrazole ring was observed downfield singlet at δ7.8 ppm. The characteristics signal obtained by hydrogen neighbourhood to nitrogen atom with unsaturated double bond in pyrazole ring was characteristics also downfield at δ 7.5 ppm. The characteristics downfield shift due to aromatic ring attached to the pyrazole ring was observed in range of δ 5-7 ppm. The characteristics peak due sp^3 carbon at methoxy group was observed at δ2.3 ppm.

3-(4-chlorophenyl)-5-phenyl-1H-pyrazole;

In the^{13}**C** NMR spectrum of compound pyrazole derivative a confirmed pattern was observed peak signals. The characteristics signal corresponding to sp^3 carbon (C-NH) in pyrazole ring was observed at δ 52.64 ppm. The characteristics signal obtained by vinyl carbon (C=C and C=N) in pyrazole ring was characteristics at 126.64 ppm and 142.42 ppm.

Biological assay of the synthesized products

Method of Antimicrobial Analysis

Methodology

The evaluation of the anti-microbial effect of Synthesized Pyrazole was done by using Agar Well Diffusion method in the Department of microbiology, ISFAL (A unit of ISF College), Moga Punjab (INDIA).

Preparation of media and media plates

Antibiotic Assay Medium No. 11 (30.5 gm/1000ml of distilled water) was dissolved and added in a conical flask. Then the flask was plugged with cotton and autoclaved for complete sterilization. The sterilized media was poured in sterile petri dishes aseptically in a laminar flow. After solidifying of Agar plates (nearly about 15 to 20 minutes) they were kept inverted in incubator at 35±2°C for overnight for checking any contamination. The ready Agar plates then transferred in zip seal plastic cover and kept in a cold room.

Procurement of cultures

The pathogenic strains of different species of *E.coli* (MTCC-1687) and *Staphylococcus aureus* (MTCC-737) bacteria and *Aspergillus niger* (MTCC-282), *Candida albicans* MTCC-227Funguswere procured from Department of Microbiology I.S.F. college of Pharmacy, Moga The cultures were in freeze dried form (i.e. in dormant state). So, their revival was necessary. For this 100 ml nutrient broth medium was made and transferred in five small conical flasks (of quantity 100ml) 20ml each. The flasks were capped with cotton plug and autoclaved at 121°C for 15 minutes at 15 lb pressure per square inch.

Spreading

For isolation of micro- organisms in pure form without contamination, streaking was done on solid media plates by applying a microbial culture with a loop to the surface of Agar in a petri plate and spreading them with a sterile spreader. Already prepared solid media plates were used for streaking process. A drop of previously made broth cultures of *E.coli*, *Staphylococcus aureus Candida albicans* and *Aspergillus niger* respectively was added at one edge of the two sets each of four agar plates and the spreading of cultures was done with sterilisedspreader. Each time the spreader was sterilised on the burner flame and cooled in to the edge of agar in the respective plate. The spread of 16 culture plates, each set of 4 loaded each of *E.coli*, *Staphylococcus aureus, Candida albicans* and *Aspergillus niger* respectively.

Loading of the plates and measurement of zone of inhibition

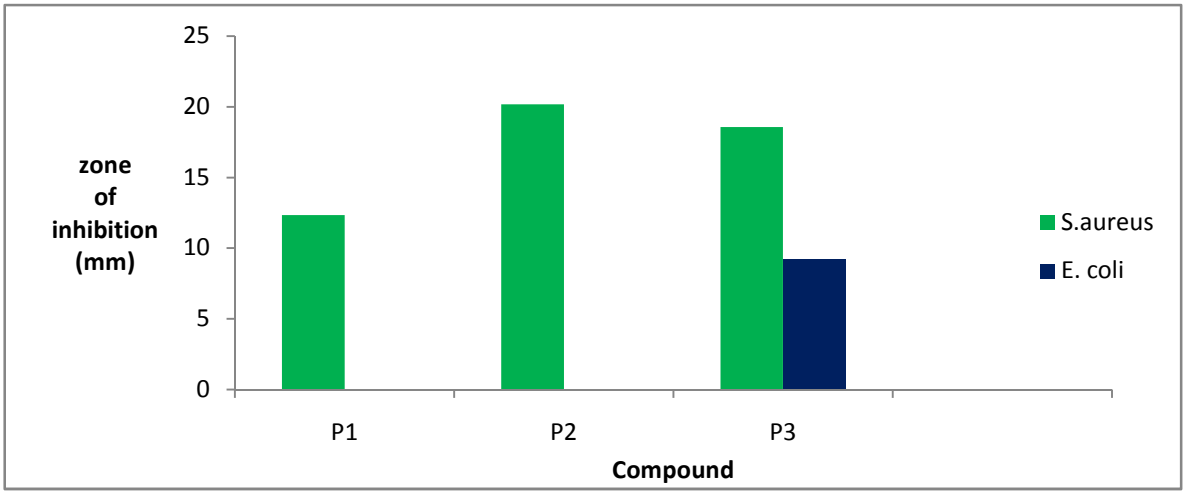
By using sterile cavity cork borer of 8mm size, wells were made in the centre of each of incubated culture plate to enable the introduction of the test sample and standard control. With the help of micropipette 100µl of concerned sample of aqueous were introduced into well of each plate streaked with different bacterial and fungal stains of *E.coli*, Staphylococcus aureus *Candida albicans* and *Aspergillus niger* respectively. For comparison one plate each for *E.coli* and Staphylococcus aures was loaded with *Ampicillin trihydrate* and for Aspergillus niger, *Candida albicans* with Ketoconazole. Then the plates were allowed to stand by for 30 minutes and were incubated for a time period of 24 hrs at the temperature of 37°C. The zone of inhibition was examined and measured with the help of antibiotic zone read. Antibacterial activity of the Pyrazole derivatives have been carried out against several types of bacteria such as, *E. coli*; and *S. aureus*; by taking standard drug Ampicillin trihydrate; and Antifungal activity have been carried out against *C.albicans* and *A.niger* by using Ketoconazole as a standard drug., using nutrient agar medium by well diffusion method (Raghuveer, 2014; Tripathi, 2000; Mehta *et al.,* 2013; Vasanthakumari, 2016). All compounds were suspended in aqueous solutions in different concentrations ranged from 10-100 mg/mL, the results are expressed on MIC (minimal inhibitory concentration), solvent blanks were run against each test organism in all assays and the experimental biological data is given in table:

Scope in future

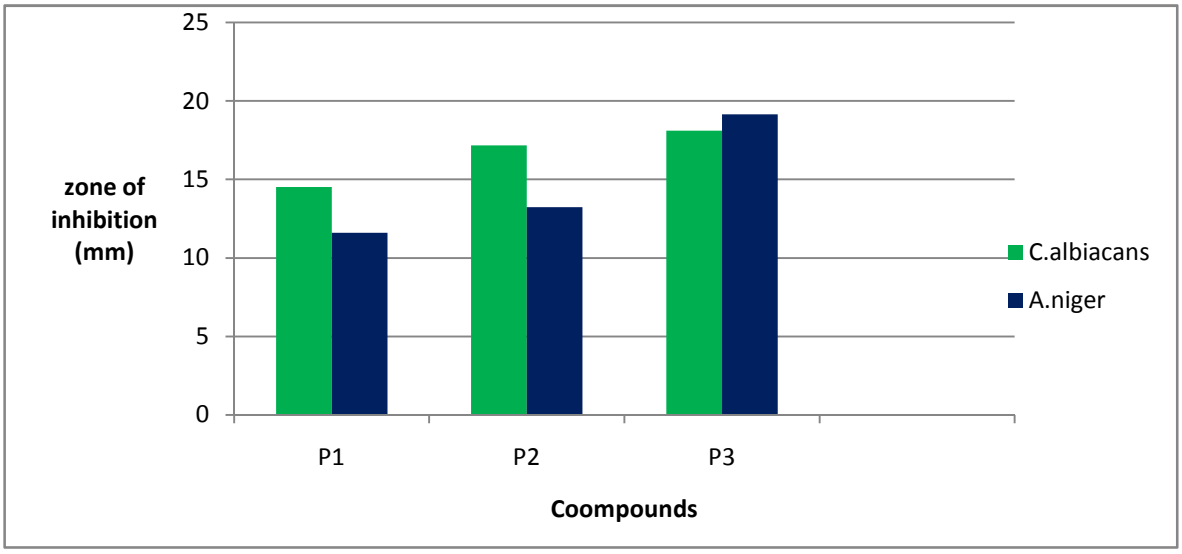
Pyrazole occupy a distinct and unique place in our life because it is an essential constituent of all living cells. This heterocyclic moiety has great biological and medicinal significance. The various pharmacological activities discussed here proves biological importance of pyrazole moiety. So it can be seen from the literature review that pyrazole ring containing heterocyclic system has wide medicinal applications. A large array of pyrazole drugs possesses a variety of medicinal properties.

Table 3. Antimicrobial activity of synthesized compounds

Comp. 100 mcg/ml	Zone of Inhibition (mm)			
	Gram positive	Gram negative	Anti-Fungal	
	S.aureus	*E.coli*	C.albicans	A.niger
P1	12.34	-	14.52	11.61
P2	20.17	-	17.16	13.24
P3	18.56	9.24	18.11	19.14
Standard drug	23.76	19.24	25.48	21.34
Solvent control	-	-	-	-



Graph 1. Comparison for anti-bacterial activity of synthesised pyrazole derivatives



Graph 2. Comparison for anti-fungal activity of synthesised pyrazole derivatives

A vast literature has been accumulated over the years and chemistry of pyrazole continues to be a blossoming field. The biological profiles of this new generation of pyrazole represent much progress with regard to the older compounds. The derivatives of pyrazoles are used as inhibitors and destructor of microbes, they have strong agent to kill all the microbes and inhibit the growth of microbes. We hope that in the future many new biological profiles will be added to it and more investigations must be carried out to evaluate more activities of pyrazole for many diseases whose treatment are challenging in the field of medical sciences. The versatile synthetic applicability and biological activity of these heterocycles will help the medicinal chemists to plan, organize and implement new approaches towards discovery of novel drugs.

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