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Topic: Pyrazole Derivatives Incorporating In The Aromatic Substitution For Physiological And Pharmacological Studies

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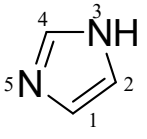
1.1 GENERAL

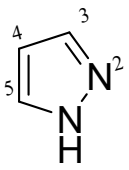
Medicinal chemistry research area focused on quality in terms of medicines in Pharma for health and aims to assured for fitness of purpose of medicinal synthetic and natural products. Many years medicinal chemistry had emerging as magnanimous field in science to synthesized and developed natural compounds as well as synthetic compounds or drugs. Medicinal chemistry is area to the intersection between in chemistry and pharmacology and medicinal chemistry involves to design, synthesized and biological evalution studies to developed pharmacological active compounds or drugs. In Medicinal chemistry several studies like as identification of active molecules for target, synthesis and develops the new chemical entities acts as therapeutic agents for disease or disorders. It also includes of computational studies following as quantitative structural-activity relationships (QSAR), docking, molecular dynamics also performed for developed new active drugs or lead molecule, and more applications of chemical research techniques to the development of active pharmaceuticals to treats against various pathological hypothesis. In early stages of to develop scientists were firstly concerned with the isolation and extraction of active chemical constituent from the plants. In current studies scientists also equally concerned with synthesized new chemical drugs or lead molecules. [1].

1.2 DIAZOLES

Diazoles are widely used as antibacterial and antifungal activities. They are of two types imidazoles and pyrazoles. Diazoles are of two isomeric forms with molecular formula $C_3H_3N_2$, having five-member cyclic aromatic ring consisting two Nitrogen (N) atoms on different positions and three carbon atoms[2].



Imidazole



Pyrazole

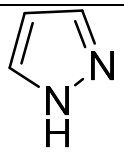
1.3 PYRAZOLES:

Pyrazoles is a aromatic five member heterocyclic compound are unique in chemical behavior. Pyrazole contains anomalous aromatic system characteristics, which are preferably pronounced in these derivatives having high ring liability that under some conditions..80 year before we known about Pyrazole derivatives, the exploration of their medicinal chemistry field purpose is very slowly. Earlier research were focused on organic molecules synthetic propose.

Recently studies focusing on the discovery and develops the synthesized derivatives for medicinal chemistry purpose against disease state to shows various pharmacological activities. Pyrazole derivatives have a various application in different fields are herbicides and insecticides are examples of agrochemicals and mainly in Pharma field to developed molecules acts as as antipyretic and anti-inflammatory and another pharmacological activities. Antipyrine is one of the earliest synthetic drug[3,4]

1.4 CHEMISTRY OF PYRAZOLE:

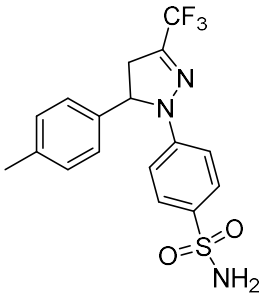
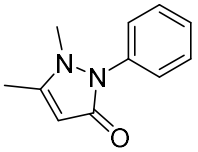
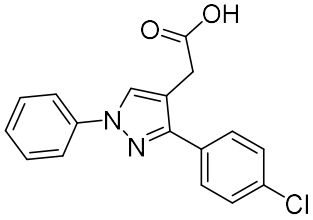
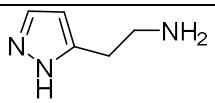
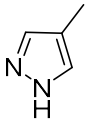
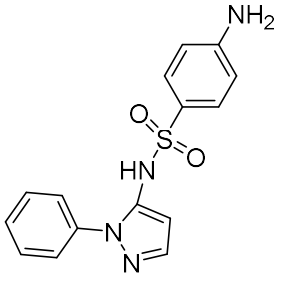
Pyrazole reactivity can be explains by the effect of each atoms or based on electronegativity and also stability. The nitrogen atom at position 2 is basic and acts as an electrophile because it has two electrons. The nitrogen atom at position 1 is unreactive, however the medium used as a base causes proton loss. Pyrazole is a Nucleophile in itself. Both N-atoms decrease the charge density of C-3 and C-5, facilitating electrophilic attack on C-4. Deprotonation happens at C-3 with in strong base, allowing the ring to open. Protonation caused pyrazolium cations to be less susceptible to electrophilic attack at C-4, but much more susceptible at C-3. Pyrazole anion has a low or non-reactive reactivity with nucleophiles, but a high reactivity with electron acceptors.[5].

Structure	 Pyrazole
Chemical Name	1,2 diazole
Molecular formula	$C_3H_4N_2$
Molecular mass	68.07 g /mol

M.P	66-70 ˚C
B.P	168-188 ˚C

Some of the marketed molecules to containing pyrazole moiety are following as:

Table 1: Pyrazole core based some marketed drugs

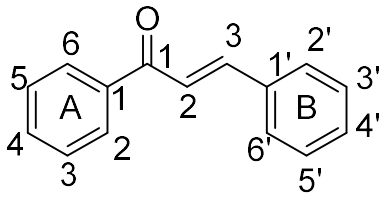
Marketed drugs	Structure
Celecoxib act as NSAIDS.	
Phenzone is used as an analgesic and antipyretic.	
Lonazolac used as NSAIDS.	
Betazole is a acts as H_2 agonist.	
Fomepizole is used as an antidote in for methanol.	
Sulfaphenazole is used as a antibacterial.	

Current research on the heterocyclic compounds to containing pyrazole core are pharmacological active lead to play key role in medicinal chemistry field. Pyrazole cores in shows various pharmacological activities to helps to develop a novel lead molecules. Extensive literature survey related to 1,3,5-trisubstituted pyrazole core based in shows a particular considerable interest in current years. **We also planned to synthesize 1,3,5-trisubstitution novel pyrazole moiety based derivatives.** Current research shows the of 1,3,5-tri substituted pyrazole core based molecules synthesis approaches by various methods and reported in publications and patents of different pharmacological activities. Various methods reported of tri substituted pyrazole moiety based molecules for the laboratory scale involves mainly,

1. Substituted hydrazines react with 1, 3-dicarbonyl compounds
2. Cycloaddition reaction of substituted hydrazines with chalcones

1.5 CHALCONES

Chalcone molecules are 1,3-diphenylpropeneone core based in this molecule two aromatic benzene ring core are connected through a 3 carbon based α,β-unsaturated carbonyl linker.

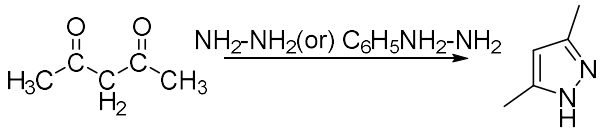


Chalcone are plethora in plants and precursors of flavonoids and isoflavonoids. Chalcone molecule contain a conjugated double bonds unsaturated and shows delocalization π-electron in benzene moiety have less intermolecular force and to goes through electron tranfer.[7]Chalcones method of preparation by catalysing the claisen-schmidt condensation reaction of an aromatic aldehyde and a ketone with a base or an acid, then dehydrating the product. Different substituents on the aromatic rings help in the backbone for the synthesis of different heterocyclic molecules to initiate different chemical reaction occur in the α, β - unsaturated part is necessary for antimicrobial property, and different substituent on aromatic rings help in the backbone for the synthesis of different heterocyclic molecules to undergo different chemical reactions. Chalcone play an key role in synthesis medicinal drugs or molecules[8,9]. Literature review of chalcone revealed molecules of shows natural or synthetic origin based to exhibit different pharmacological evalution activities following as

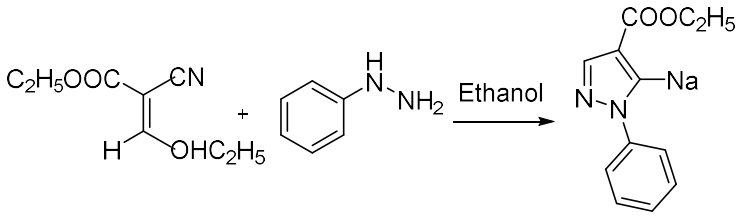
antioxidant, antimicrobial agents, anti-inflammatory activity, cytotoxic activity, hypoglycemic activity, antihepatotoxic, antimalarial, antileishmanial, tyrosine inhibitors and antitumor activities.

1.6 GENERAL METHODS FOR SYNTHESIZING PYRAZOLE RING [3] :

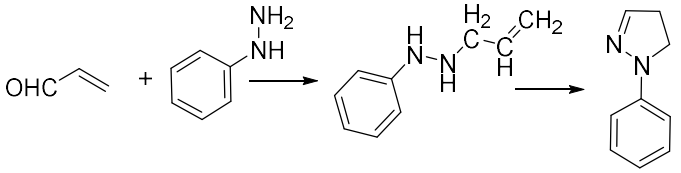
1. **From dicarbonyl compounds:** Direct method of pyrazole synthesis involved reaction in 1,3-dicarbonyl compound and hydrazine or its derivatives forms various substituted pyrazoles.



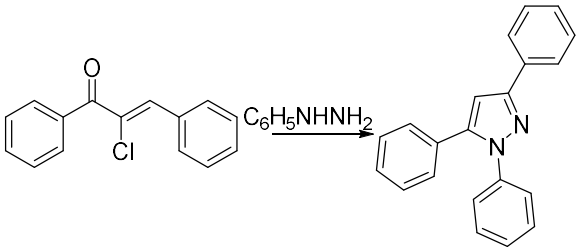
2. **From ethyl ethoxy methyleno acetate:** Synthesis of pyrazole moiety involves condensation of ethyl ethoxy methyleno acetate and phenyl hydrazine in presence of ethanol.



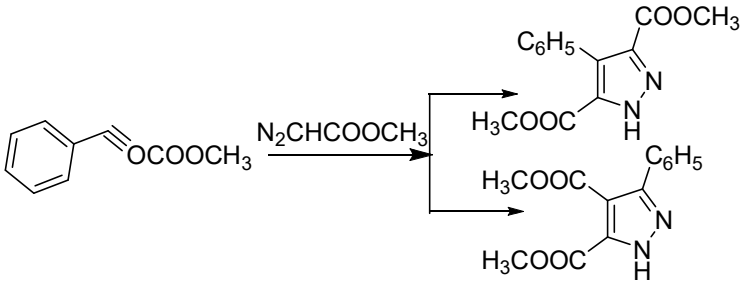
3. **From acrolein:** 1-phenylpyrazoline molecule was synthesized from the reaction between phenyl hydrazine and acrolein.



4. **From α,β-ethylene carbonyl compounds:** Reaction between an α,β-ethylene carbonyl derivative and hydrazine to gives trisubstituted pyrazole.

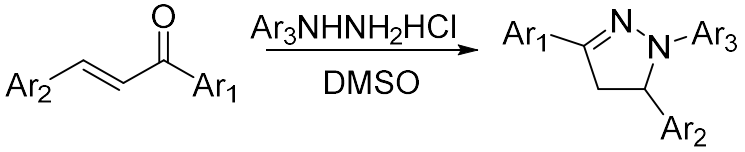


5. **From 1,3-dipolar addition:** azo molecule adds an acetylenic derivative in this reaction acetylenic triple bond activated by an electron withdrawing group, methyl diazoacetate and methyl phenyl propionate yields the isomeric pyrazoles in equimolar amounts.

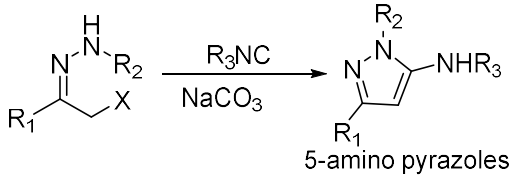


1.7 SYNTHESIS OF PYRAZOLE MOIETY

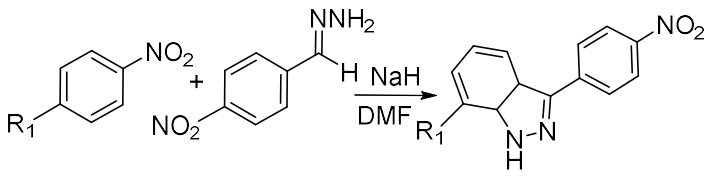
Ying R. Huang *et al* have synthesized dihydropyrazole by condensing of α,β - unsaturated ketones with aryl hydrazines.



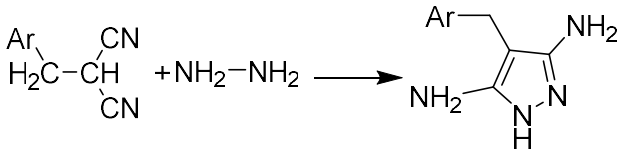
Altan *et al* to have synthesized 5-aminopyrazoles from α- halogenoketone hydrazone reacted with isocyanides .



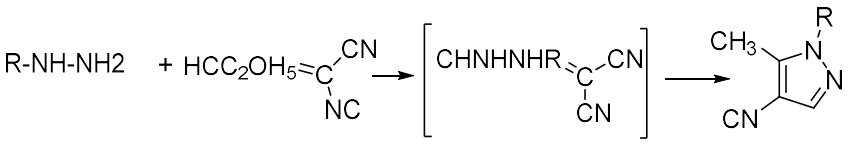
Takehiko *et al* to synthesized reaction of nitrobenzene with aryl imines to gives aryl pyrazoles.



Ram *et al* have to synthesised and reported diamino substituted pyrazole.

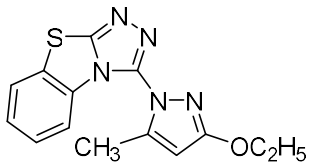


Cheng *et al* synthesis of substituted cyanopyrazole from mono-substituted hydrazine, was reacted with ethoxymethylenemalanonitrile in presence of boiling alcoholic solution.

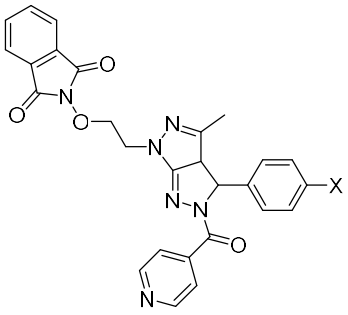


R=Alkyl (or) Aryl

Kapratwar *et al* reported the synthesies of subsitued heterocyclic and benzothiazolyl]-3-ethoxy-5methypyrazole and triazolo(3,4-b)benzothiazolyl]-3,5-dimethyl pyrazole.

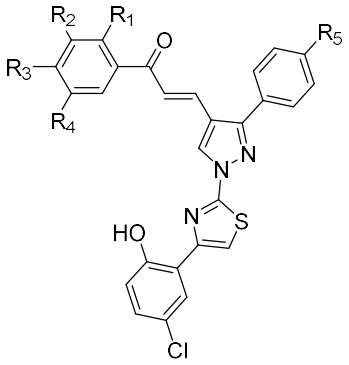


Talesara *et al* reported synthesis of substituted -thiocarbamoyl-3-3′dihydropyrazolo[3,4-c]pyrazoles.



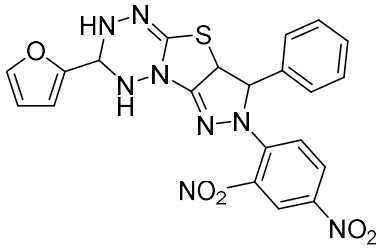
X=H, Cl, OCH3, N(CH3)2

Dawane *et al* reported of some substituted thiazolyl and pyrazolines derivatives, were prepared through base catalyzed condensation reaction , derivatives acts as antibacterial and antifungal activity.

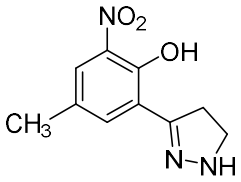


	R1	R2	R3	R4	R5
A	OH	H	H	Cl	OH
B	OH	Br	H	Cl	OH
C	OH	I	H	Cl	OH

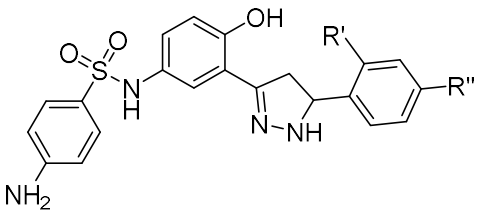
Mohan *et al* in have synthesized derivatives of tetrahydro-4H-pyrazolothiazolotetrazines for antibacterial and antifungal activity.



Desai *et al* reported of substituted-pyrazolines, condensation of chalcones with hydrazine hydrate presence of ethanol to gives derivatives. Synthesized derivatives acts as effective against *S. aureus* and *E. coli*.

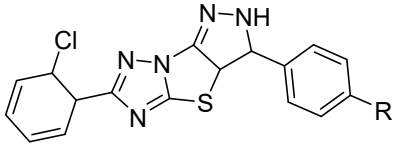


Palkar *et al* in 1999 have synthesized of 3-(5′- aminobenzenesulphanilamido-2′-hydroxyphenyl)-5-substitutedphenylpyrazoles shows antimicrobial activity.

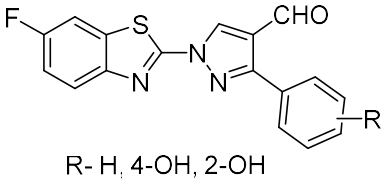


	R'	R"
A	H	H
B	H	CH_3
C	Cl	H

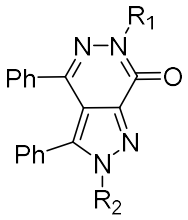
Kumar *et al* to synthesized 3-aryl-6-o- chlorophenyl based trans-3,3a-dihydropyrazolothiazolo[3,2-b]-s-trizoles derivatives shows activity against fungus.



Kumar *et al* to reported a series of synthesized pyrazol-1-yl and benzothiazoles based derivatives acts as antibacterial.



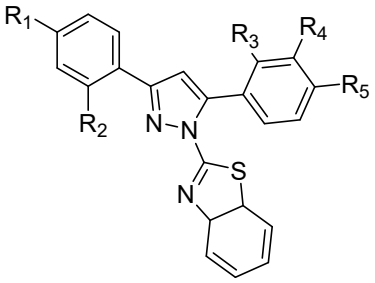
Berber *et al* to synthesized substituted pyrazolpyridazine. Pyrazolpyridazine derivatives acts shows activity against microbial infection.



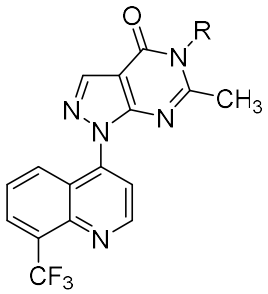
	R_1	R_2

A	H	CH_3
B	Ph	CH_3
C	CH_3	CH_3
D	$C_6H_4COOH(4)$	CH_3

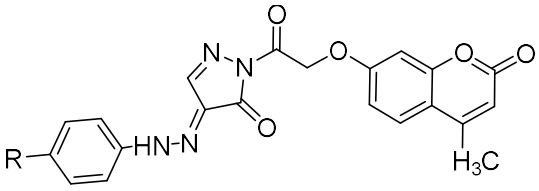
Sharma *et al* to synthesized substituted benzothiazolo pyrazoline derivatives acts as antimicrobial.



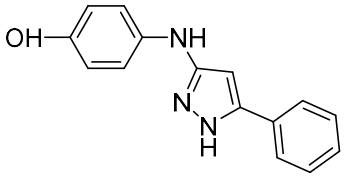
Holla *et al* in to synthesized pyrazolo pyrimidine derivatives acts as bacterial and fungal infection.



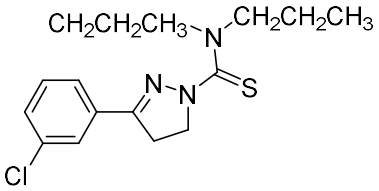
Kumar *et al* to synthesized substituted pyrazoles and substituted pyrazolin-5-ones derivatives were active against bacterial infection and acts as antioxidant.



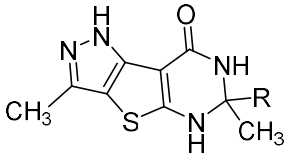
Sahu *et al* reported the series of novel substituted dihydropyrazole-3-yl-amino) phenols acts as antimicrobial.



Abid *et al* to synthesized a substituted pyrazoline derivatives were effective as for their antiamoebic affect.

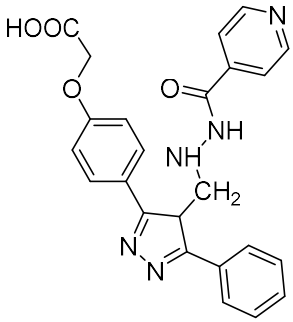


Shah *et al* to synthesized novel substituted pyrazolothienopyrimidin-8-ones core based derivatives evaluated act as antitubercular activity.

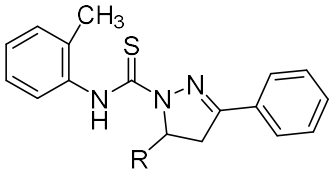


R=Phenyl, 2-hydroxy phenyl, 4-fluoro phenyl

Pattan *et al* synthesized some novel substituted pyrazolo phenoxy acetic acid and biological evalution as a antitubercular activity and synthesized derivatives activity compared by streptomycin.

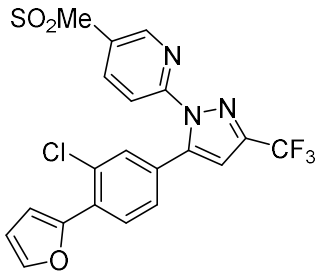


Patel *et al* reported the synthesis of substituted dihydropyrazole-1-carbothioamide nucleus based derivatives and molecules biological evalution studies performed as for anti-tubercular activity.

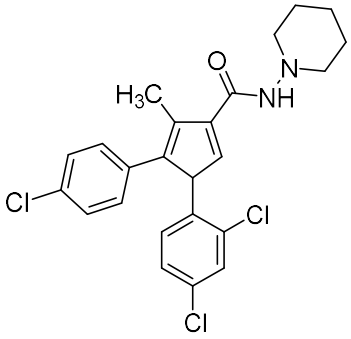


R=Chloro-Phenyl, p-hydroxy phenyl

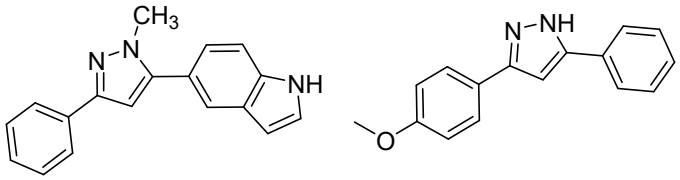
Cheng *et al* to reported SAR based of pyrazole molecules as selective against canine COX-2 inhibitors.



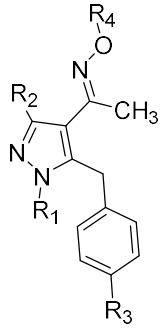
Thomas *et al* in 2005 have synthesized derivatives based of alkyl carboxamide analog of N-(piperidinyl)-and substituted pyrazole-3-carboxamide.



Cocconcelli *et al* to synthesized of aryl azoles in this substituent of phenyl hydrazine is prepared through reaction with the α,β-unsaturated compounds to gives of 4,5-dihydro-1-H-pyrazole shows good neuroprotective activity.

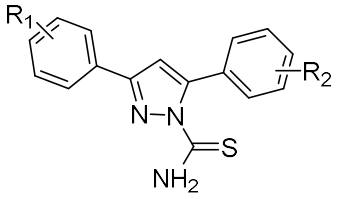


Park *et al* to synthesized a pyrazole oxime ether derivatives biological evalution studies against cancer.

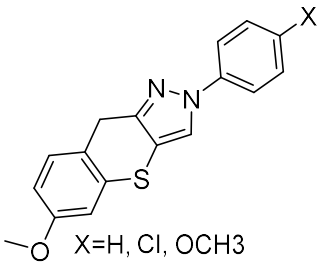


R_1 and R_2 are alkyl or aromatic groups.
R_3 is phenoxy and R4 is benzyl group.

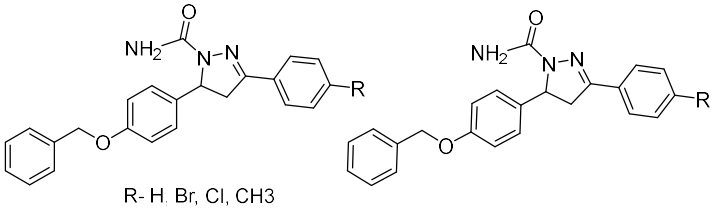
Cheng *et al* to synthesized of substituted dihydro-(1H)-pyrazole through cyclization of chalcones with thiosemicarbazide to gives pyrazole derivatives contained thiourea moiety ,these derivatives act against as anticancer agents.



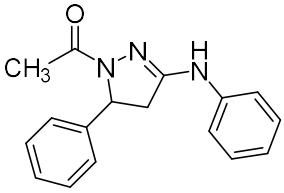
Marini *et al* to synthesized 1,4-dihydrobenzothiopyranopyrazole molecules and biological evalution studies confirms act as antiproliferative.



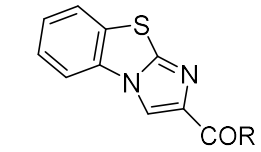
Sabbagh *et al* to synthesized of derivatives to pyrazolothiazol-4(5H)-ones and pyrazolothiazoles these derivatives were acts as antiviral activity.



Singh *et al* synthesized chalcones of anilide and their corresponding substituted pyrazol-1-yl)] ethanone derivatives, characterized by IR, H^1NMR, Mass spectra and biological evalution studies against convulsant activity.

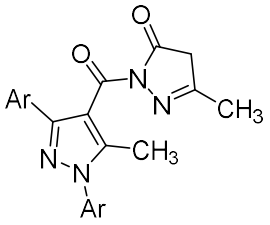


Trapani *et al* to synthesized a substituted imidazo-benzothiazoles derivatives and evalution studies against affinity at central benzodiazepine receptors.

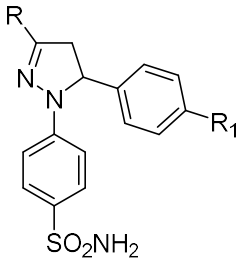


R=OCH_3, OC_2H_5, NH_2

Umesha *et al*., to synthesized a substituted dihydro-pyrazol-3-one derivatives ,these derivatives acts as antimicrobial and antioxidant activity.

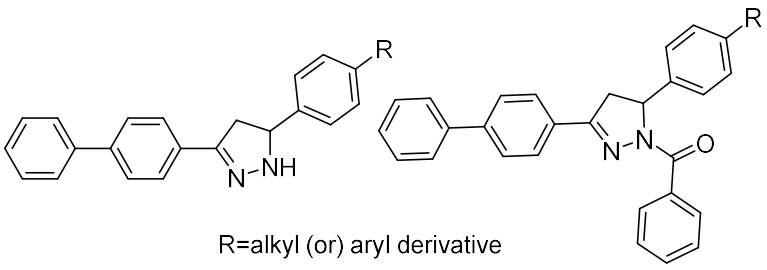


Rathish *et al* to synthesized derivatives 2-pyrazoline to contained benzene sulfonamide to prepared by condensing chalcones reacts hydrazinonbenzenesulfonamide hydrochloride shows activity against inflammation.



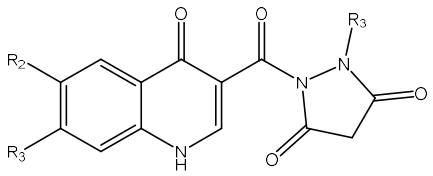
R=R'=alkyl, aryl derivative

Amir *et al* ., to reported a derivatives of substituted phenyl-2- pyrazolines and -substituted phenyl-2-pyrazolines derivatives and were screening against inflammation and analgesic affect.



R=alkyl (or) aryl derivative

Suma *et al* ., reported the synthesis of Quinolone and pyrazolindinedione based derivatives shows antibacterial and anti-inflammatory action.



	R_1	R_2	R_3
A	Cl	F	H
B	H	H	H
C	H	F	H
D	H	Cl	H
E	H	Br	H

METHODOLOGY

4.1 SYNTHESIS

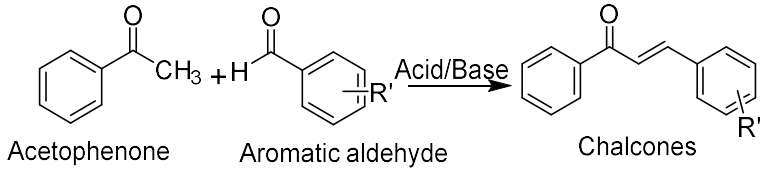
GENERAL PROCEDURE:

The following steps are used to synthesize 1,3,5-trisubstituted pyrazole moiety based derivatives:

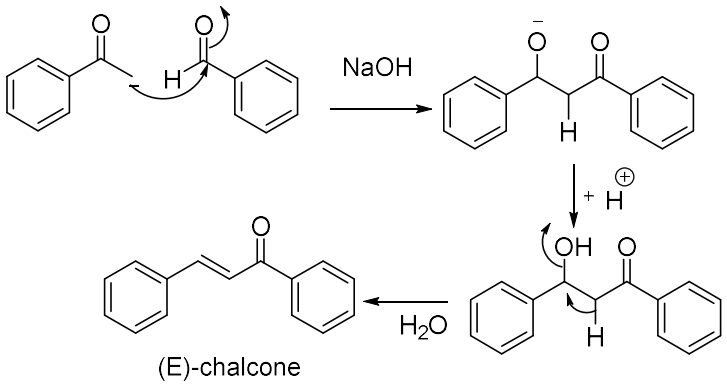
1. Synthesis of chalcones moiety
2. Synthesis of succinichydrazide from corresponding ester.
3. Final step involves the reaction of succinichydrazide with chalcones to form 1,3,5-trisubstituted pyrazole moiety based compounds.

Step I: Synthesis of chalcones and its derivatives

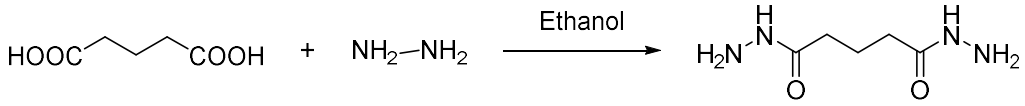
Chalcones and its derivatives synthesized by reaction of **claisen-schmidt condensation**. It is a type of aldol condensation called crossed aldol condensation, where a molecule of aldehyde containing □-hydrogen atom condenses with a ketone without α-hydrogen, in the presence of strong acidic or basic catalyst to form chalcones.



Base catalysed reaction mechanism:



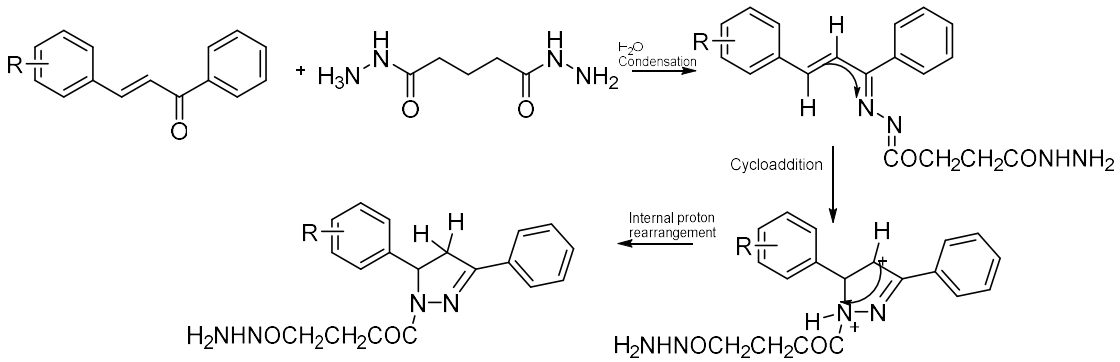
Step 2: Synthesis of succinichydrazide: Succinic acid and hydrazine hydrate reacts presence of alcohol can be converted to succinichydrazide, and reaction mixture was cooling at room temperature, succinichydrazide recrystallization in presence of ethanol to separates as solid .



Step 3: 1,3,5-tri substituted pyrazole moiety based derivatives synthesis :

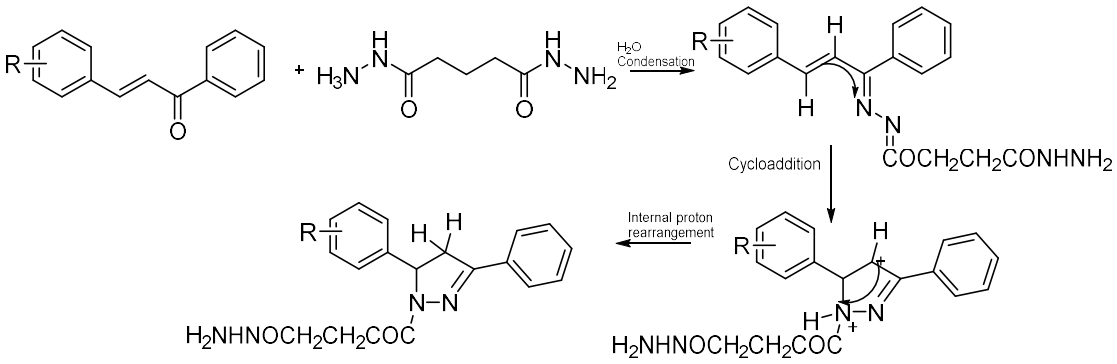
Synthesis of pyrazole derivatives goes through the cycloaddition reaction of substituted hydrazide with chalcones. Equimolar quantities of hydrazide and chalcones are mixed together and refluxed in the presence of suitable solvent.

Reaction Scheme:



R= Substituted aromatic aldehydes

Reaction Mechanism:



R= Substituted aromatic aldehydes

4.1.1 Experimental procedure:

Step 1: Procedure for the synthesis of chalcones and its derivatives :
In an ice bath, equimolar amounts of acetopenone (0.01 mol) and aromatic aldehydes (0.01 mol) in ethanol were cooled to 10-15°C. On a TLC plate, the reaction progress was monitoring using chloroform: petroleum ether (8:2) as the mobile phase. The reaction mixture (cooled solution) was placed on a magnetic stirrer, and 40 percent NaOH was added drop by drop to the reaction mixture while stirred continuously for 30 minutes and then left overnight. The reaction mixture was placed into a beaker with broken ice and dilute HCl to acidify it. The solid was filtered and rinsed in ice cold water before being dried and recrystallized in the presence of ethanol to obtain derivatives (C1-C15).

Table 2: Substitutions of derivative (C_1-C_{15}):

Compound	R
C_1	Benzaldehyde
C 2	2-chloro benzaldehyde
C 3	4-methoxy benzaldehyde
C 4	4-dimethylaminobenzaldehyde
C 5	Furan-2-carbaldehyde
C 6	Cinnamaldehyde
C 7	4-dimethylaminocinnamaldehyde
C 8	3-methoxy-2-hydroxy benzaldehyde
C 9	2-hydroxy benzaldehyde
C 10	2-nitro benzaldehyde
C 11	3,4,5-trimethoxy benzaldehyde
C 12	3,4,-dimethoxy benzaldehyde
C 13	4-chloro benzaldehyde
C 14	4-hydroxy benzaldehyde
C 15	4-methyl benzaldehyde

Step2: Procedure for synthesis of 1,3,5-trisubstituted pyrazole derivatives:

A mixture of chalcone (C_1-C_{15}) (0.01mol), succinichydrazide (0.01mol) and acetic acid (5ml) in ethanol was reflux for 8 hrs and reaction progress was determine by using TLC. and mixture was cooled and poured in over ice water to show solid separates, which were then filtered and washed with water.

Reaction scheme:

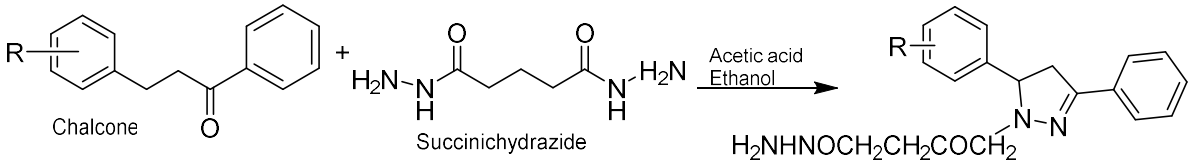


Table 4 : Substitutions of derivative (P_1-P_{15}):

Compound	R
P_1	Benzaldehyde
P_2	2-chloro Benzaldehyde
P_3	4-methoxy Benzaldehyde
P_4	4-dimethylaminobenzaldehyde
P_5	Furan-2-carbaldehyde
P_6	Cinnamaldehyde
P_7	4-dimethylaminocinnamaldehyde
P_8	3-methoxy-2-hydroxy Benzaldehyde
P_9	2-hydroxy Benzaldehyde
P_{10}	2-nitro Benzaldehyde
P_{11}	3,4,5-trimethoxy Benzaldehyde
P_{12}	3,4-dimethoxy Benzaldehyde
P_{13}	4-chloro Benzaldehyde
P_{14}	4-hydroxy Benzaldehyde
P_{15}	4-methyl Benzaldehyde

4.1 IDENTIFICATION AND CHARACTERIZATION:

The synthesized compounds were scaled for yield and purified using a suitable solvent system and recrystallization. The following approaches are used to characterize purified compounds:

1. Physical properties
2. Melting point evalution
3. Thin layer chromatography
4. Infrared spectroscopy for functional groups
5. Nuclear magnetic resonance for C and H
6. Mass spectroscopy for mass of compounds

Melting point evalution:

M.P of the synthesized molecules was determined using a open capillary tube and recorded in °C without correction, m.p. is physical constant in the characterized of an organic compound.

Thin layer chromatography:

TLC was done with polar or nonpolar mobile phase on precoated silica gel plates (604 GF 254 Merck). TLC is used to monitor the reaction's progress and determine the purity and impurities in end product.

Infrared spectroscopy:

The infrared spectra for compounds to identification of identification of functional groups in the molecule spectra recorded by **SHIMADZU FTIR 8400** spectrometer using KBr pellet.

Nuclear magnetic resonance:

The synthesized compounds ^{1}H NMR spectra by using a BRUKER SPECTROSPIN-400MHz spectrometer and TMS as a reference. Chemical shift unit as delta values related to TMS in ppm and we choose DMSO or $CDCl_3$ as solvent for ^{1}H NMR

Mass spectroscopy:

The mass spectra of derivatives was using GCMS-QP5050 SHIMADZU instrument.

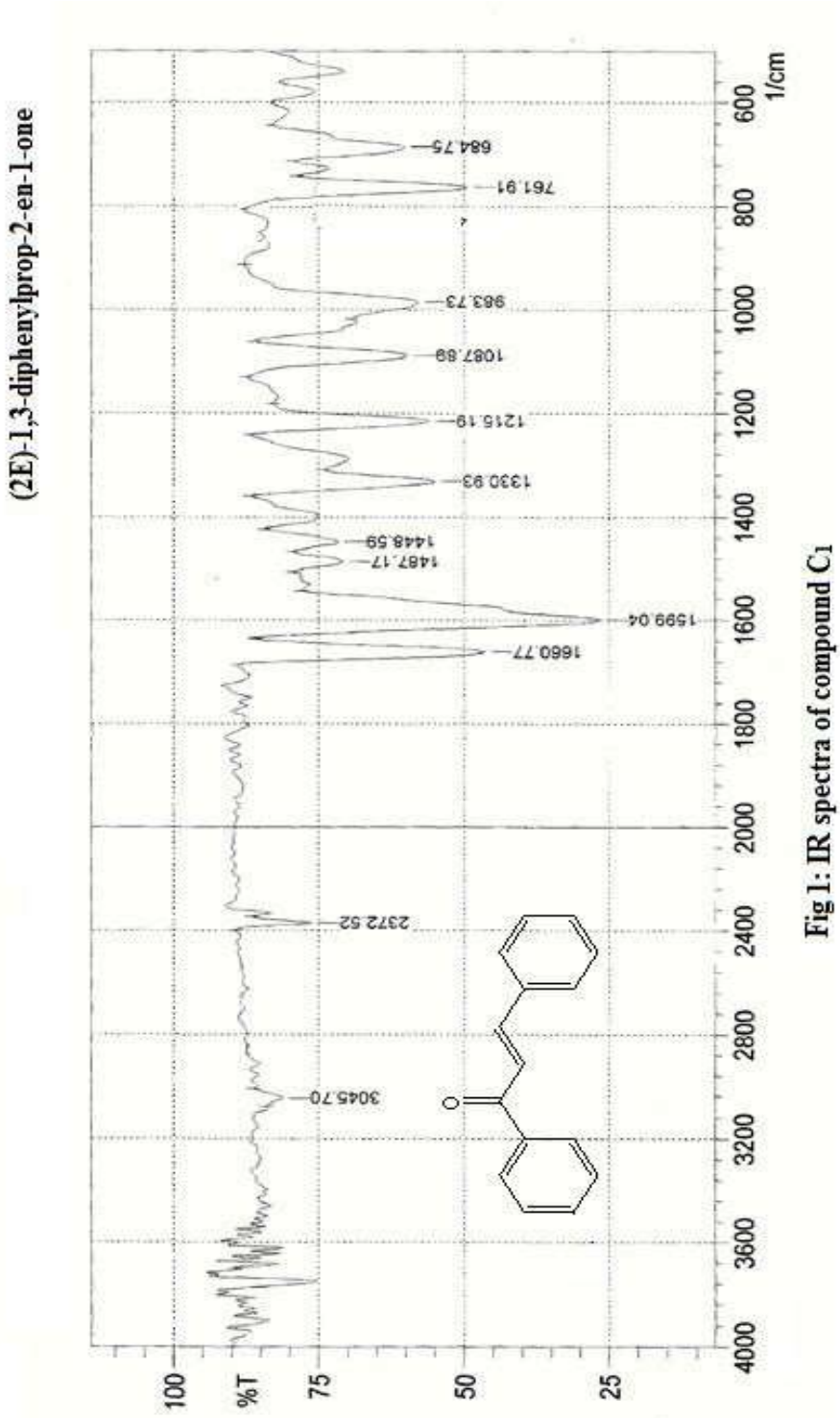


Fig 1: IR spectra of compound C_1

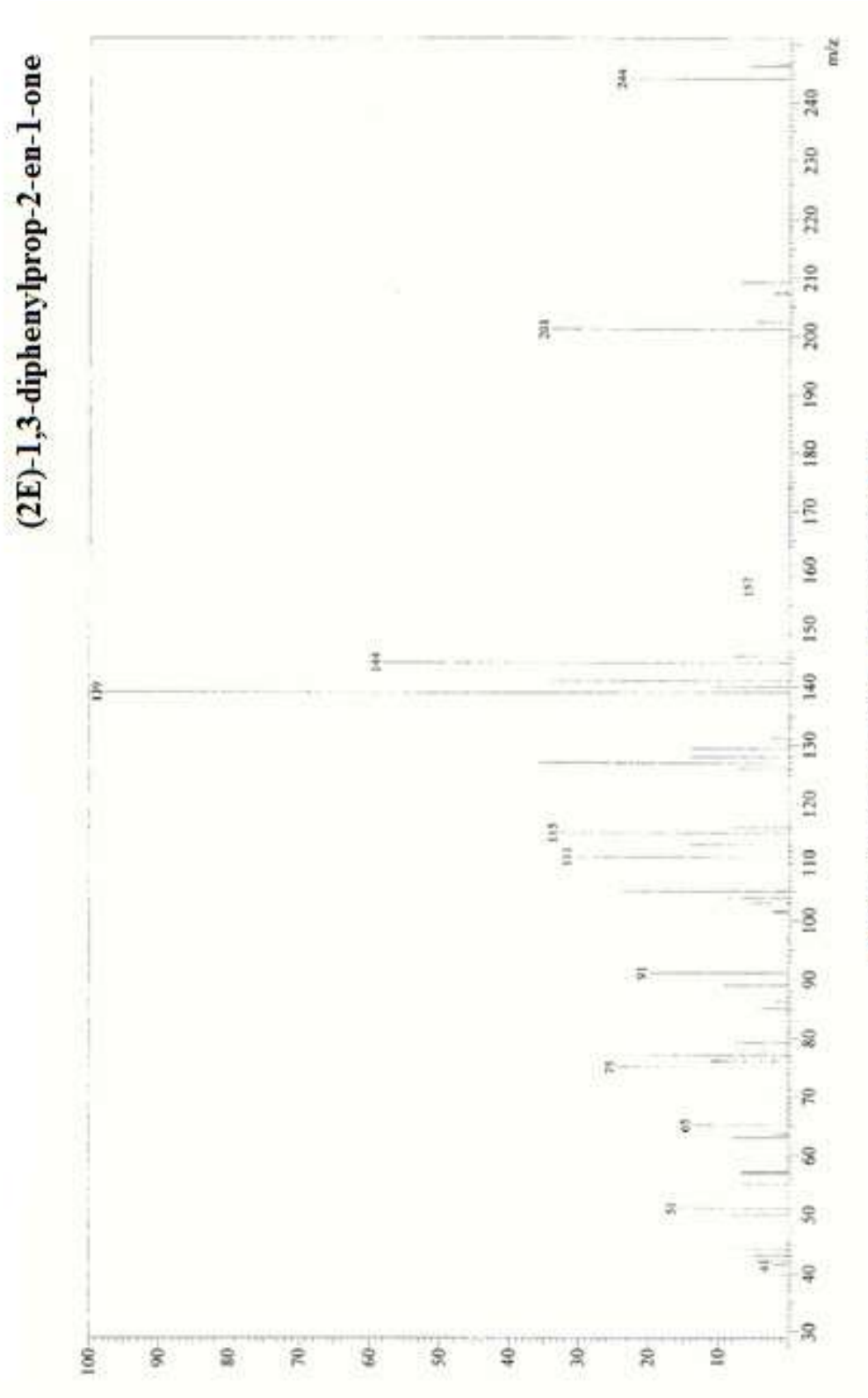


Fig 3: Mass spectra of compound C_1

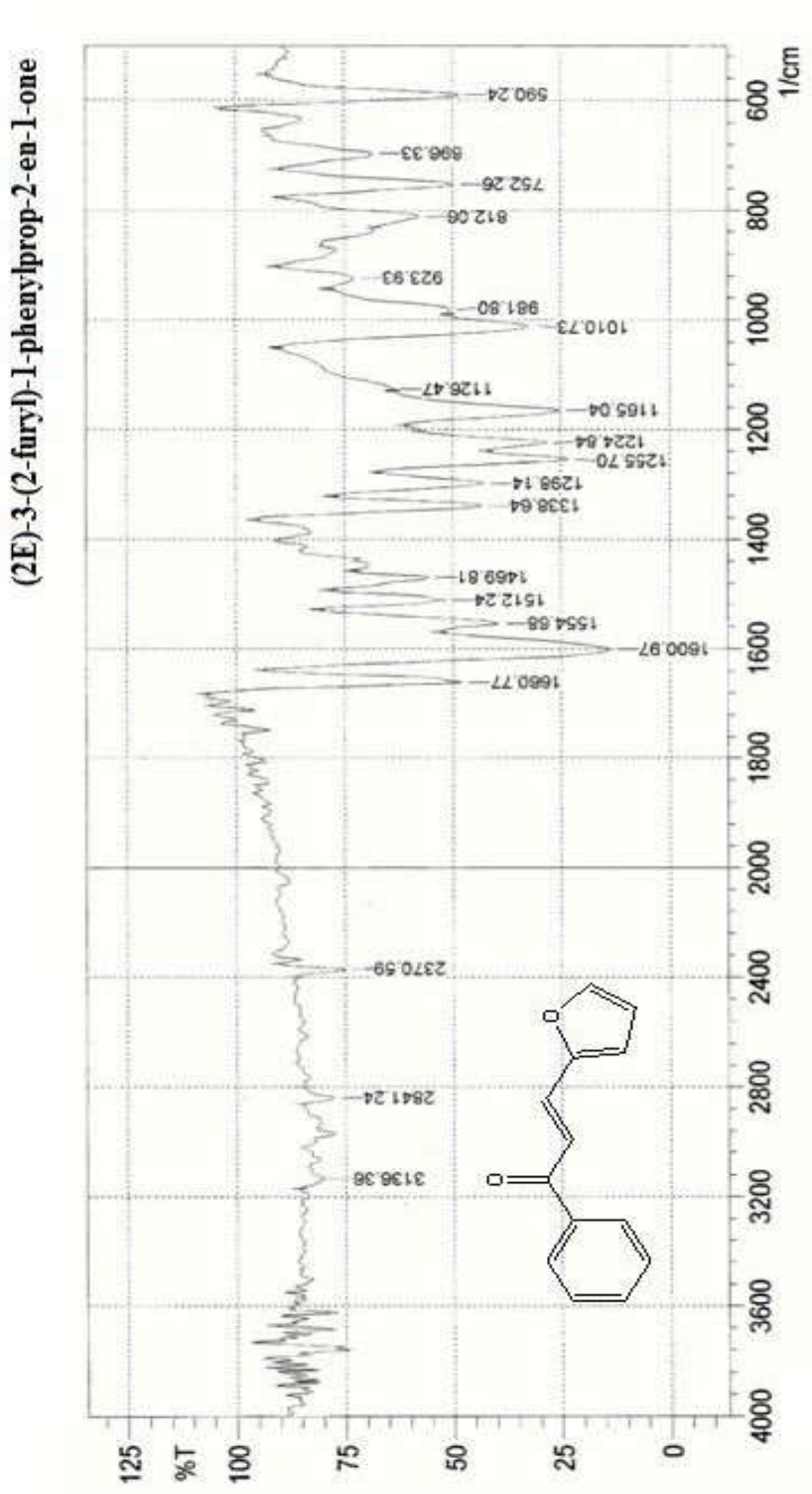


Fig 4: IR Spectra of compound C_5

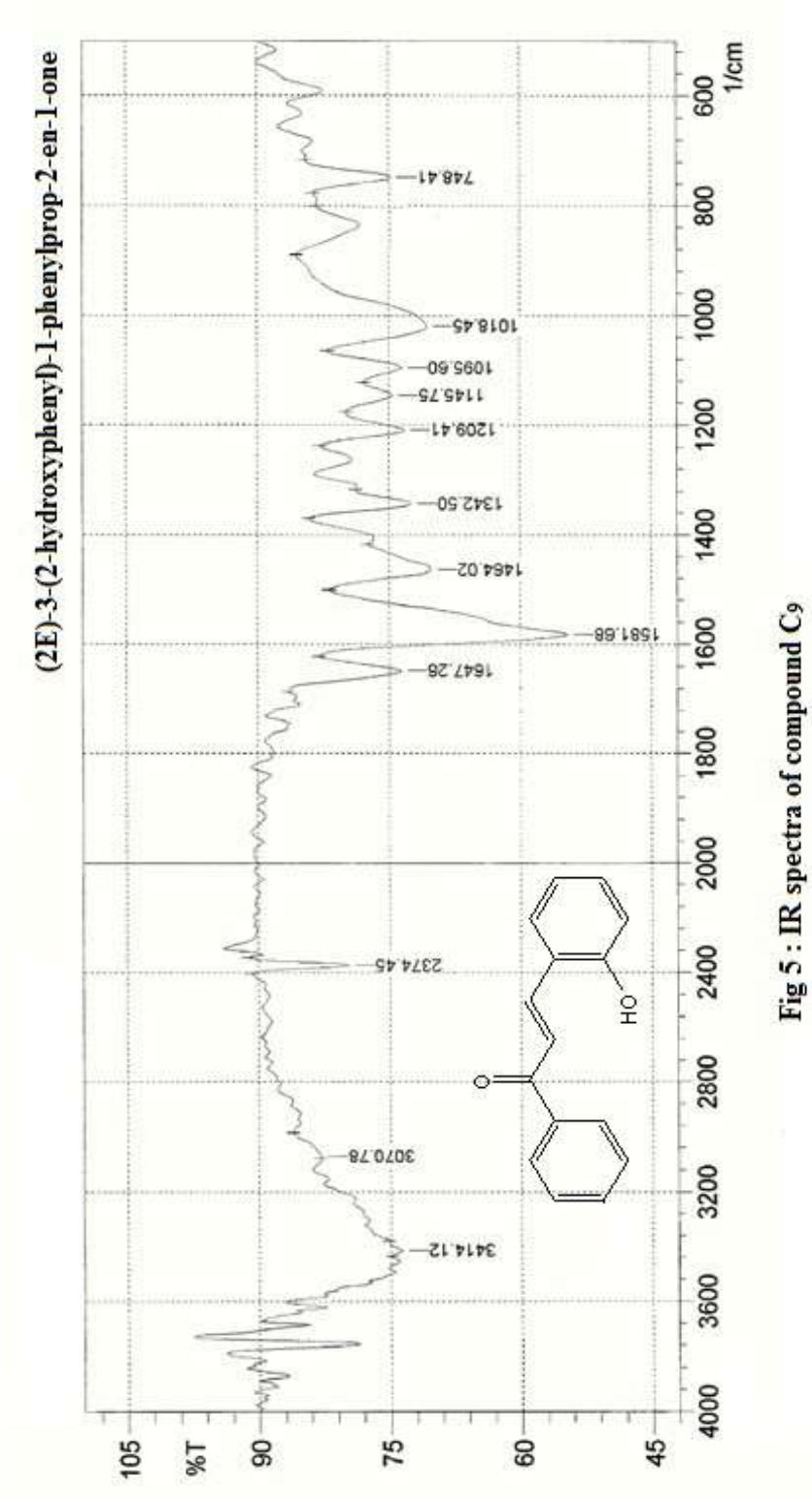


Fig 5 : IR spectra of compound C_9

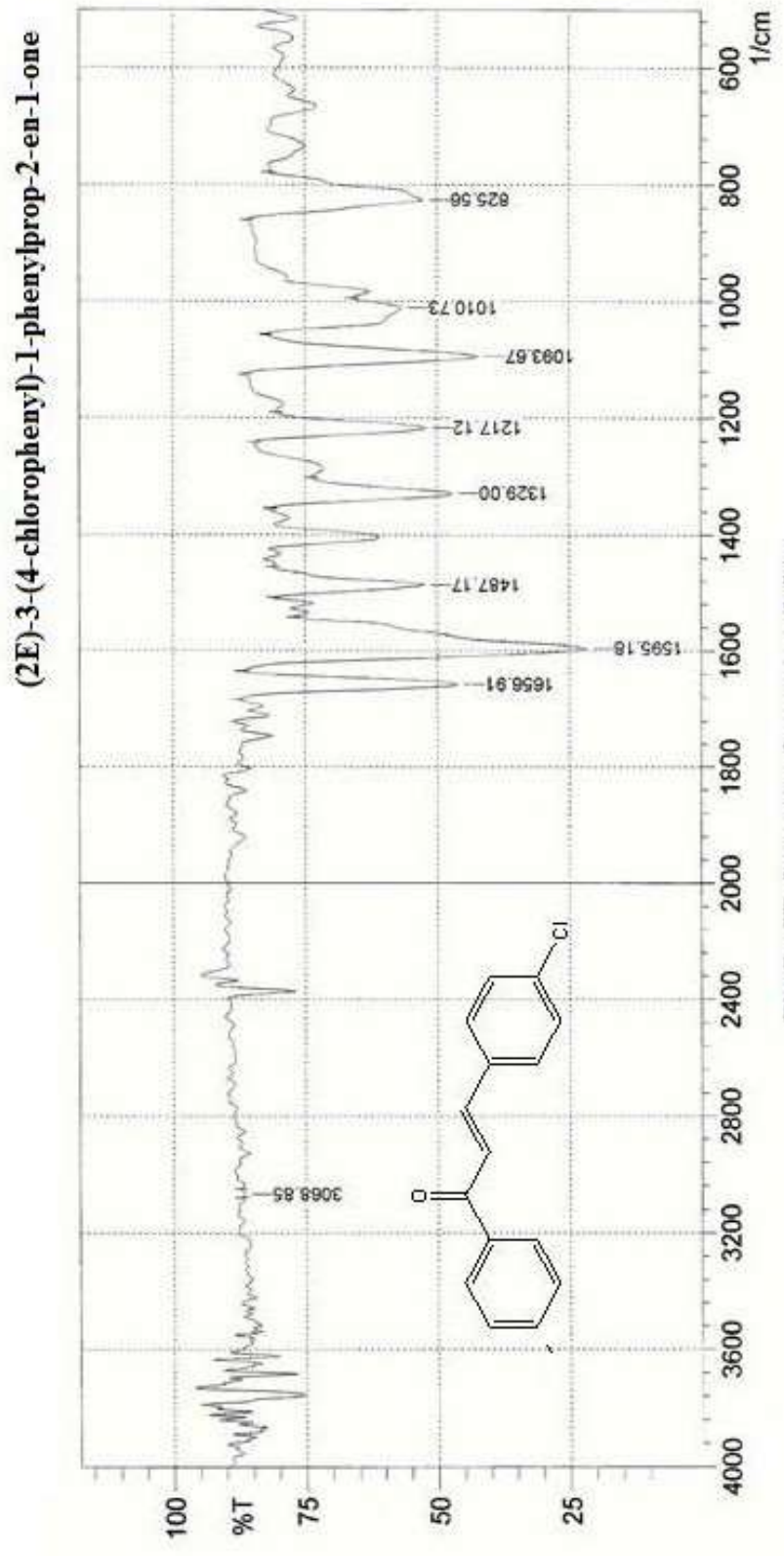


Fig 7 : IR Spectra of compound C_{13}

4.2.1 Characterization of 1,3,5-trisubstiuted pyrazoles:

Table 7 :Spectral data of derivative (P_1):

IR Spectral Data		
Group and mode of vibration	**Frequency in cm^{-1}**	**Expected range in cm^{-1}**
C-H stretch aromatic	3205.69	3200-3010
C-H stretch	3047.53	3000-2840
C=O amide	1660.77	1680-1630
C=N	1489.75	1580-1540
C=C aromatic	1415.75	1600-1475
$-NH_2$ stretch	3313.62	3500-3100
-NH stretch	3501.89	3500-3100
Mono substituted phenyl ring	750.33	700-800
^{1}H NMR spectral data		
Proton of carbon/peak splitting	**ppm value (δ)**	**Expected range**
4H,d,methyene of pyrazoline	1.26, 1.68	1.8-2.1
1H,s,methane of pyrazoline	5.30	4.5-5.5
4H,d,methylene side chain	4.81	4.5-5.5
10H,m,Ar-H	6.82-7.37	6.0-7.6
2H,s,NH_2	1.53	1.5-2.5
1H,s,NH	8.33	8-12
Total no. of protons		**20 Protons**

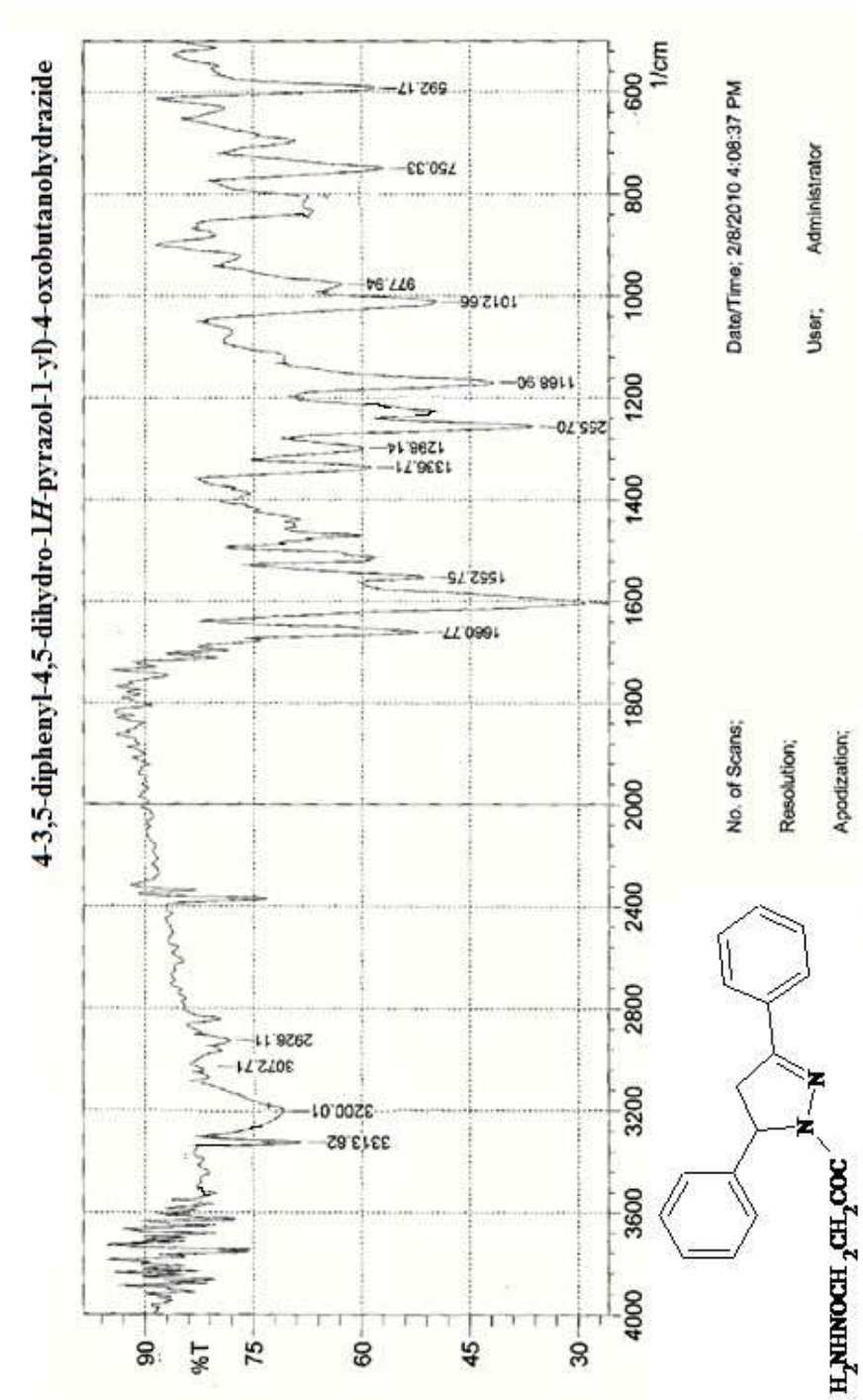


Fig 8: IR Spectra of derivative P_1

Table 8: Spectral data of derivative (P_2):

IR Spectral Data		
Group and mode of vibration	**Frequency in cm^{-1}**	**Expected range in cm^{-1}**
C-H stretch aromatic	3026.69	3200-3010

C-H stretch	2926.01	3000-2840
C=O amide	1666.50	1680-1630
C=N	1552.75	1580-1540
C=C aromatic	1602.85	1600-1475
-NH_2 stretch	3474.64	3500-3100
-NH stretch	3501.89	3500-3100
Ar-Cl	873.75	885-540
Mono substituted aromatic ring	758.12	700-800
^{1}H NMR spectral data		
Proton of carbon/peak splitting	**Ppm value (δ)**	**Expected range**
1H,s,Ha,pyrazoline ring	3.7	3.5-4.5
1H,s,Hb,pyrazoline ring	3.8	3.5-4.5
1H,s,methane of pyrazoline	5.10	4.5-5.5
4H,d,methylene side chain	2.35-2.49	2.1-2.5
9H,m,Ar-H	6.96-7.76	6.0-7.6
2H,s,NH_2	1.58	1.5-2.5
1H,s,NH	8.95	8-12
Total no. of protons		**19 Protons**

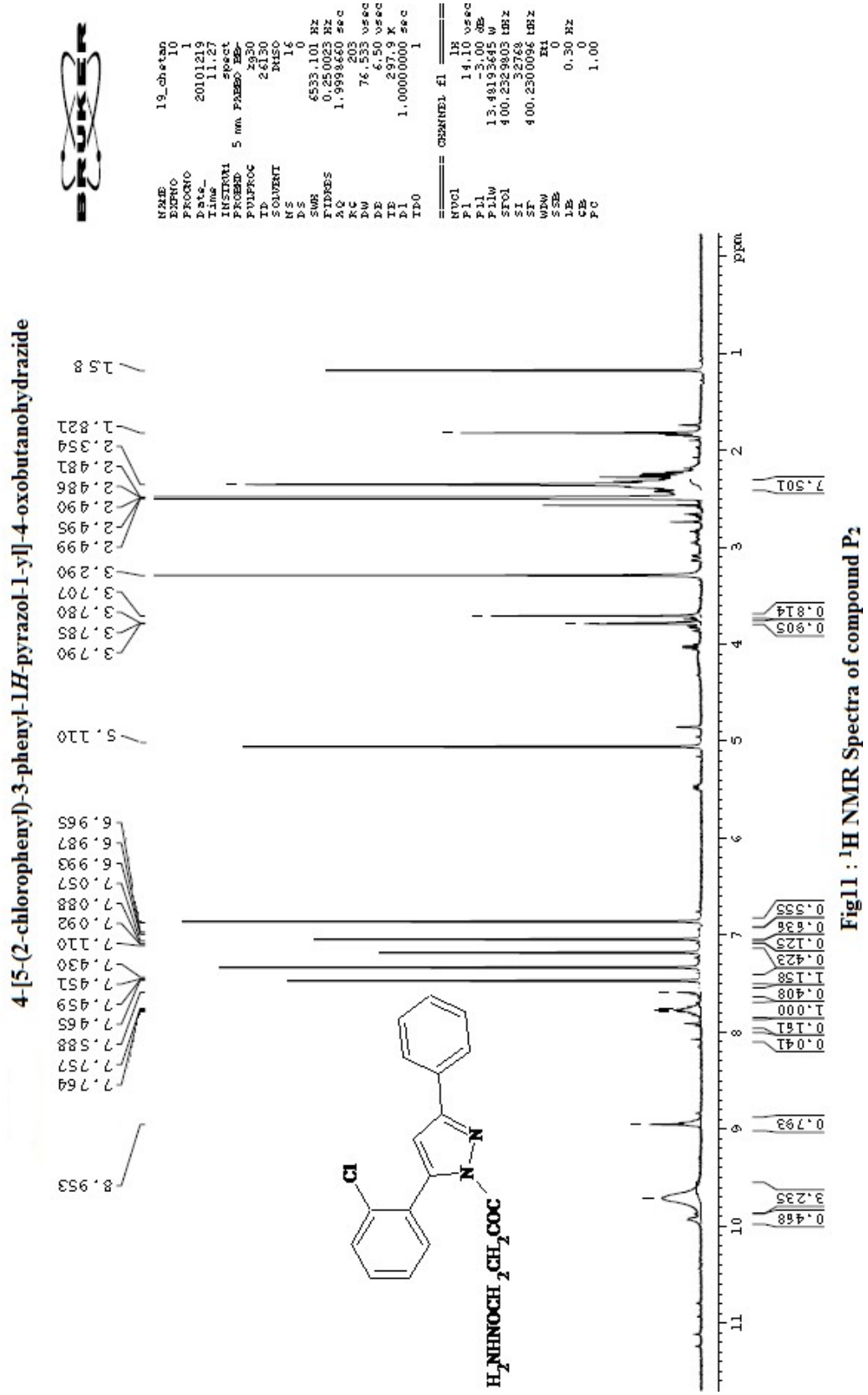


Fig11 : ^{1}H NMR Spectra of compound P_2

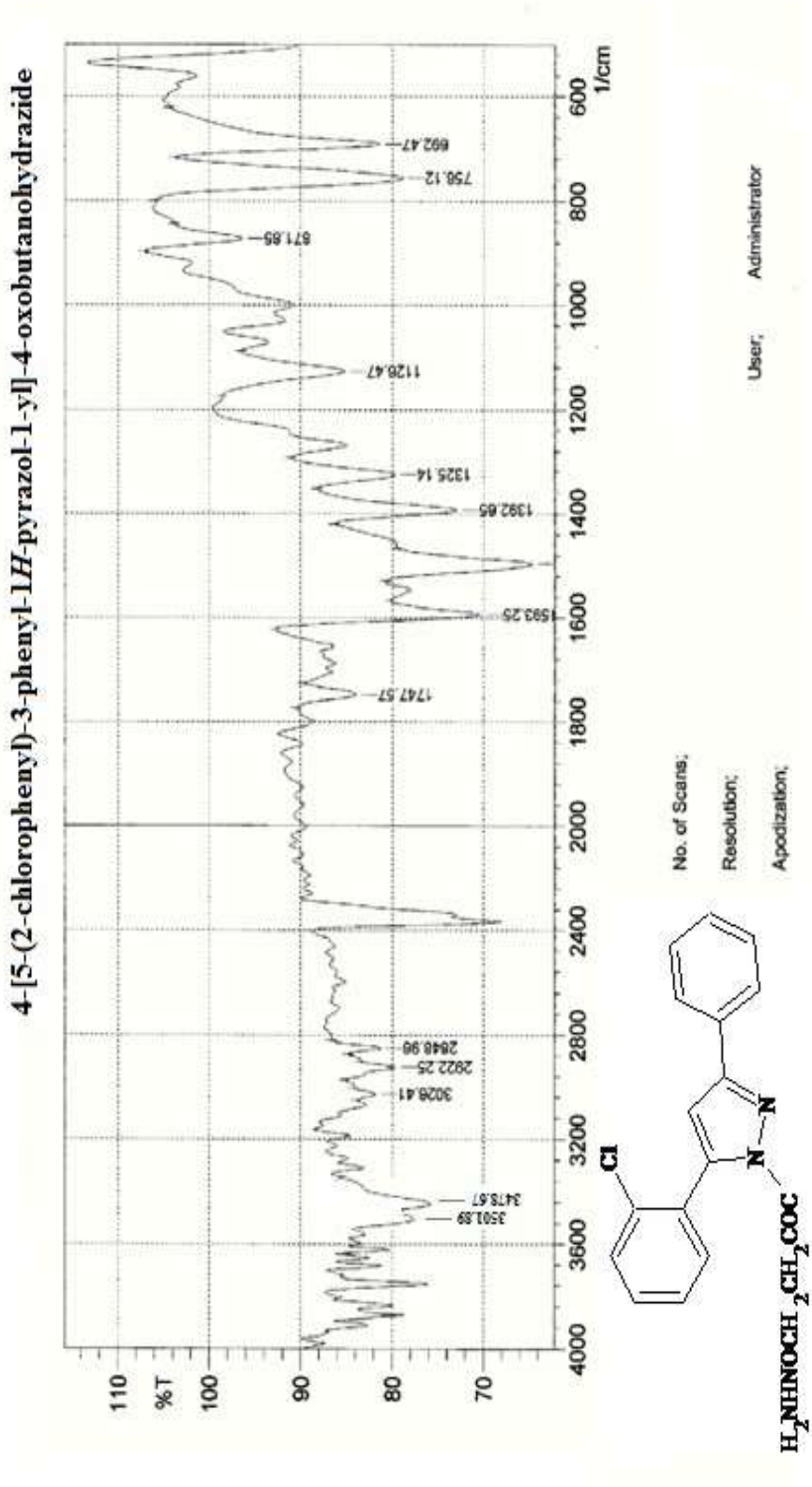


Fig 10: IR Spectra of derivative P_2

Table 9 : Spectral data of derivative(P_3):

IR Spectral Data		
Group and mode of vibration	**Frequency in cm^{-1}**	**Expected range in cm^{-1}**
C-H stretch aromatic	3064.99	3200-3010
C-H stretch	2993.62	3000-2840
C=O amide	1658.84	1680-1630
C=N	1575.89	1580-1540
C=C aromatic	1458.23	1600-1475
-NH_2 stretch	3362.23	3500-3100
-NH stretch	3479.89	3500-3100
Ar-O-C	1213.27	1300-1000
Ar-O-CH_3	1276.92	1300-1000
Mono substituted ring	779.27	700-800
Mass m/z		
Molecular ion peak	345	
Base peak	297	
Other prominent peaks	315,269,241,229,155,93,77	

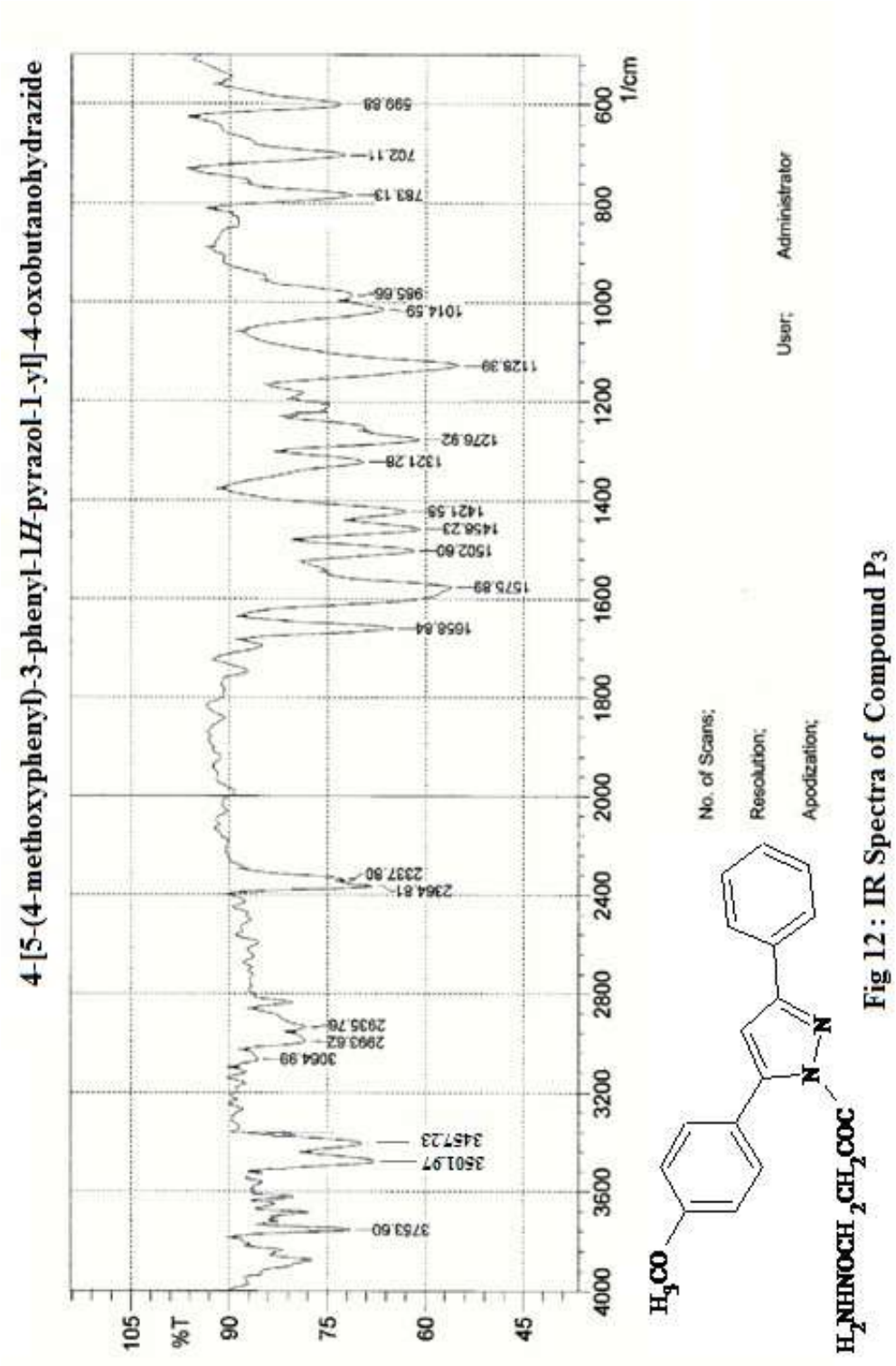


Fig 12 : IR Spectra of Compound P3

User : Admin
Sample : 3
Inj. Volume : 5.000
Data Name : C:\LCMSsolution\User\Data\VIA-APCI-POS1.qld
Method Name : C:\LCMSsolution\User\Method\esi.qlm

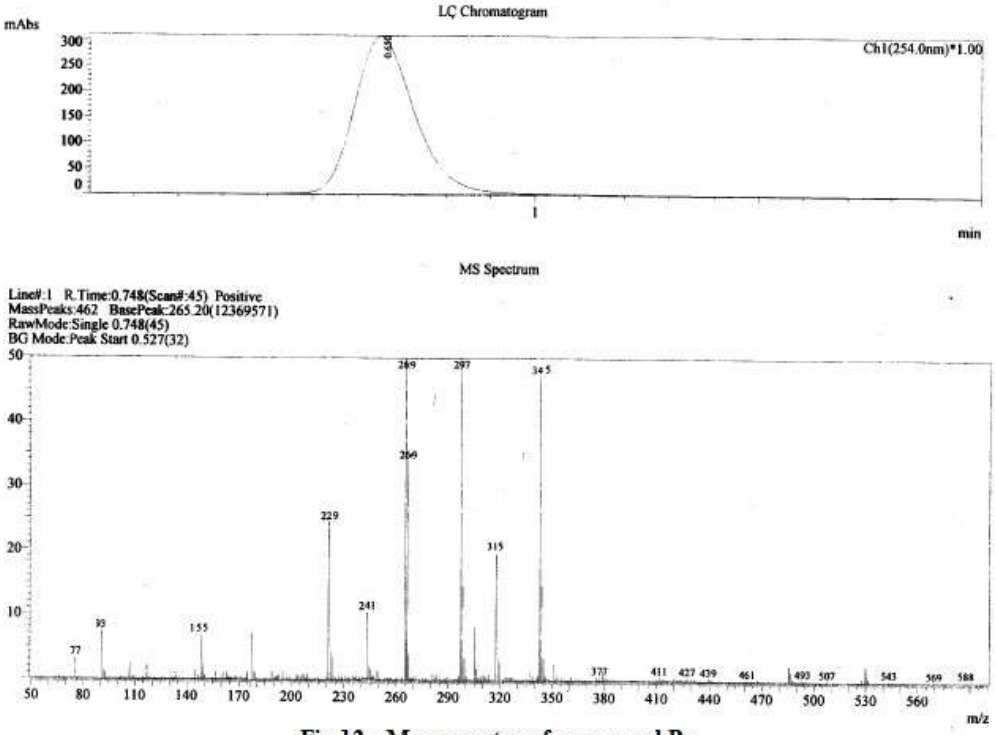


Fig 13 : Massspectra of compound P_3

Table 10: Spectral data of synthesized1,3,5-trisubstituted pyrazole derivatives:

Compound	IR (KBr) in (cm^{-1})	^1H NMR($CDCl_3$) δ in ppm
P_5	3064.99(Ar C-H), 2984.63(C-H), 1660(C=O), 1600.97(C=N), 1585.54(C=C), 3362.23(-NH_2), 3479.89(-NH), 1069.82(C-O-C), 779.27(substituted aryl or hetero aryl ring).	-
P_9	3047.63(Ar C-H), 2924.18(C-H), 1666.69(C=O), 1616.47(C=N), 1456.23(C=C), 3498.63(-NH_2), 3548.52(-NH), 3317.28(-OH), 744.55(mono substituted aromatic ring).	-
P_{12}	3072.71(Ar C-H), 2926.11(C-H), 1600.77(C=O), 1552.75(C=N), 1598.75(C=C), 3424.50(-NH_2), 3498.45(-NH),1168.90(Ar-O-CH_3), 750.33(mono substituted aromatic ring).	1.23,1.54(2H,d, methylene of pyrazoline) 3.04(9H,m,methoxy), 5.16(1H,d,methane of pyrazoline), 6.52-7.66(7H,m,Ar-H), 2.43(4H,d,methylene side chain)
P_{14}	3102.87(Ar C-H), 2963.59(C-H), 1708.99(C=O), 1660.77(C=N), 1516.10(C=C), 3473.74(-NH_2), 3421.83(-NH), 3396.74(-OH), 748.48(mono substituted aromatic ring).	-

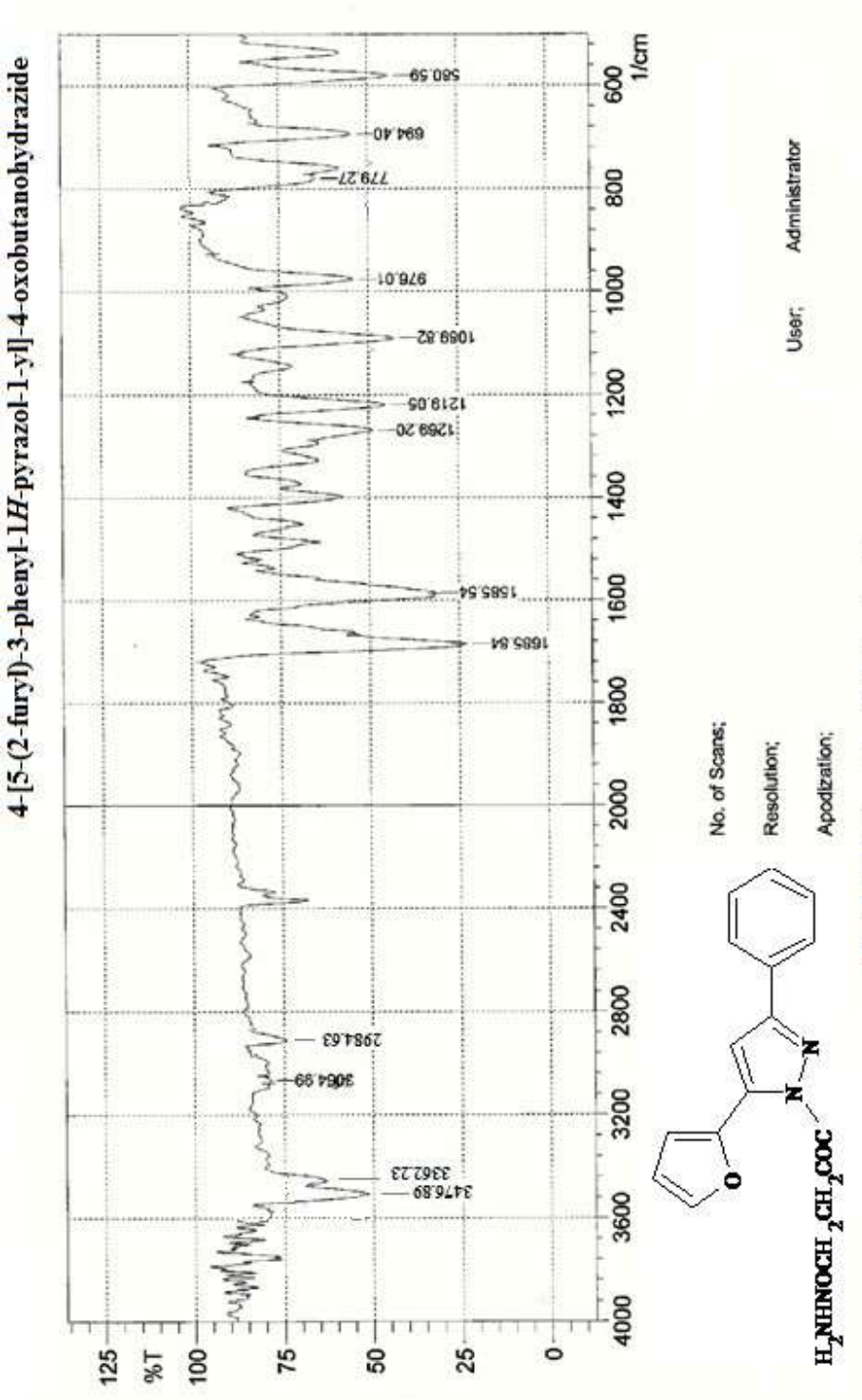


Fig 14 : IR Spectra of derivative P5

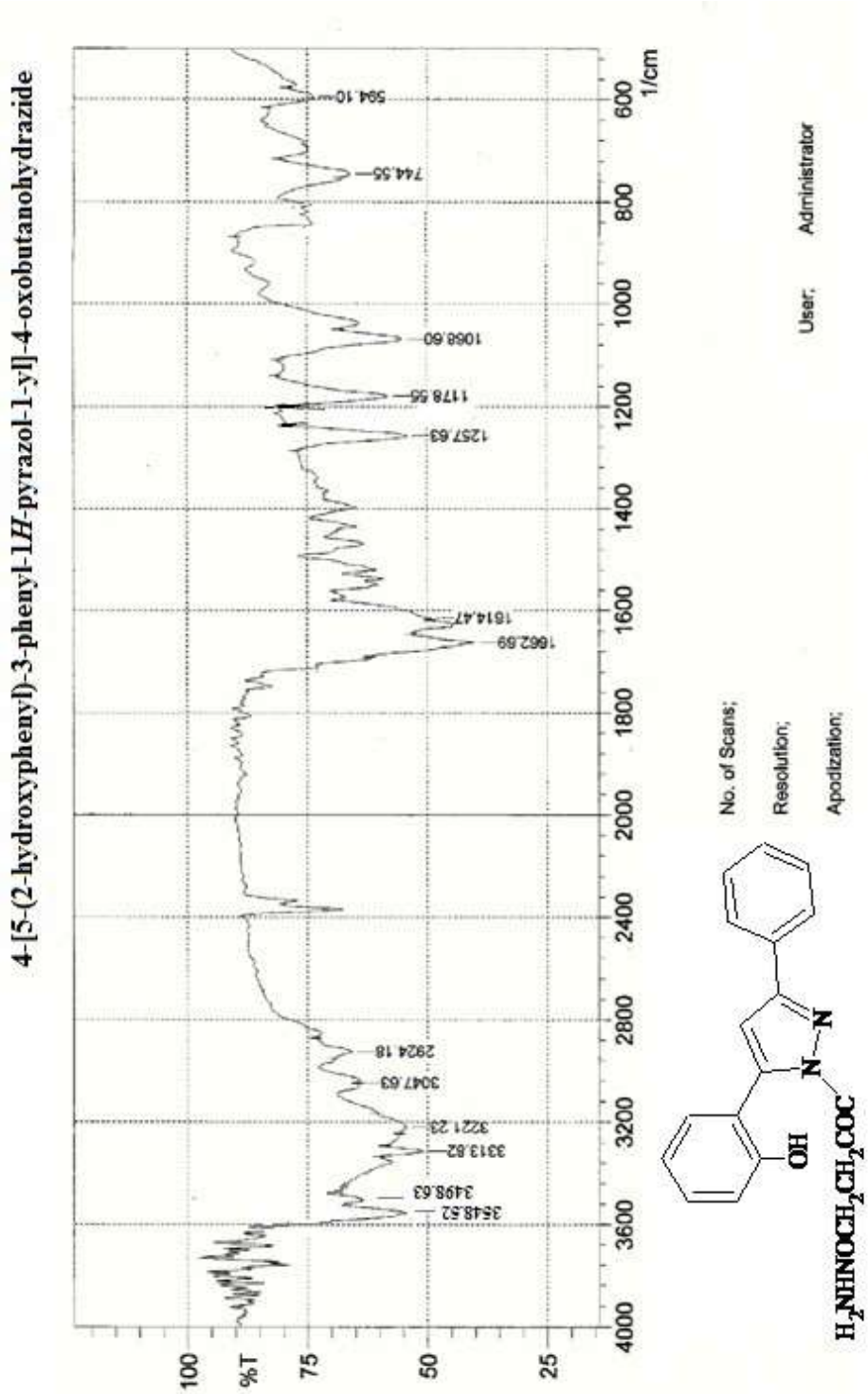


Fig 15 : IR Spectra of compound P9

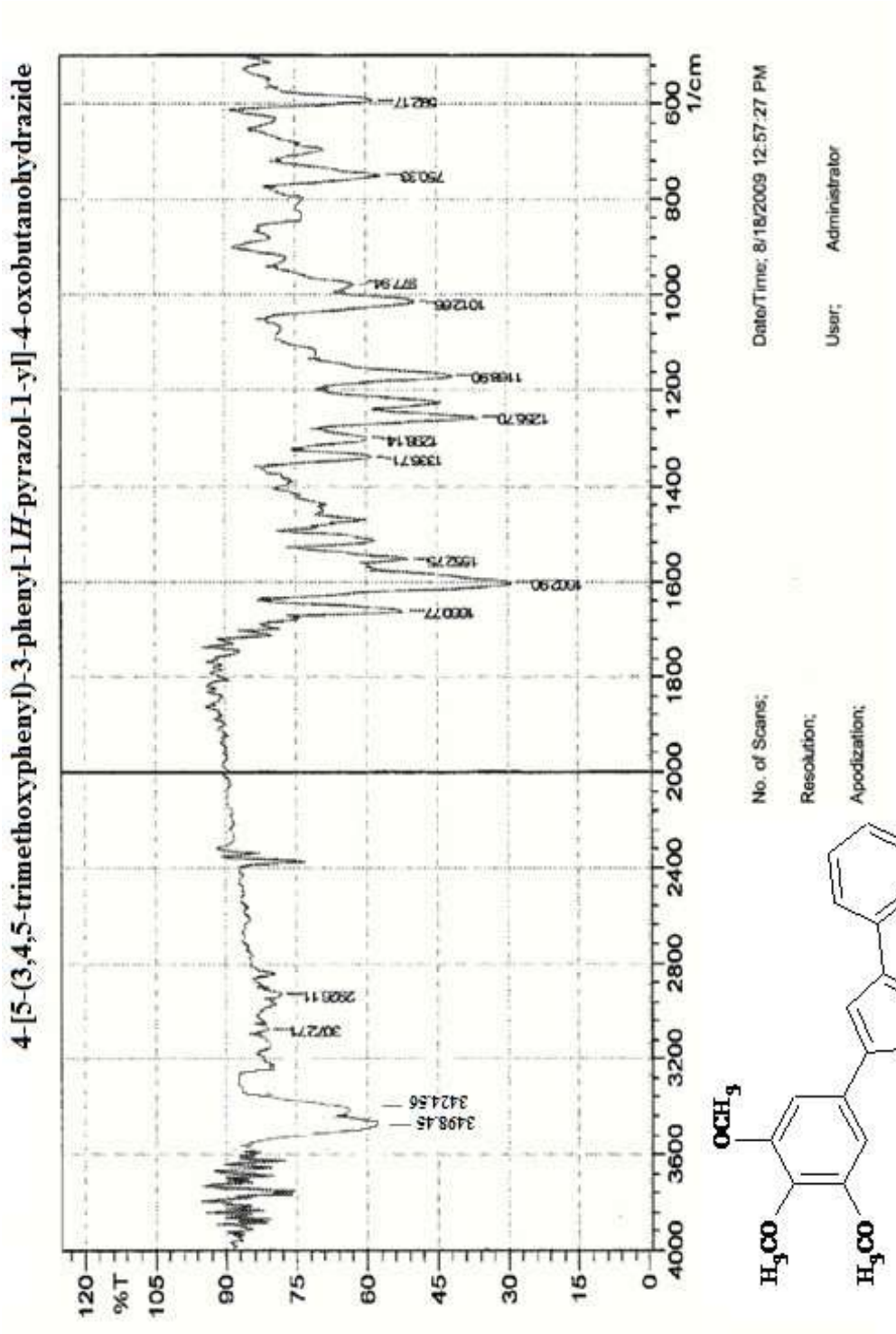


Fig 16 : IR Spectra of derivative P12

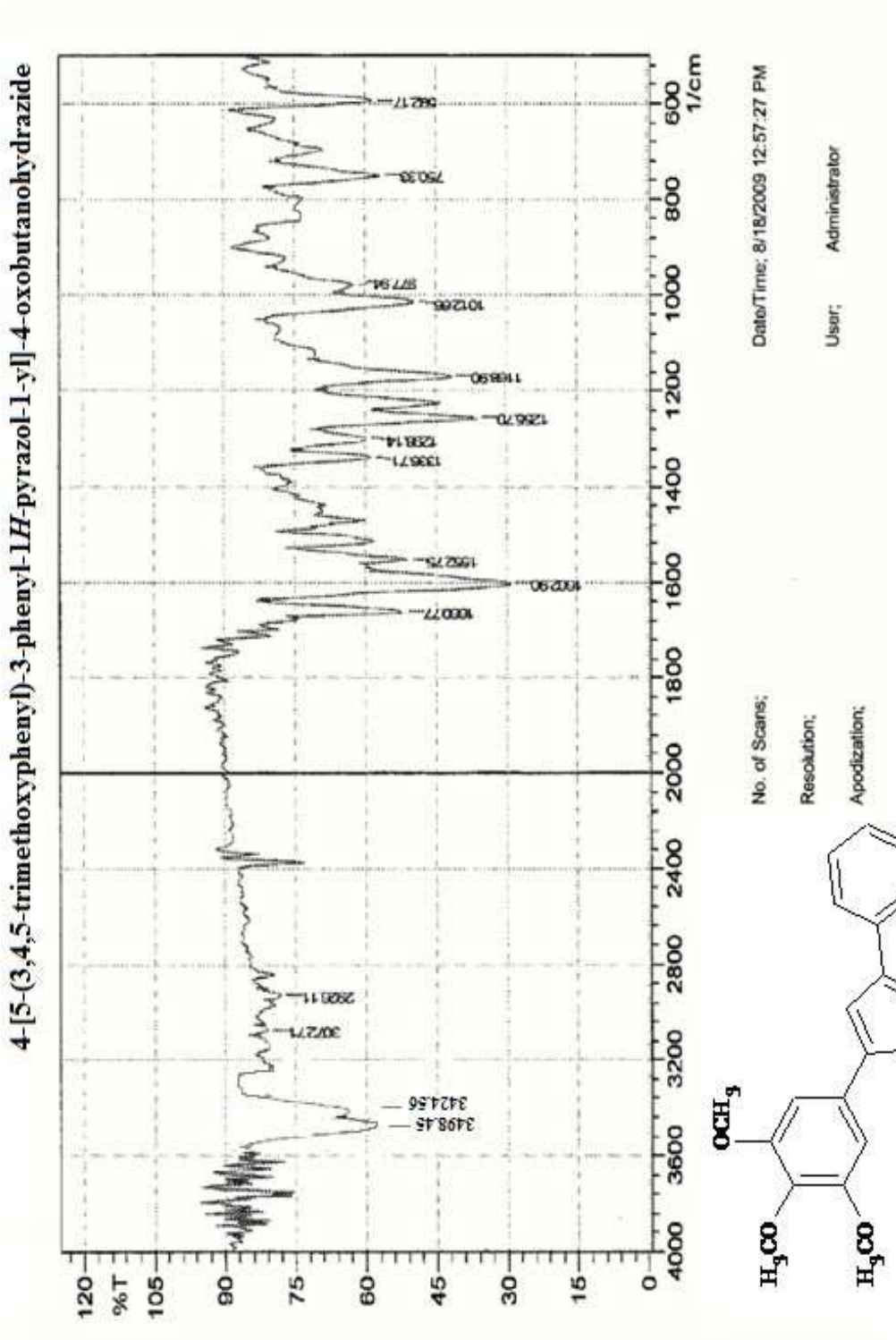


Fig 16 : IR Spectra of derivative P12

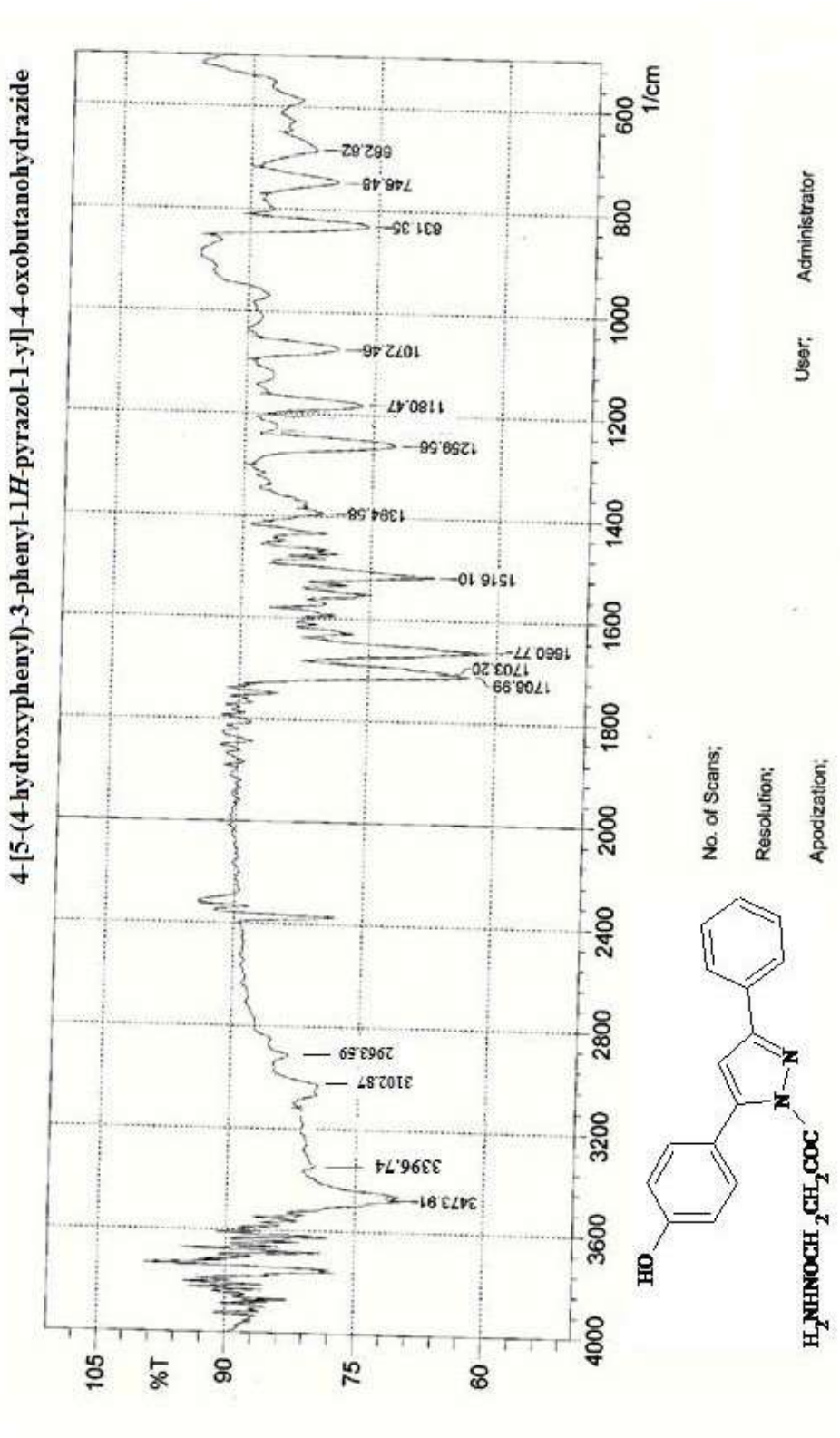


Fig 18 : IR Spectra of derivative P_{14}

BIOLOGICAL ACTIVITY

5.1 ANTIMICROBIAL STUDY:

Chemotherapeutic agents was initially restricted to antibiotics, but now since microbial metabolites have been isolated for their antimicrobial activity. Synthetic and microbiologically produced drugs or lead molecules to need to be included together. Antimicrobial agent to design as synthetic and natural products obtained drugs that effective against growth of microorganisms.

Antimicrobial agents occupied of uniqueness in history of medicine. Exponential develops in antibacterial field is higher and effective results shows by sulfonamides and penicillin's antibiotics.

Antimicrobial agent screening can be done in two ways:

1. Dilution or turbid metric technique.
2. Diffusion of agar

Method of turbidimetry:

Agar diffusion method:

The agar diffusion method determines the microorganism's amount of proliferation after inoculation into a solid nutritional agar bed by an antibiotic agent. The test chemical or molecules are placed in an agar bed cup, where they diffuse and impede microbial growth. In comparison, the diameter of the zone of inhibition is proportional to the concentration of drug ingredients added to the drug or the potency of the reference drug. The diffusion coefficient of antimicrobials in the agar cup, the sensitive of the microbe to the test chemical, and a proper temperature are all determined by the thickness of the agar bed and the diameter of the cup. Sterilization and chilling at 42°C, incubation with test organism in suitable fluid, properly mixed, then placed in a petri dish and chilled. Bores are bored into it, and a test solution is injected before it is allowed to sit at a temperature of 24 degrees Celsius for 30 minutes. Zone of inhibition is measures in millimetres after a 24-hour incubation period at 37°C.

Methods of obtained pure cultures

- Spreading bacteria over a sterile solid surface, such as an agar plate, to choose and transfer offspring of a single cell to sterile medium is the streak plate technique for achieving pure culture.

- Pour plate method of obtain pure culture entailed serial dilution, which was then transferred to melt agar, where a particular volume of the dilution contained organisms and cells were picked out from the agar colony.

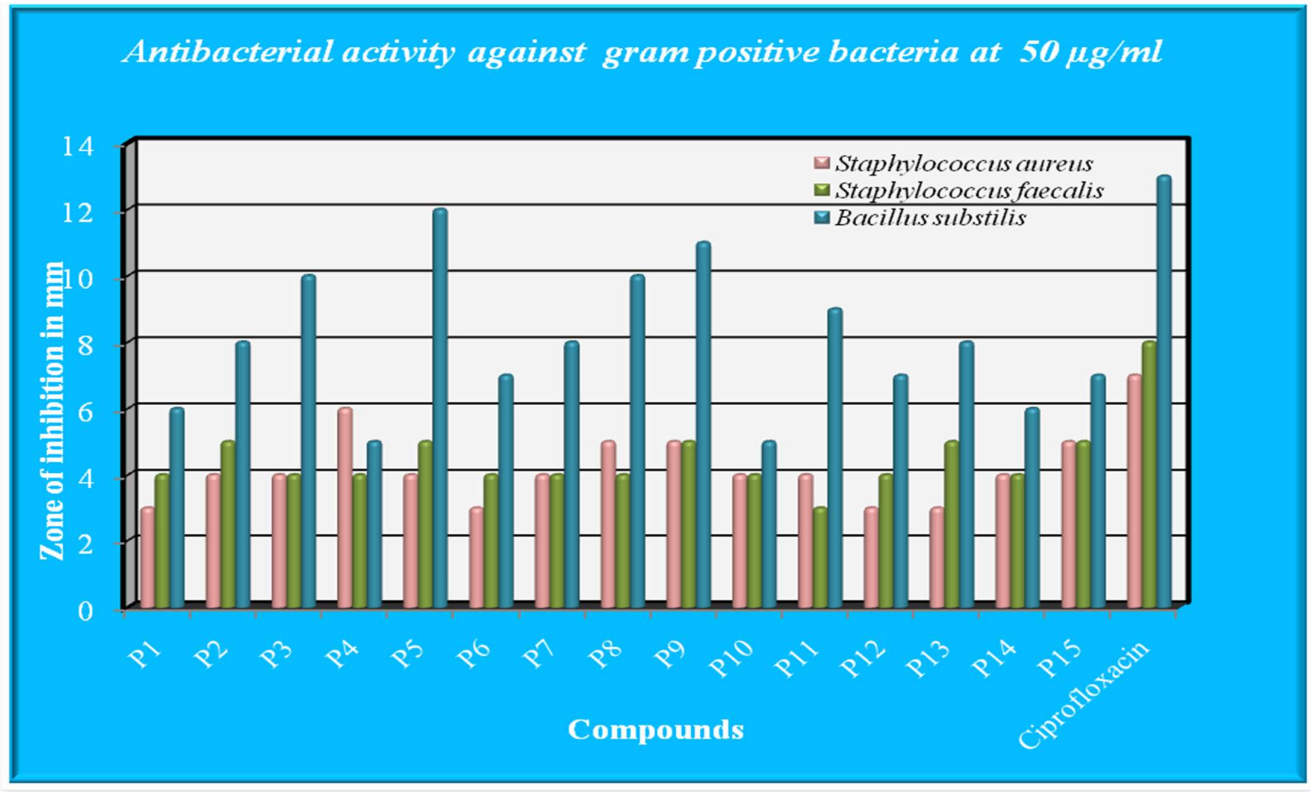


Fig 19: Graph showing Zone of inhibition of the molecules against gram Positive bacteria

Fig 20: Zone of inhibition of derivatives against *Staphylococcus aureus*

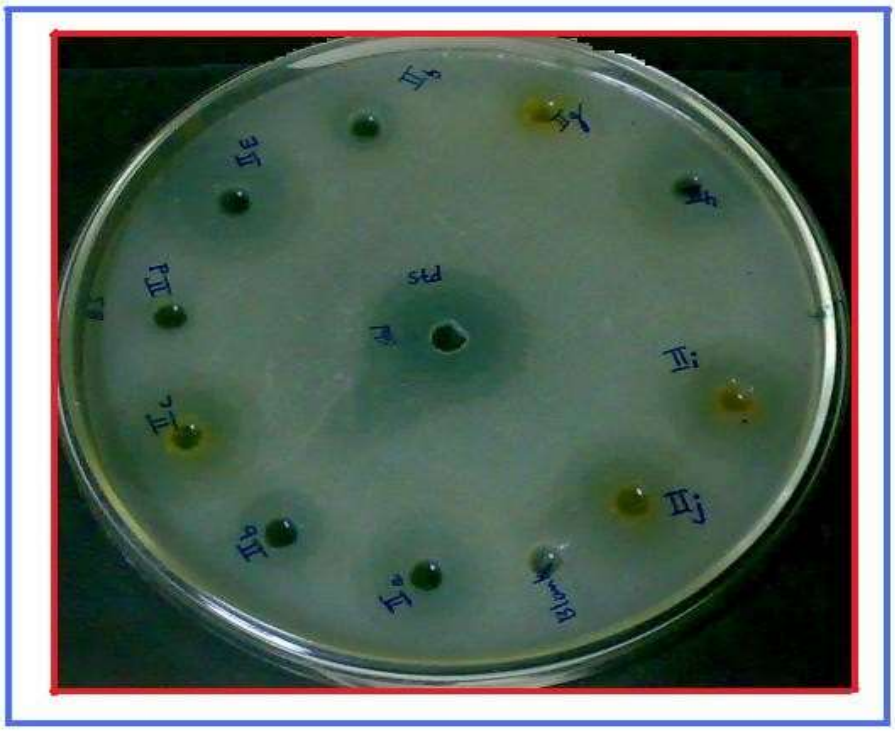


Fig 21 : Zone of inhibition of derivatives (P_1-P_{10}) against *Bacillus subtilis*.

Table 12: Antibacterial activity of substituted pyrazole moiety based derivatives against gram negative bacteria

COMPOUND	Zone of inhibition in mm			
	E. coli		*K. penumoniae*	
	50µg	**100µg**	**50µg**	**100µg**
P_1	3	5	4	5
P_2	5	7	5	6
P_3	4	4	4	5
P_4	5	6	4	4
P_5	4	4	5	6

P_6	3	5	4	4
P_7	5	8	6	10
P_8	5	6	4	6
P_9	5	5	5	6
P_{10}	4	4	4	6
P_{11}	6	8	3	7
P_{12}	3	5	4	7
P_{13}	3	6	5	8
P_{14}	6	9	4	6
P_{15}	5	6	5	7
DMSO	-	-	-	-
Ciprofloxacin	7	10	8	12

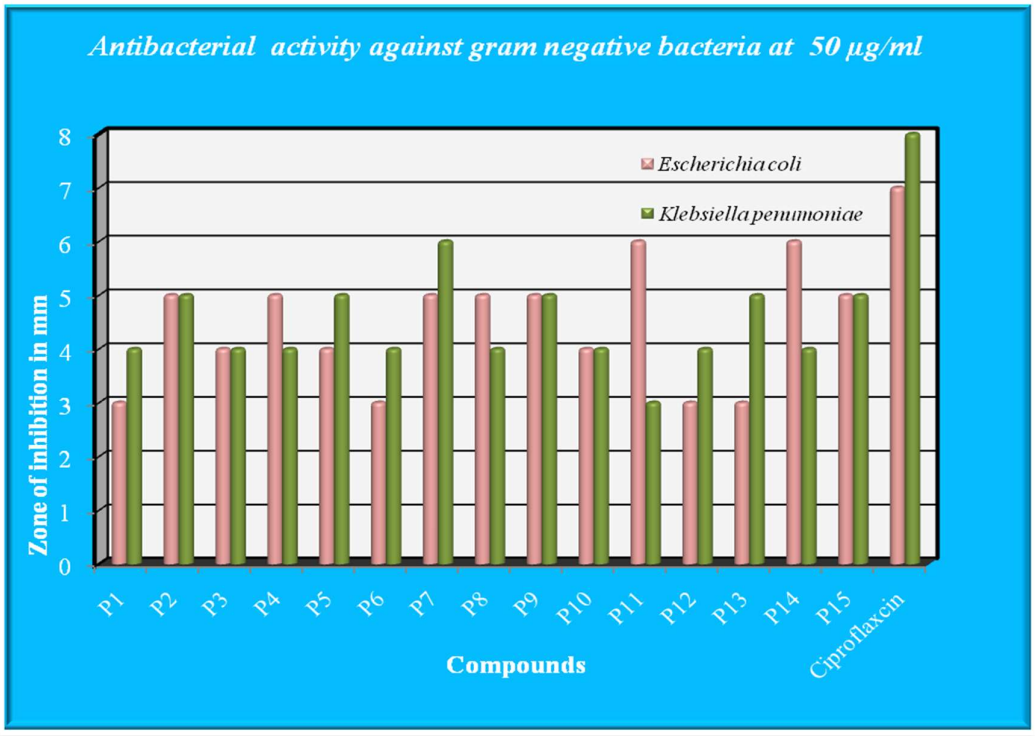


Fig 22 : Graph showing Zone of inhibition of the molecules against gram negative bacteria

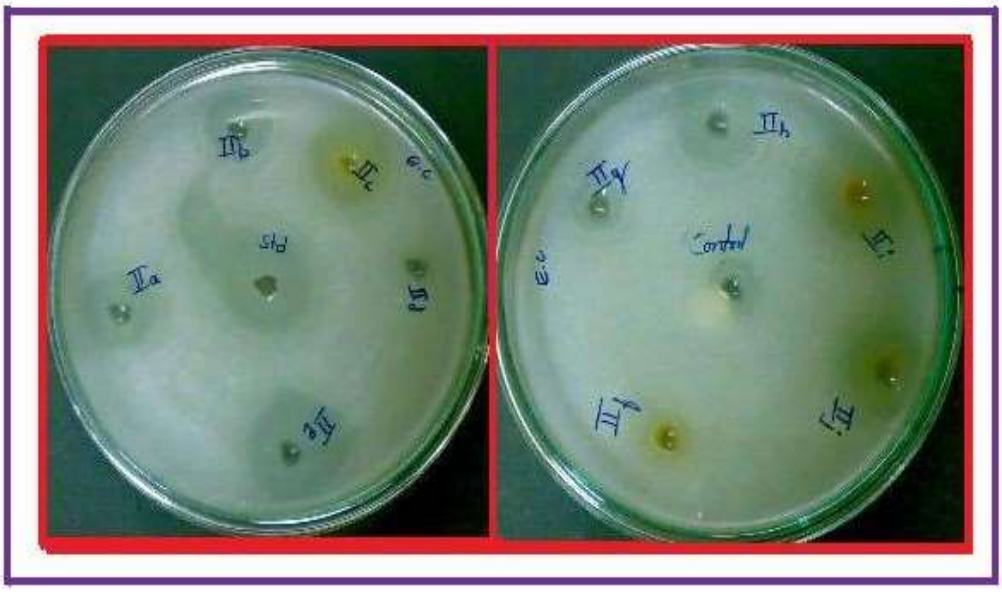


Fig 23: Zone of inhibition of derivatives against *E.coli*

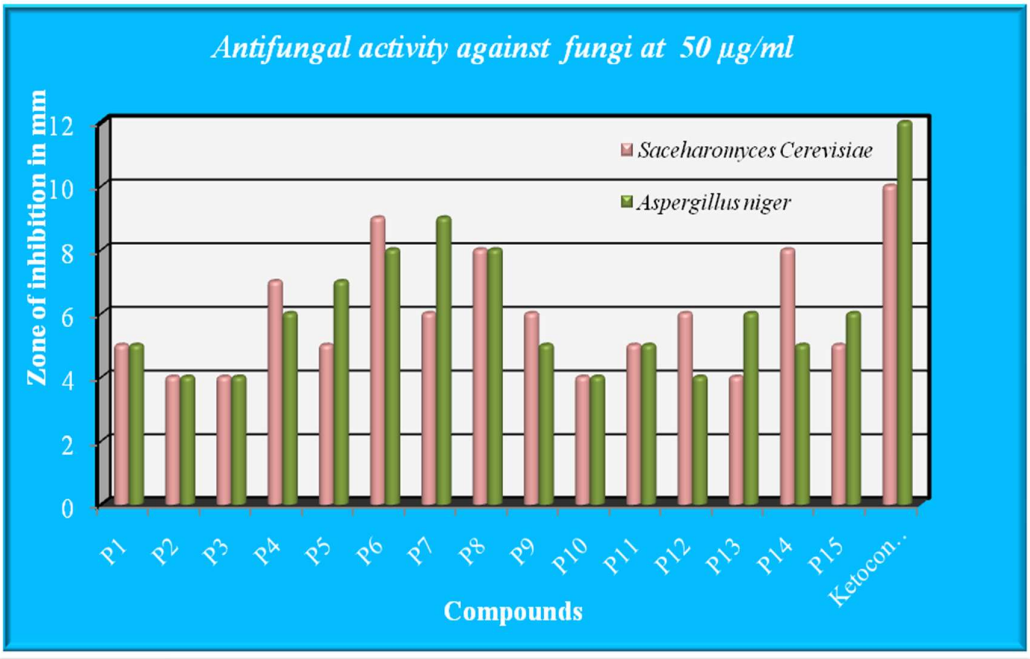


Fig 24 : Graph showing Zone of inhibition of the synthesized derivatives against fungi

RESULTS AND DISCUSSION

Azoles are mostly shows antibacterial and antifungal activities. Major antibacterial and antifungal drugs present in market to contain azoles moiety core. Some of molecules are in clinical trials for development of new leads against various pharmacological activities..

Synthesis of pyrazole derivatives required refluxing of two moieties in alcohol used as solvent for 5-15 hrs depends on their reactivity, hence time consuming, it is important to develop a simple methodology techniques and procedure to speed up the synthesis of pyrazoles for their biological activities.

6.1 BIOLOGICAL ACTIVITY:

Antibacterial activity:

According to data found from antibacterial evalution results compounds (P_1-P_{15}) have showed mild to good effective activity against microorganisms for use as testing, compound P_1, P_3, P_9, P_{10} and P_{12} shows mild activity and P_2, P_6, P_8, P_{13} showed moderate activity and P_4, P_{11}, P_{14} showed good activity against gram positive bacteria. Compound P_1, P_3, P_9, P_{12}, P_{13} showed mild activity P_2, P_4, P_8, P_9, P_{12} showed moderate activity and P_7, P_{11}, P_{14}, P_{15} showed good activity against gram negative bacteria.

Antifungal activity:

Antifungal activity data suggests that synthesized compounds (P_1-P_{15}) have showed mild to good effective activity against tested organisms.Compound P_1, P_2, P_{13} shows mild activity and P_4, P_{10}, P_{11}, P_{12} showed moderate activity and P_5, P_6, P_7, P_8, P_9, P_{14} showed good activity against fungi.

However, further studies on activity and long term toxicity are to be carried out before any conclusion are drawn, as these categories of drug are known to have potential antimicrobial activity. Testing on different models can further substantiate the antimicrobial activity of the synthesized analogues.

SUMMARY

New drugs are development and discovery against various disease state or disorders through following techniques Computer aided drug design(CADD) techniques as QSAR, Molecular modeling and helpful combinatorial chemistry in the synthesis for drugs largely minimum time required for the primary screening of the drug molecules.

Mostly marketed drugs antibacterial and antifungal drugs are azoles are mostly widely used and some molecules for evalution in clinical trials for antimicrobial agents or other pharmacological activities. Azoles resistant strains leads to discovery a new antimicrobial compounds. 1, 3, 5 trisubstituted pyrazole molecules in current years research for extensively evalution for the development of others lead molecules for antimicrobial activity.

Synthesis:

Condensing acetophenone with various aromatic aldehydes and 40 % NaOH and ethanol at 5-10 °C reaction mechanism goes through Clasien-Schmidt condensation produced a series of chalcones (C_1-C_{15}).Succinichydrazide was produced by combining succinic acid and hydrazine hydrate in ethanol then refluxing both along. Acetic acid was used as a catalyst in the cycloaddition reaction of chlacones with succinichydrazide to yield 1,3,5-trisubstituted pyrazole moiety derivatives (P_1-P_{15}).

Biological activity:

Antibacterial activity: According to data obtained from antibacterial activity all synthesized molecules of 1,3,5-trisubstituted pyrazole core based (P_1- P_{15}) have shows mild to good activity against various gram positive and gram negative microbes used for testing. Among 1,3,5- trisubstituted pyrazole compounds, P_4 bearing 4-dimethylamino aryl group at 5th position of the pyrazole ring has shown good activity against *Staphylococcus aureus*, compound P_{13} bearing 4-chloro aryl at this position has shown good activity against *Staphylococcus faecalis* and compound P_5 containing furan ring at this position has shown good activity against *Bacillus substilis* for gram positive bacteria.

Antifungal activity: According to we found our biological data from antifungal evalution activity results trisubstituted pyrazole moiety based compounds (P_1-P_{15}) have shows mild to good activity against organisms used in testing. Compound P_7 bearing 4-dimethylamino aryl group at 5th position carbon of the pyrazole ring has shown good activity against both *Saceharomyces Cerevisiae* and *Aspergillus niger* and compound P_8 bearing 3-methoxy-4-hydroxy group at this position has shown good activity against *Aspergillus niger*.

CONCLUSION

1,3,5-trisubstituted pyrazoles moiety are pharmacologically importance class of heterocyclic derivatives. The method used in the current studies to develop the best methodology for introduce the substitution at 1,3,5 positions of the pyrazole moiety.

Cycloaddition reaction of chalcones reacts with hydrzides in presence of different reagent and condition to gives 1,3,5- trisubstituted pyrazole compounds (P_1-P_{15}). Cycloaddition was successfully in the reaction was carried by catalyst (acetic acid) and solvent (ethanol). Through conventional method pyrazole derivatives (P_1-P_{15}) were obtained in a good yield.

The antibacterial activity, of derivatives, P_4, P_5 and P_7, P_{14} were shows effect against gram positive microorganism and gram negative microorganisms .

Evalution of antifungal activity, of the synthesized derivatives P_7 and P_8 showed effective results against fungi.

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