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Original article

Synthesis of Pyrazole Derivative Incorporating Fluorine in the Aromatic Substitution for Physiological and Pharmacological Studies

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ABSTRACT

Over the past decade, drug resistance has become a growing problem in the treatment of infectious diseases caused by bacteria, fungi and viruses. In particular, resistance of bacterial pathogens to current antibiotics has emerged as a measure of health problems. This is especially true in the case of infectious diseases such as pneumonia, meningitis and tuberculosis, which would once have been easily treated with antibiotics, is no longer so readily treated. At present, all widely used antibiotic, including some of the agent such as streptogramins and new generation fluoroquinolones are subjected to bacterial resistance. The search for new antimicrobial agent is one of the most challenging tasks to the medicinal chemist. A search for new antimicrobial compounds with potent and minor toxicity continues to be a region of exploration in medicinal chemistry. It has been well recognized that nitrogen containing heterocyclic molecule is a seat of diverse medicinal activities. Heterocyclic nucleus Pyrazole constitutes an important class of compounds for new drug development. The synthesis of these derivatives and investigation of their chemical and biological behavior have gained more importance in recent decades. Owing to the pharmacological importance of Pyrazole, and its derivatives, in the present work, it has been contemplated to combine various biologically active moieties with these heterocycles through active functional systems to form new molecular framework. Prompted by the therapeutic importance of these heterocyclic derivatives, in the present research work we have synthesized Pyrazole with fluorinated aromatic rings, and its derivatives through multistep reaction. Appropriate synthetic methodologies have been developed for the synthesis of new molecules. Their purification techniques have been established. All the newly synthesized compounds have been characterized by 1H NMR, 13C NMR, mass spectral, IR and elemental analyses. Also structures of a few molecules have been confirmed by X-ray crystallographic analysis. Further, the target molecules have been evaluated for their in vitro antibacterial and antifungal activity. From the pharmacological evaluation, it has been observed that some of Pyrazole, derivatives showed good antimicrobial activity.

Keywords: *Pyrazole; Antibacterial; Antifungal studies; Thin layer chromatography; HPTLC*

Introduction

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 [1].

Pyrazole is versatile leading compound have five membered heterocyclic ring which is bioactive agent after designing. Pyrazole follow both class of simple aromatic ring organic compound of heterocyclic diazole series distinguished by a five-member ring structure made up of 3 carbon atom and 2 nitrogen atoms in joining position and to unsaturated parent compound show in figure 1.

For the pharmacological effect on human, they are composed from alkaloid, even if they are rare in nature.

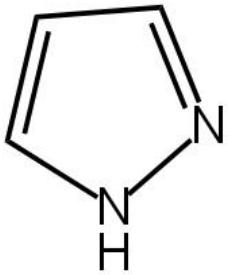


Figure 1: Pyrazole

Highly valuable compounds are heterocyclic compounds. These compounds exhibit a broad spectrum of physical chemical and pharmacological characteristics [2,3].

Heterocyclic compounds are an important part in metabolism owing to their structural occurring in so many natural products include alkaloid, vitamin and many others [4-6]. Heterocyclic compound and nitrogen containing heterocyclic compound showed several involvements in natural science and pharmacological activity [6]. Also heterocyclic compounds and nitrogen containing heterocyclic structure are found in natural plants hormones and alkaloids [7,8].

In chemically basic structure of pyrazole has 5 membered ring and possess 2 nitrogen atoms at joining position $C_3H_4N_2$ is molecular formula of pyrazole and possess 6π electrons over the ring that formed aromatic system. Pyrazole is linked to oxidizing form like as pyrazoline [9-11].

In synthetic chemistry the development of new approaches for pyrazole derivatives synthesis with bioactive evaluation is acknowledged as an important challenge.

Chemical and Physical properties of pyrazole

By the effect of individual atoms that explain the pyrazole chemical reactivity, it is moderately basic in nature and nitrogen atom at 2 positions along with lone pair of electrons reacts with electrophilic centered reagents, but at 1 position of nitrogen atom was not show reactive but give up H^+.

Pyrazole 1 with unsubstituted compound show colorless solid and possess range 96-70^{0}C melting point, and boiling point range is 186-188^{0}C that is attributed to intermediate molecule H-bonding, 9.15eV is the ionization potential of substituted pyrazole 1.

The center of pyrazole to the bond between 2nd and 3rd atom with action and similarity with 1,3 diaza-2,4 cyclopentadiene's is main event and divergence possible. Pyrazoles are 5 membered heterocyclic compound that constitute a compound important in organic structure synthesis (Figure 2).

Now a day, Pyrazole compound structure system with substituted fluorine and aromatic fluorinated compounds as a bioactive achieved attention due to their relevant pharmacological activities. This heterocycle is well established medicine belonging to various groups with pharmacotherapeutic effect [12-15].

Pyrazole nucleus access method

Pyrazole is aromatic heterocyclic compound and substitution reaction of electrophilic obtain at position 4th and attack of nucleophilic at position 3rd and 5th.

Biological significant role of pyrazoles

Pyrazole ring is a unsaturated 5 member ring containing 2 adjacent nitrogen atoms it is synthesis procedure have been studied and such studies have been trigger by many of application especially in pyrazole derivatives substitution. Genuinely some substituted pyrazoles are anticancer [16], antimicrobial [17], antidepressant [18], anticonvelsant [19], antipyretic [20], anti-inflammatory [21], antibacterial [22], anti-arthritic and antifungal activities [23].

Diazoles

Diazoles are widely used as antibacterial and antifungal activities. They are of two types imidazole's and pyrazoles (Figure 3). Diazoles are of two isomeric forms with molecular formula $C_3H_3N_2$, having a five-member cyclic aromatic ring consisting of two Nitrogen (N) atoms on different positions and three carbon atoms [24].

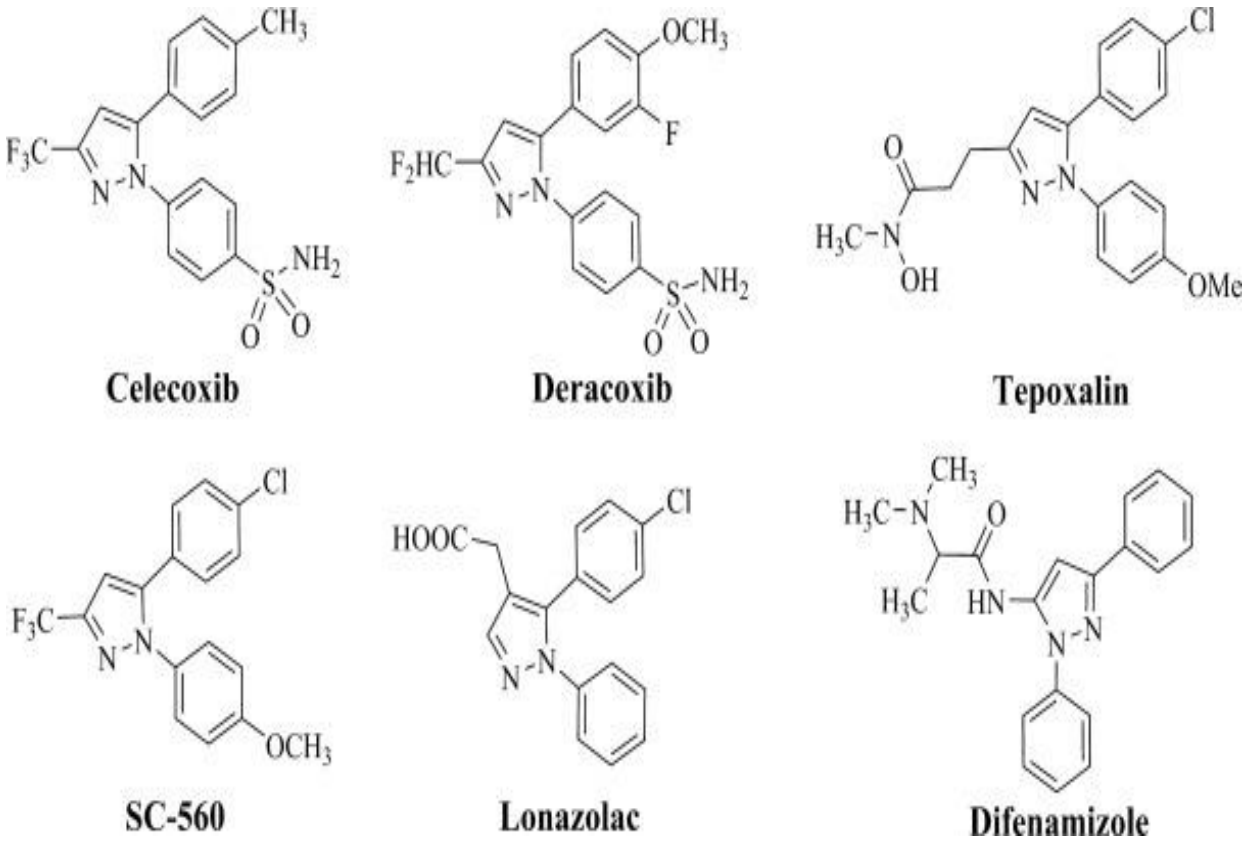


Figure 4: Pharmaceutical drugs containing pyrazole derivatives.

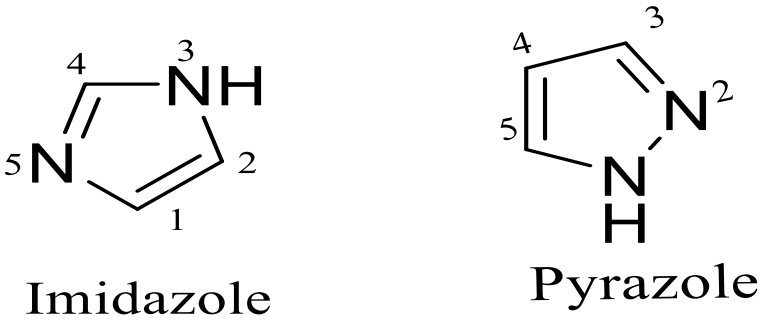


Figure 3: Structure of Imidazole and Pyrazole.

Pyrazoles

Pyrazoles is an aromatic five-member heterocyclic compound 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 years before we known about Pyrazole derivatives, the exploration of their medicinal chemistry field purpose is very slowly.

Earlier research was 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 antipyretic and anti-inflammatory and another pharmacological activity. Antipyrine is one of the earliest synthetic drug.

Pyrazole belongs azole family and possess 5 membered rings extensively have nitrogen and carbon atom. N-substitution at 1 position in pyrazole exhibit annular tautomer's. These two tautomeric forms are the 2 nitrogen atoms are identical. As compared to pyrrole, pyrazole is less reactive toward electrophilic (Figure 4).

In strong acid medium pyrrole, pyrazole is less reactive toward electrophilic, in strong acid medium pyrazole cation are formed and show deactivation for sulfonation, nitration. According to localization energy of electrons electrophilic attach on C-4 position in pyrazole and attack on other position is rare.

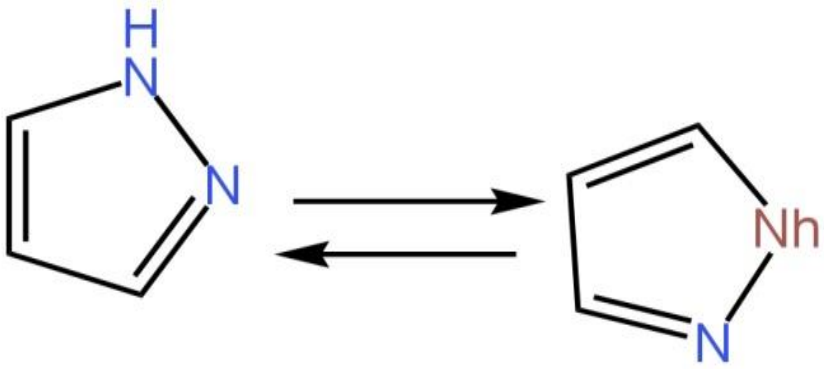


Figure 4: Structure of Pyrazoles.

Synthetic access and pharmacological action of pyrazoles. Pyrazole and its derivative have broad spectrum pharmacology activities.

In previous studies remarkable report evidence has collected to illustrated the pyrazole derivatives efficacy including antibacterial & antifungal, antitumor, antiviral, antidiabetic, analgesic and antidepressant.

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 (Figure 5).

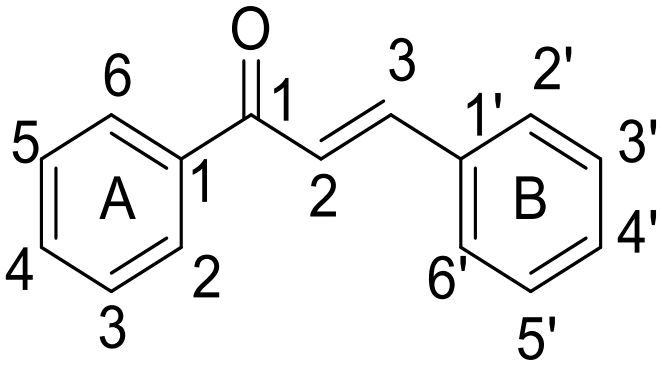


Figure 5: Structure of chalcones.

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 transfer.

Chalcones method of preparation by catalyzing 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 a key role in synthesis medicinal drugs or molecules [25,26].

Literature review of chalcone revealed molecules of shows natural or synthetic origin based to exhibit different pharmacological evaluation activities following as antioxidant, antimicrobial agents, anti-inflammatory activity, cytotoxic activity, hypoglycemic activity, antihepatotoxic, antimalarial, antileishmanial, tyrosine inhibitors and antitumor activities.

Methodology

Synthesis

General Procedure

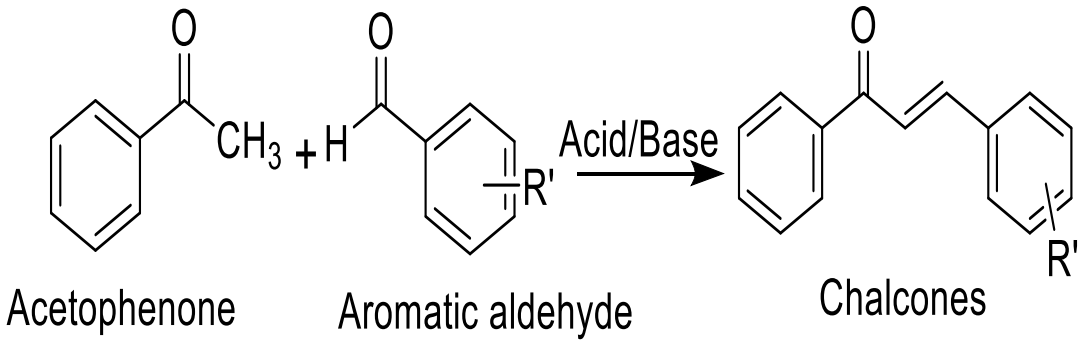
The following steps are used to synthesize 1,3,5-trisubstituted pyrazole moiety-based derivatives:

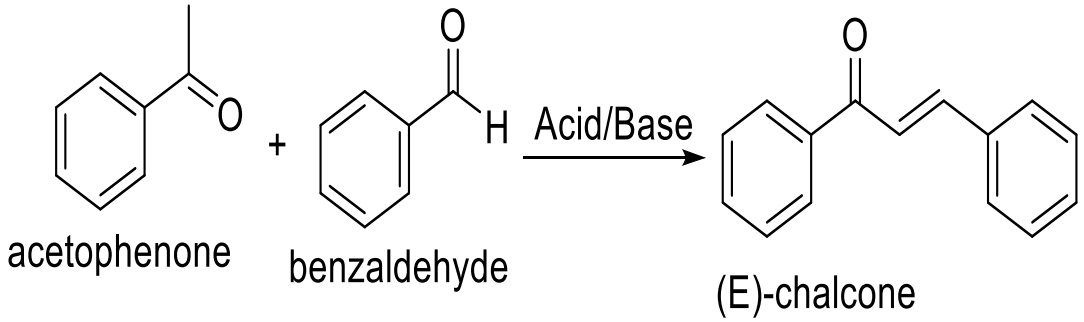
1. Synthesis of chalcones moiety
2. Synthesis of succinic hydrazide from corresponding ester.
3. Final step involves the reaction of succinic hydrazide with chalcones to form 1,3,5-trisubstituted pyrazole moiety based compounds.

Step 1: Synthesis of chalcones and its derivatives.

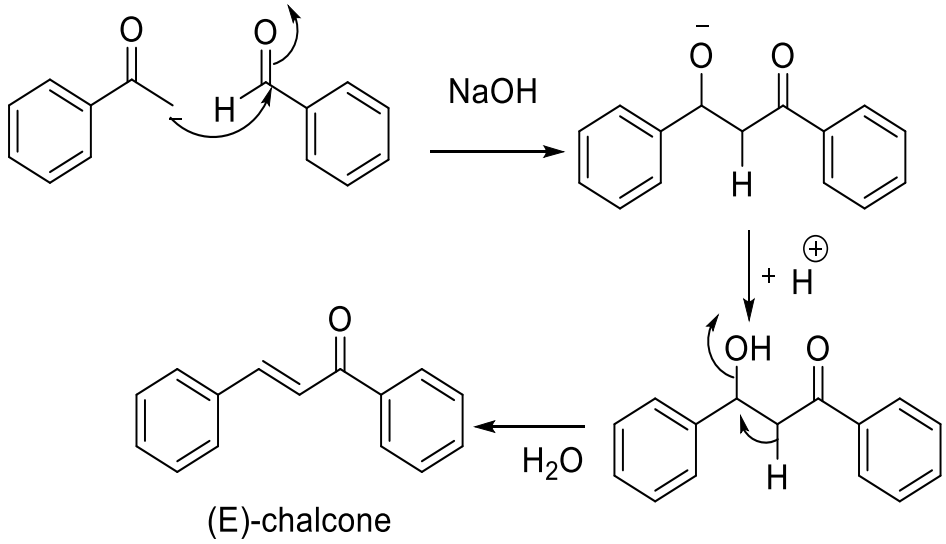
Chalcones and its derivatives synthesized by reaction of Claisen Schmidt condensation.

It is a type of aldol condensation called crossed aldol condensation, where a molecule of aldehyde containing δ-hydrogen atom condenses with a ketone without α-hydrogen, in the presence of strong acidic or basic catalyst to form chalcones.



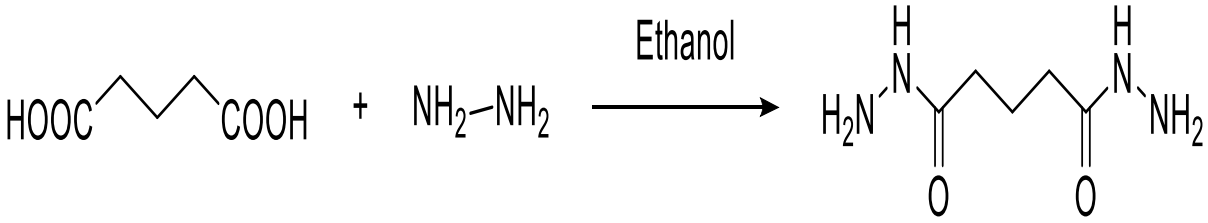


Base catalysed reaction mechanism:



Step 2: Synthesis of succinic hydrazide:

Succinic acid and hydrazine hydrate reacts presence of alcohol can be converted to succinic hydrazide, and reaction mixture was cooling at room temperature, succinic hydrazide 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 and refluxed in the presence of suitable solvent.

Experimental procedure

Step 1: Procedure for the synthesis of chalcones and its derivatives:

The reaction mixture was placed into a beaker with broken ice and dilute HCl to acidify it. The solid was

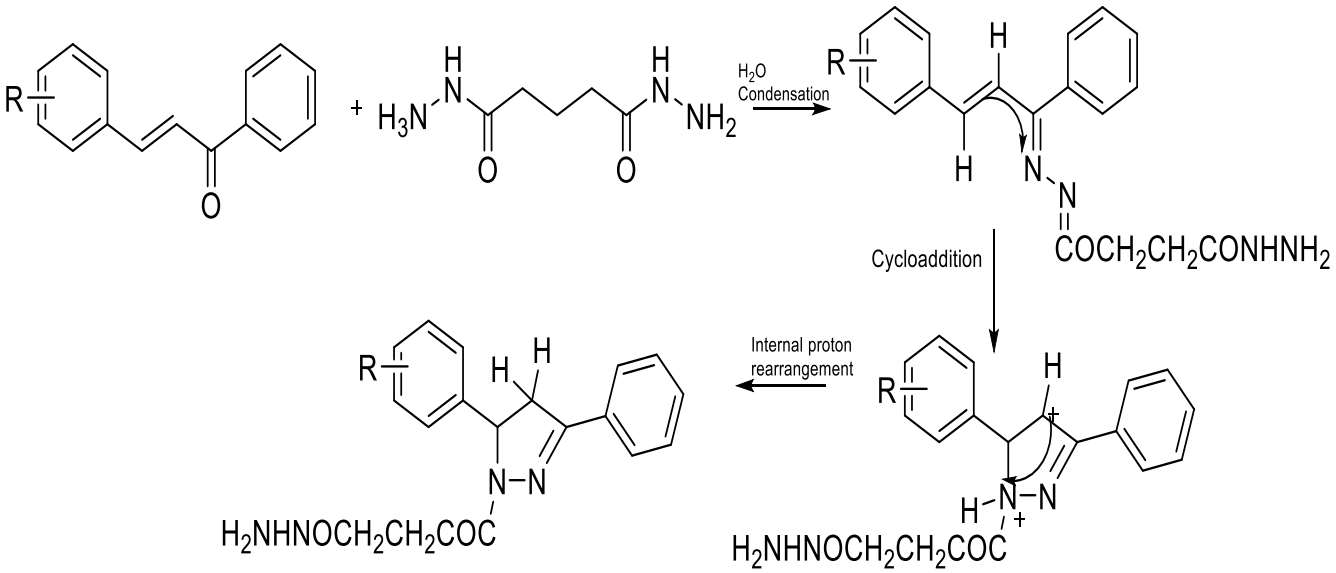
In an ice bath, equimolar amounts of acetophenone (0.01 mol) and aromatic aldehydes (0.01 mol) in ethanol were cooled to 10-15°C.

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.

filtered and rinsed in ice cold water before being dried and recrystallized in the presence of ethanol to obtain derivatives (C1-C15).

Reaction Scheme:



Reaction Mechanism:

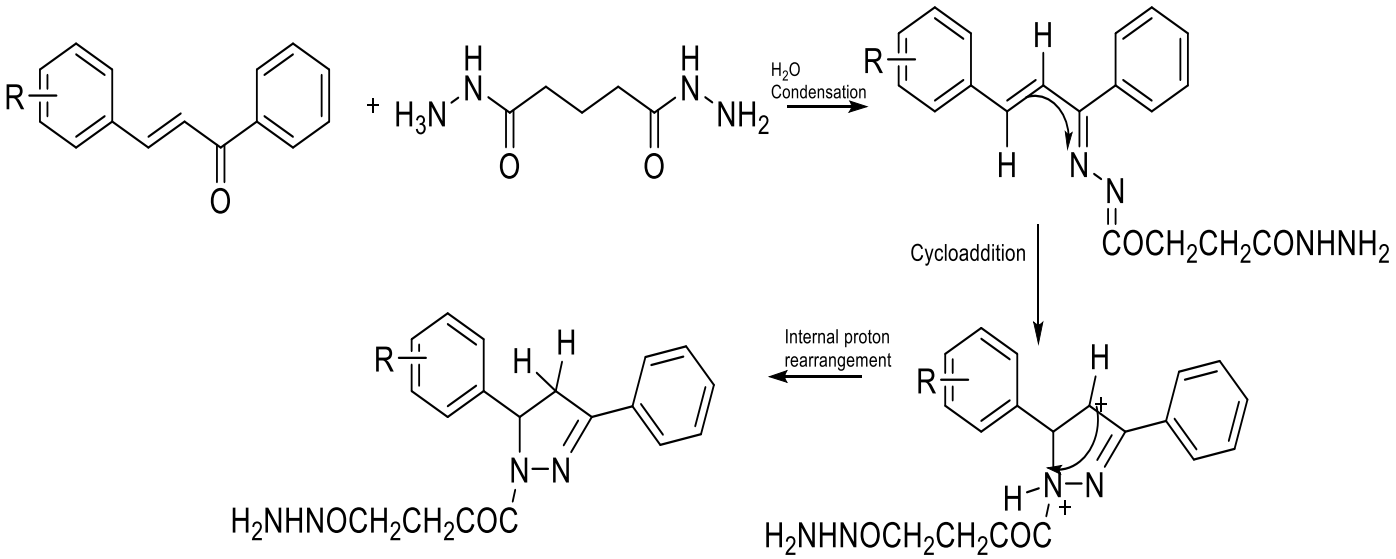


Table 1: Substitutions of derivative (C_1-C_{15}).

Compound	R
C_1	Benzaldehyde
C_2	2-Flouro benzaldehyde
C_3	3-Fluoro-4-methoxybenzaldehyde
C_4	4-(dimethylamino)-3-fluorobenzaldehyde
C_5	4-fluorofuran-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

Step 2: 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:

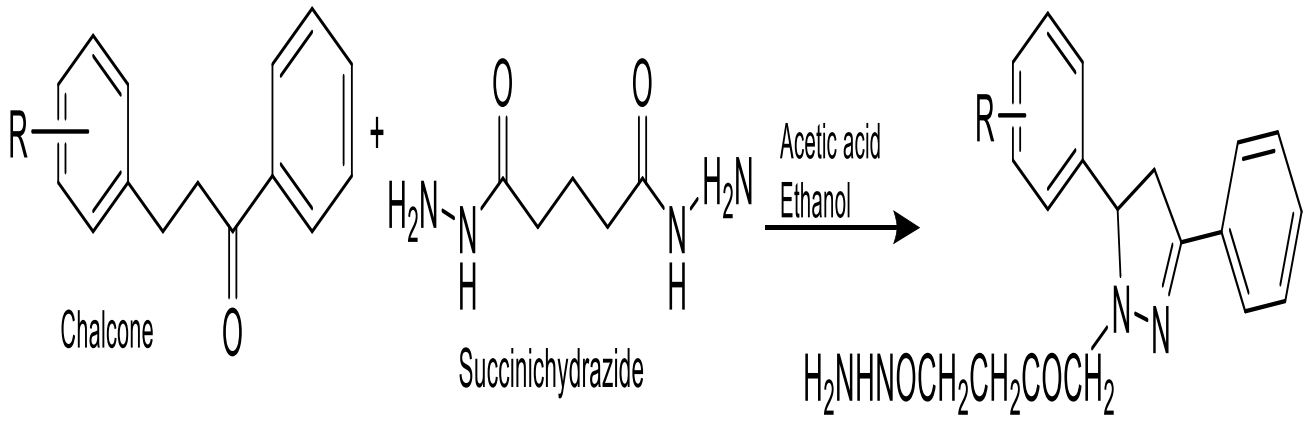
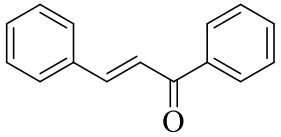
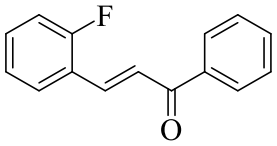
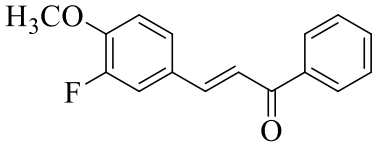
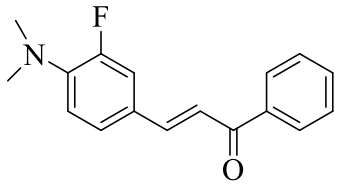
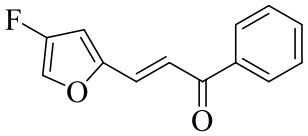
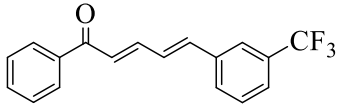
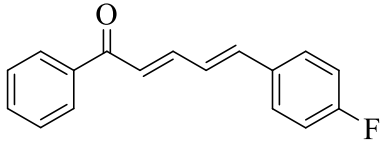
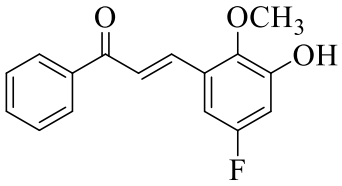
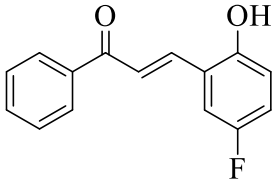
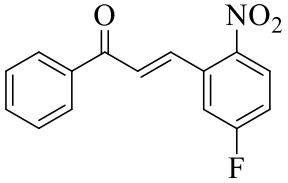
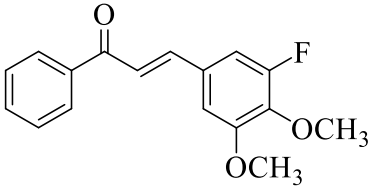


Table 2: Physical characterisation data of synthesized chalcones (C_1-C_{15}).

Compound	Chemical Structure and Name	Mol Formula	Molecular Weight	Yield (%)	Colour	Rf Value
C1	 (*E*)-chalcone	$C_{15}H_{14}O$	208.26	86.31	Yellow	0.81
C2	 (*E*)-3-(2-fluorophenyl)-1-phenylprop-2-en-1-one	$C_{15}H_{11}FO$	226.25	82.29	Yellow	0.65
C3	 (*E*)-3-(3-fluoro-4-methoxyphenyl) -1-phenylprop-2-en-1-one	$C_{16}H_{13}FO_2$	256.28	68.00	Yellow	0.75

C4	 (*E*)-3-(4-(dimethylamino)-3-fluorophenyl) -1-phenylprop-2-en-1-one	$C_{17}H_{16}FNO$	269.32	50.00	Yellow	0.68
C5	 (*E*)-3-(furan-2-yl)-1-phenylprop-2-en-1-one	$C_{13}H_9FO_2$	216.06	67.5	Brown	0.71
C6	 (2*E*,4*E*)-1-phenyl-5-(3-(trifluoromethyl)phenyl) penta-2,4-dien-1-one	$C_{18}H_{13}F_3O$	302.30	94.44	Orange	0.72
C7	 (2*E*,4*E*)-5-(4-fluorophenyl) -1-phenylpenta-2,4-dien-1-one	$C_{18}H_{13}FO_3$	302.30	83.47	Orange	0.66

C8	 (*E*)-3-(5-fluoro-3-hydroxy-2-methoxyphenyl) -1-phenylprop-2-en-1-one	$C_{16}H_{13}FO_3$	272.28	64.34	Colourless	0.57
C9	 (*E*)-3-(5-fluoro-2-hydroxyphenyl) -1-phenylprop-2-en-1-one	$C_{16}H_{13}FO_2$	242.25	54.93	Brown	0.62
C10	 (*E*)-3-(5-fluoro-2-nitrophenyl) -1-phenylprop-2-en-1-one	$C_{15}H_{10}FNO_3$	271.25	44.32	Blue	0.86
C11	 (*E*)-3-(3-fluoro-4,5-dimethoxyphenyl) -1-phenylprop-2-en-1-one	$C_{17}H_{15}FO_3$	286.30	64.83	Yellow	0.69

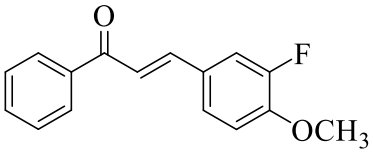
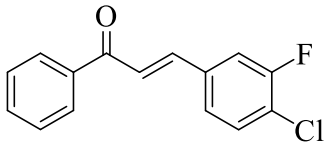
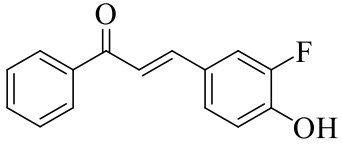
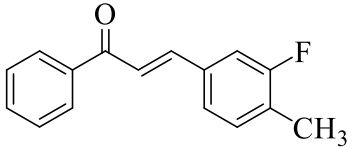
C12	 (*E*)-3-(3-fluoro-4-methoxyphenyl) -1-phenylprop-2-en-1-one	$C_{16}H_{13}FO_2$	256.28	71.81	Yellow	0.63
C13	 (*E*)-3-(4-chloro-3-fluorophenyl) -1-phenylprop-2-en-1-one	$C_{15}H_{10}ClFO$	260.69	81.03	Yellow	0.77
C14	 (*E*)-3-(3-fluoro-4-hydroxyphenyl) -1-phenylprop-2-en-1-one	$C_{15}H_{11}FO_2$	242.25	64.83	Yellow	0.83
C15	 (*E*)-3-(3-fluoro-4-methylphenyl) -1-phenylprop-2-en-1-one	$C_{16}H_{13}FO$	240.28	64.83	Yellow	0.79

Table 3: Substitutions of derivative (P_1-P_{15}).

Compound	R
C_1	Benzaldehyde
C_2	2-fluorobenzaldehyde
C_3	3-fluoro-4-methoxybenzaldehyde
C_4	4-(dimethylamino)-3-fluorobenzaldehyde
C_5	4-fluorofuran-2-carbaldehyde
C_6	3-(3-(trifluoromethyl)phenyl)acrylaldehyde
C_7	3-(4-fluorophenyl)acrylaldehyde
C_8	5-fluoro-3-hydroxy-2-methoxybenzaldehyde
C_9	5-fluoro-2-hydroxybenzaldehyde
C_{10}	5-fluoro-2-nitrobenzaldehyde
C_{11}	3-fluoro-4,5-dimethoxybenzaldehyde
C_{12}	3-fluoro-4-methoxybenzaldehyde
C_{13}	4-chloro-3-fluorobenzaldehyde
C_{14}	3-fluoro-4-hydroxybenzaldehyde
C_{15}	3-fluoro-4-hydroxybenzaldehyde

IDENTIFICATION AND CHARACTERIZATION:

Characterization of chalcone derivative

Table 4: Spectral data of synthesized chalcones (C_1-C_{10}).

	IR (KBr) in (cm^{-1})	^{1}H NMR($CDCl_3$) δ in ppm	Mass (m/z)
C_1	3046(Ar C-H), 1661(C=O), 1600(C=C), 761(mono substituted Ar- ring)	8.05(1H,d,=CH-Ar), 7.60(1H,d,-CO-CH=), 7.14-7.80(10H,m,Ar-H)	208(Molecular ion peak) 129(Base Peak) 157,144,111,91,77(0ther prominent peaks)
C_5	3130(Ar C-H) 1661(C=O), 1600(C=C), 1165(C-O-C), 752(mono substituted Ar-ring)	-	-
C_9	1647(C=O), 1579(C=C), 3456(OH)	8.08(1H,s,=CH-Ar), 7.50(1H,s,-CO-CH=), 4.4(1H,s,-OH), 6.4-7.7 (9H,m,Ar-H)	-
C_{13}	1656(C=O), 1595(C=C), 825(Ar-Cl)	-	-

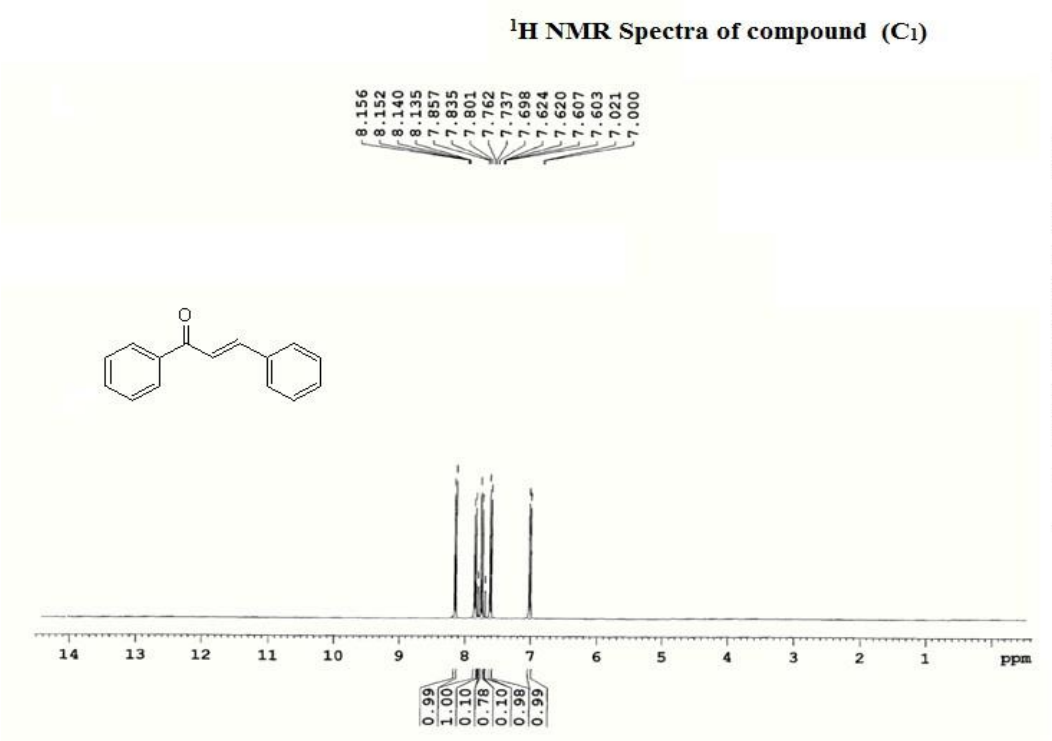


Figure 6: (2E)-1,3-diphenylprop-2-en-1-one (C1).

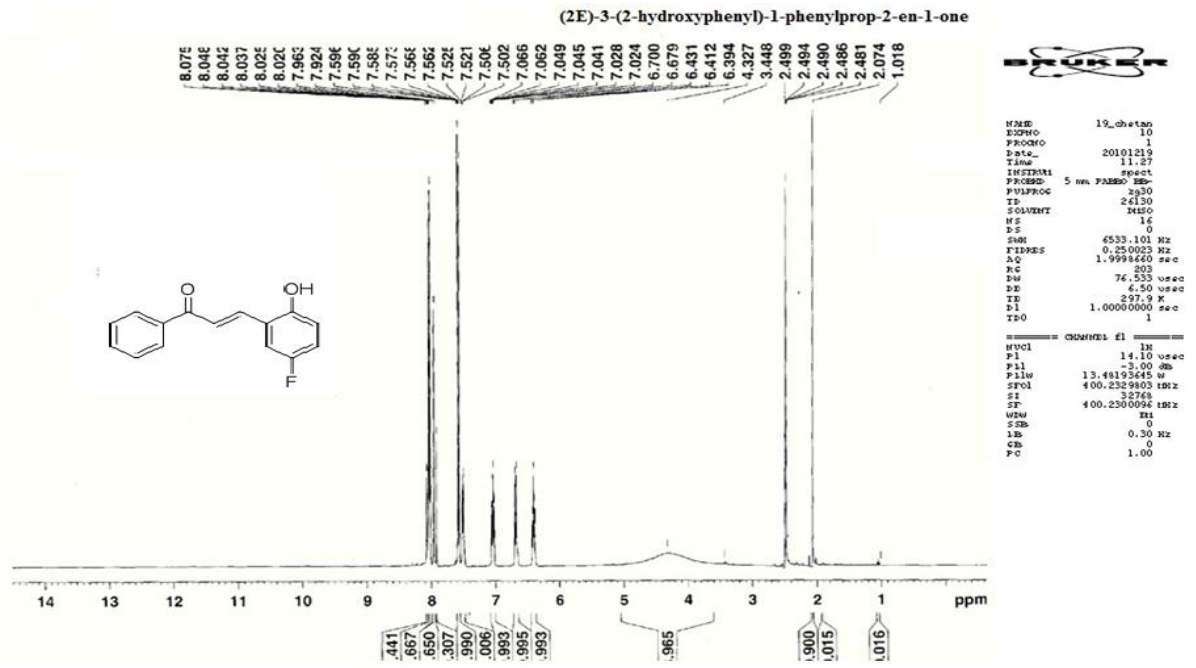


Fig 6 : ¹H NMR Spectra of compound C9

Figure 7: ¹H NMR Spectra of Compound C_9

Characterization of 1,3,5-trisubstiuted pyrazoles

Table 5: 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 (□)**	**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**	

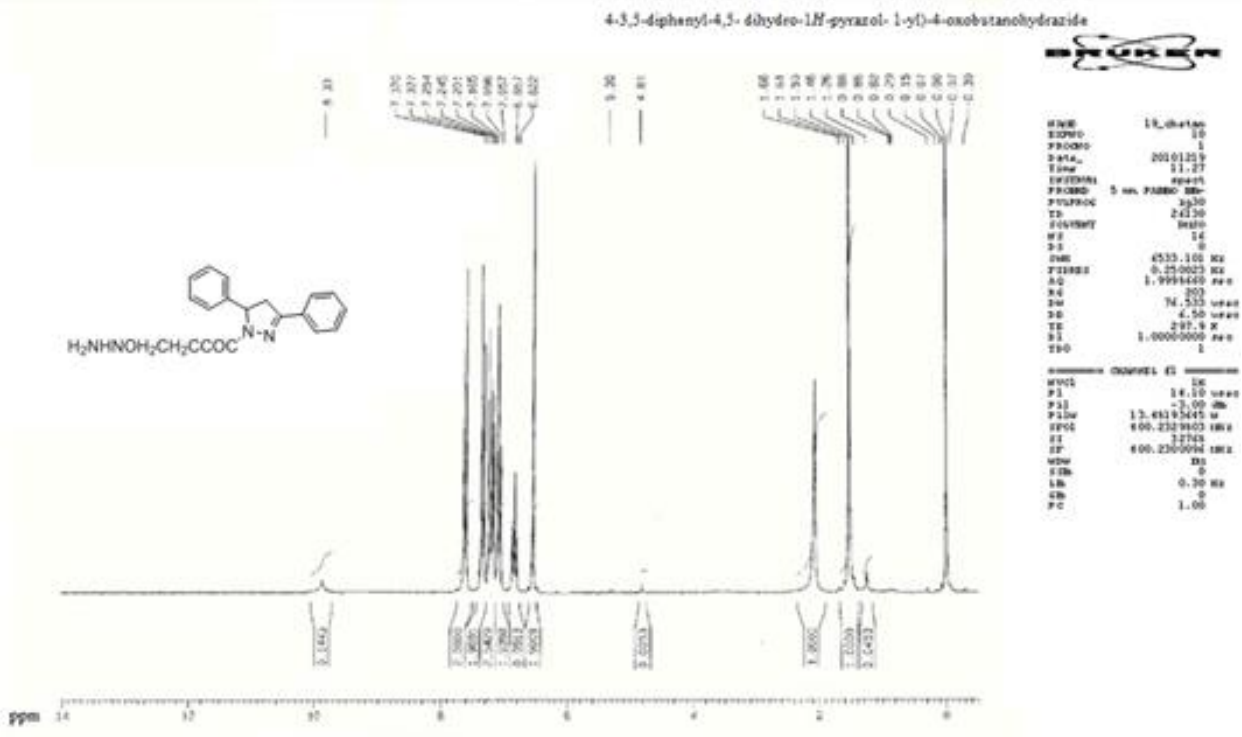


Figure 8: ^{1}H NMR Spectra of Compound P_1

Table 6: 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 (□)**	**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**	

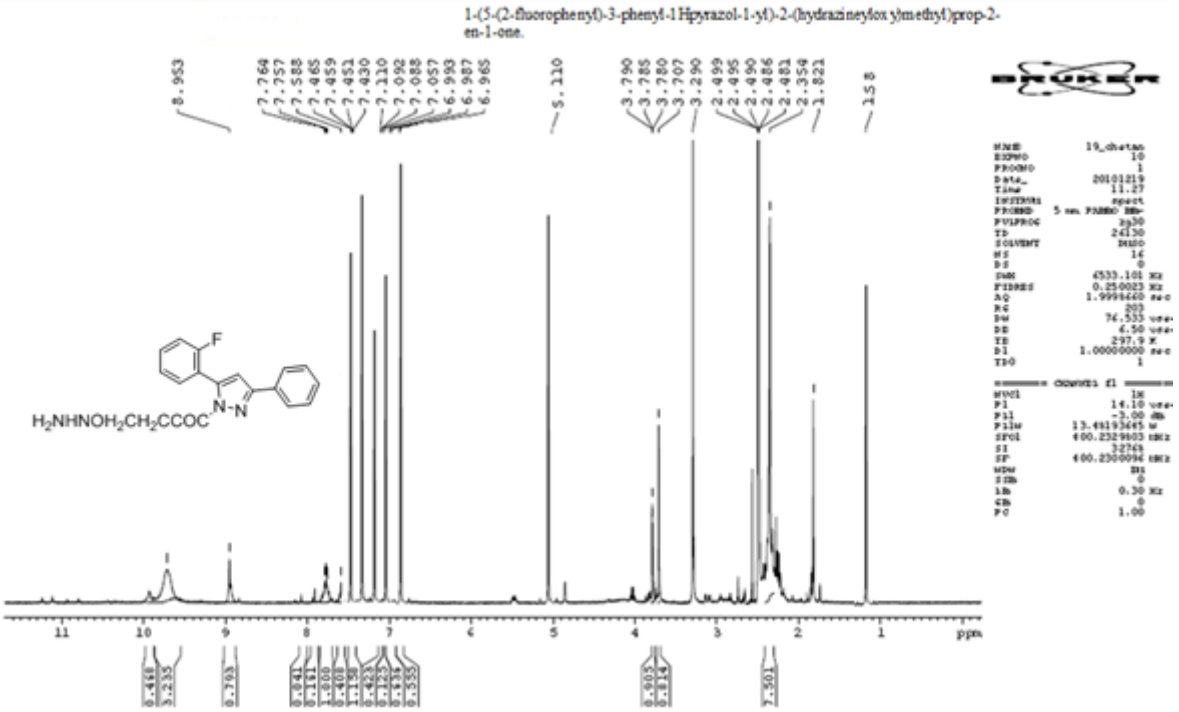


Figure 9: ^{1}H NMR Spectra of Compound P_2

Table 7: 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	

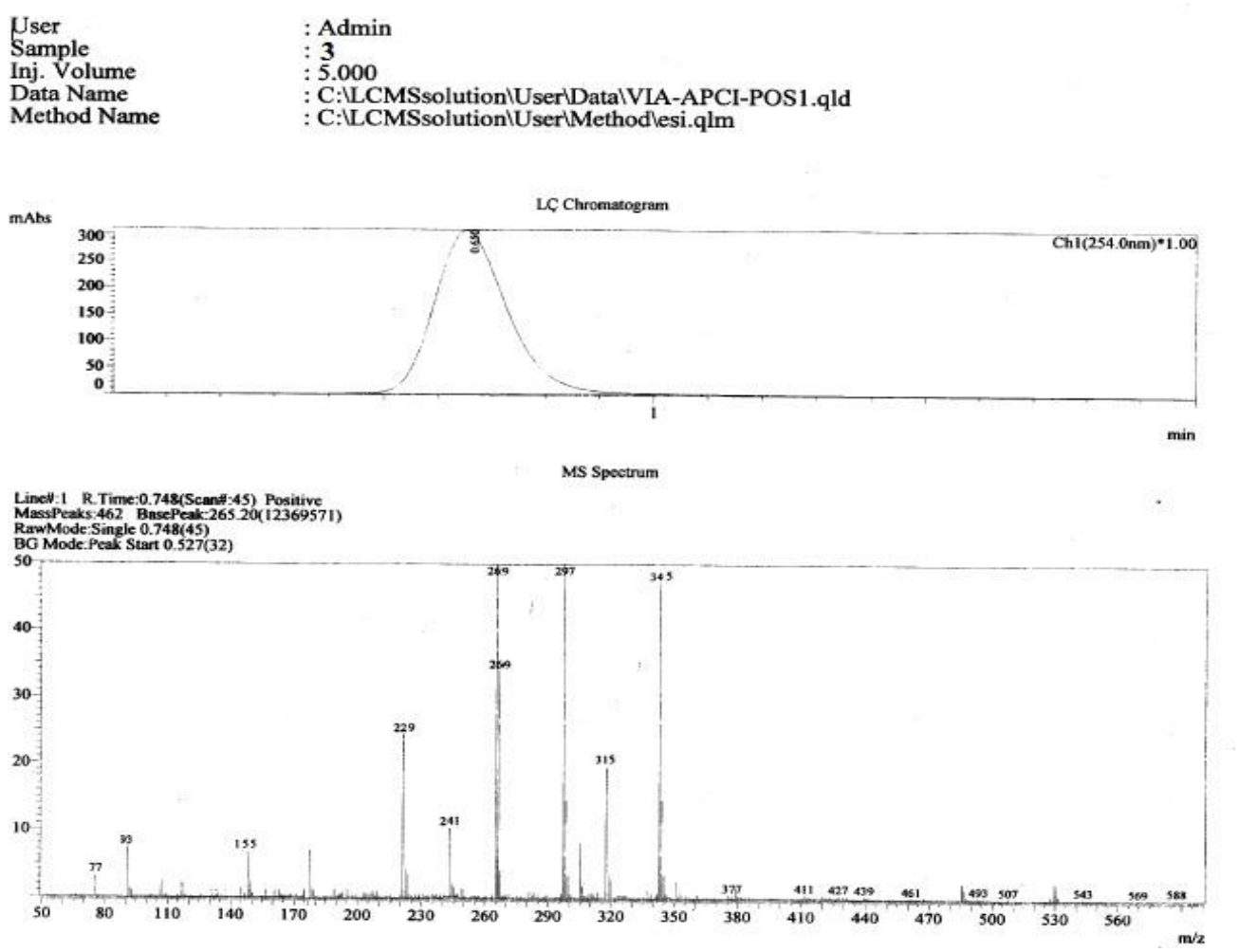


Figure 10: Mass spectra of compound P_3.

Table 8: 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).	-

Biological Activity

Method of turbidimetry:

The minimal dose needed to prevent microbe development in a homogeneous bacteriological fluid medium containing the specified microbial culture is calculated using through turbidimetric method.

The Minimum Inhibitory quantity is the lowest concentration necessary to stop the growth. In sterile fluid nutritional media, prepare a graded concentration of the antibacterial ingredient. Inoculation using a specific microorganism's loop. A blank, a positive control, and a negative control were included.

They are incubated at for 24 hrs at 37°C in some conditions depends on organism were chosen. Among the various concentrations of material, at least one inhibits microorganism development and is visually noted or evaluated as a percentage of transmittance or absorbance against a blank at 530 nm wavelength.

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 °C 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.

Culture media: Microbes can grow in a variety of environments, including water, soil, and living or dead organic matter.

Microbes grow in artificial media

1. Synthetic media: A predetermined amount of nutrients are available in synthetic media.

2. Complex media: Contains many nutrients with a relatively well-known composition that changes throughout batch.

In most common lab cultures, peptones or digested meats were applied. Yeast extracts, serum, casein, entire blood, or heated whole blood are among the options (enriched media) are some of the additional cultured media used [27,28].

3. Diagnostic media

Microbes can grow in a variety of environments, including water, soil, and living or dead organic matter.

Different types of media

• Selected media: to encourage the growth of certain microorganisms while inhibiting the growing of others.
• Differential media: allows different types of colonies to exist on the same plate while remaining distinct.
• Enriched media: to offer a nutrient that promotes an organism's growth.

Material and Method

Cup plate method: Petri plates, cork borers, glass syringe, inoculated loop, cotton all are required for the process.

Methods for working: The synthesised compounds and a reference medication were prepared as stock solutions in DMSO at a concentration of 100 g/ml.

Procurement of Microbes

Staphylococcus aureus, Staphylococcus faecalis, Bacillus subtilis, Escherichia coli, and Klebsiella penumonia standard culture media and *Saceharomyces cerevisiae, Aspergillus niger* species were obtained from Department of Microbiology, Mangalayatan University, Aligarh. Staining technique methods and bio-chemical reaction identify for microorganism.

Microbes were maintaining by sub culturing and was used at properly intervals in nutrient agar medium.

Table 9: Antibacterial activity evaluation of substituted pyrazole based derivatives against gram positive bacteria.

Compound	Zone of Inhibition in mm					
	S.aureus		***S.faecalis***		***B. substilis***	
	50µg	**100µg**	**50µg**	**100µg**	**50µg**	**100µg**
P_1	3	5	4	5	3	6
P_2	4	5	5	6	5	8
P_3	4	4	4	5	7	10
P_4	6	9	4	4	3	5
P_5	4	4	5	6	6	12
P_6	3	5	4	4	4	7
P_7	4	5	4	5	4	8
P_8	5	6	4	6	7	10
P_9	5	5	5	6	7	11
P_{10}	4	4	4	6	4	5
P_{11}	4	8	3	7	6	9
P_{12}	3	5	4	7	5	7
P_{13}	3	6	5	8	5	8
P_{14}	4	9	4	6	3	6
P_{15}	5	6	5	7	5	7
DMSO	-	-	-	-	-	-
Ciprofloxacin	7	10	8	12	9	13

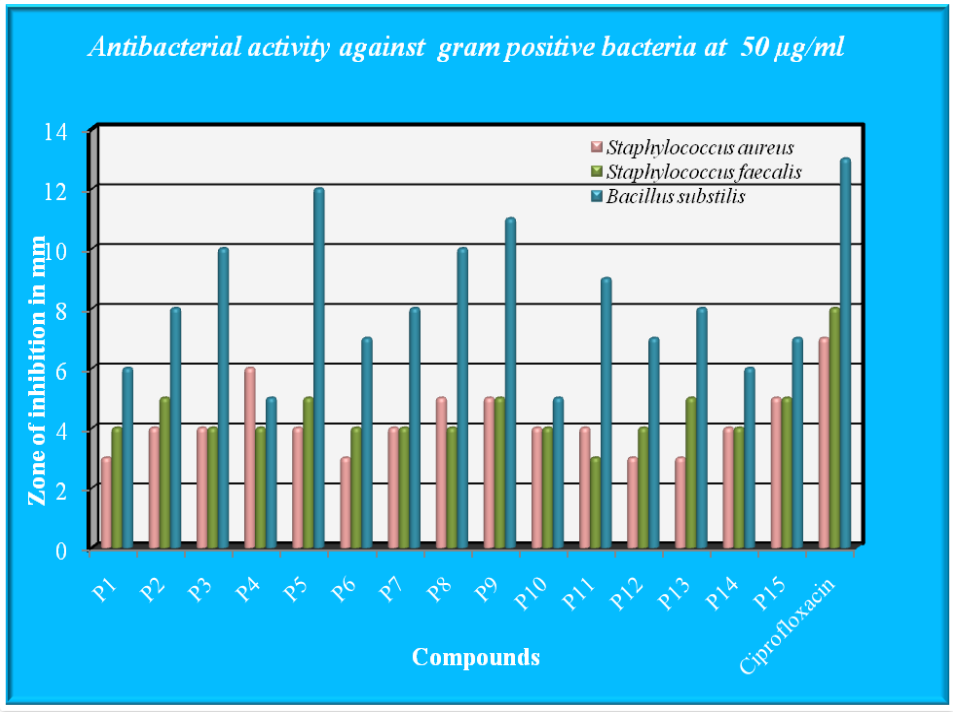


Figure 11: Graph showing Zone of inhibition of the molecules against gram Positive bacteria.

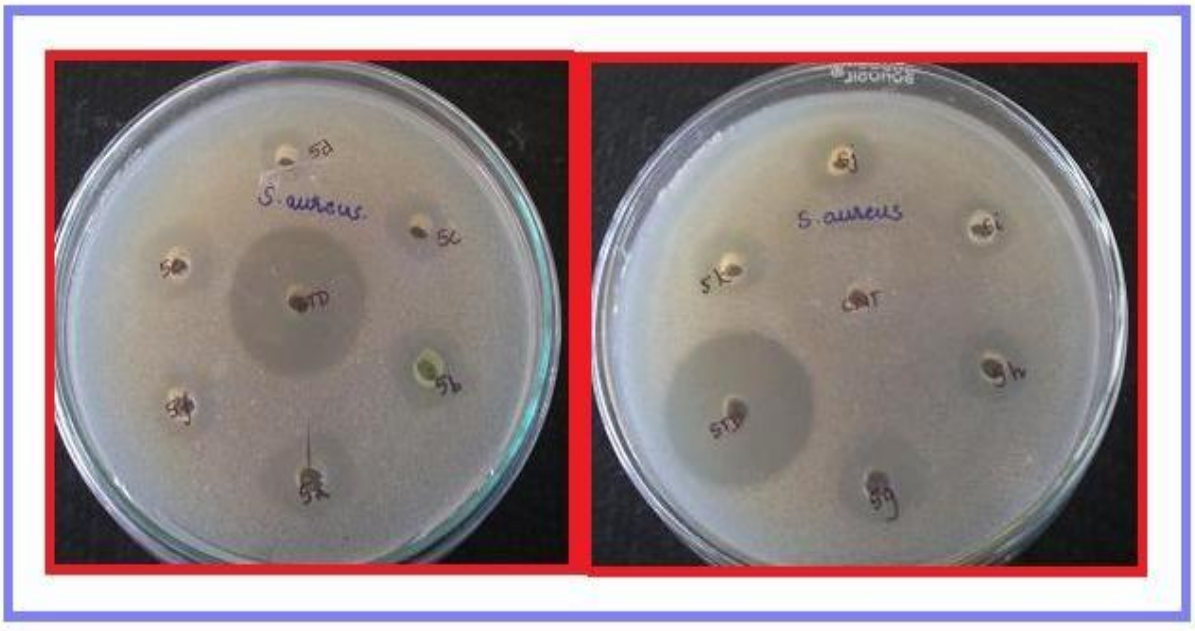


Figure 12: Graph showing zone of inhibition of derivatives against *Staphylococcus aureus*.

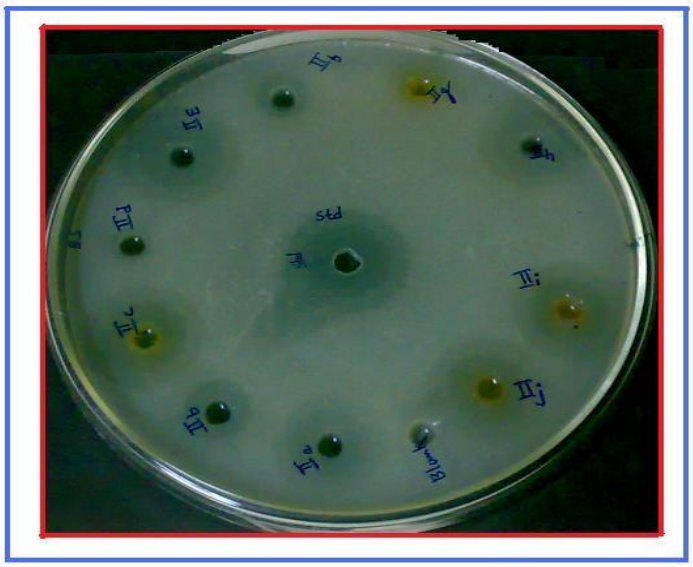


Figure 13: Zone of inhibition of derivatives (P1-P10) against Bacillus subtilis.

Table 10: 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**
Z	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

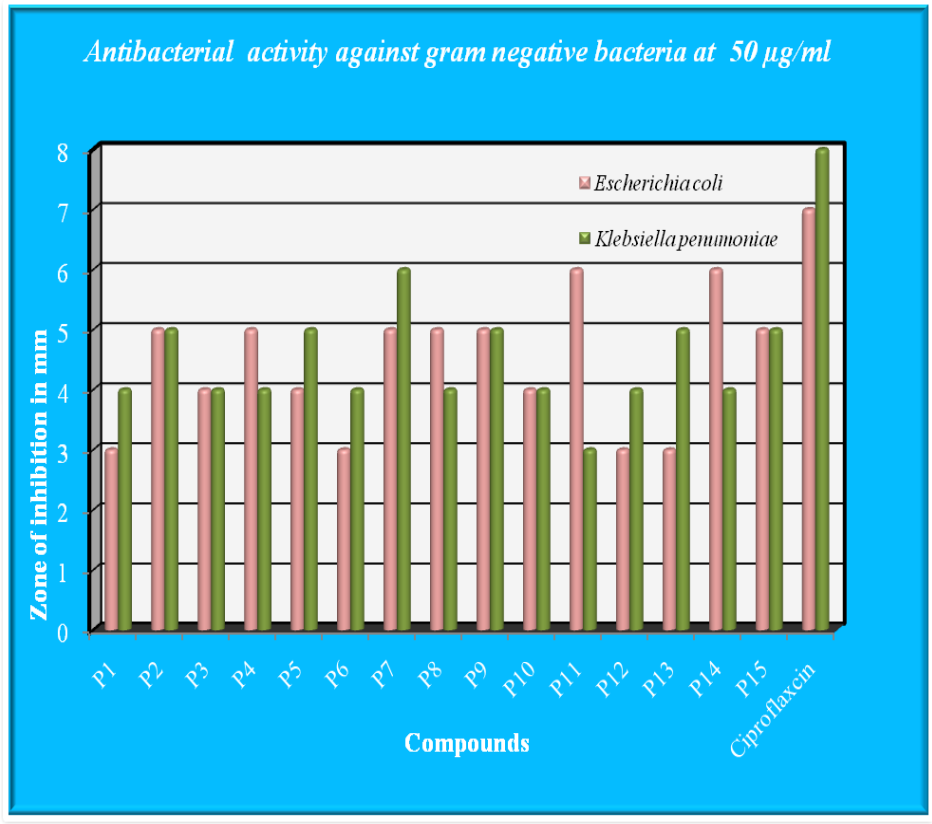


Figure 14: Graph showing Zone of inhibition of the molecules against gram negative bacteria.

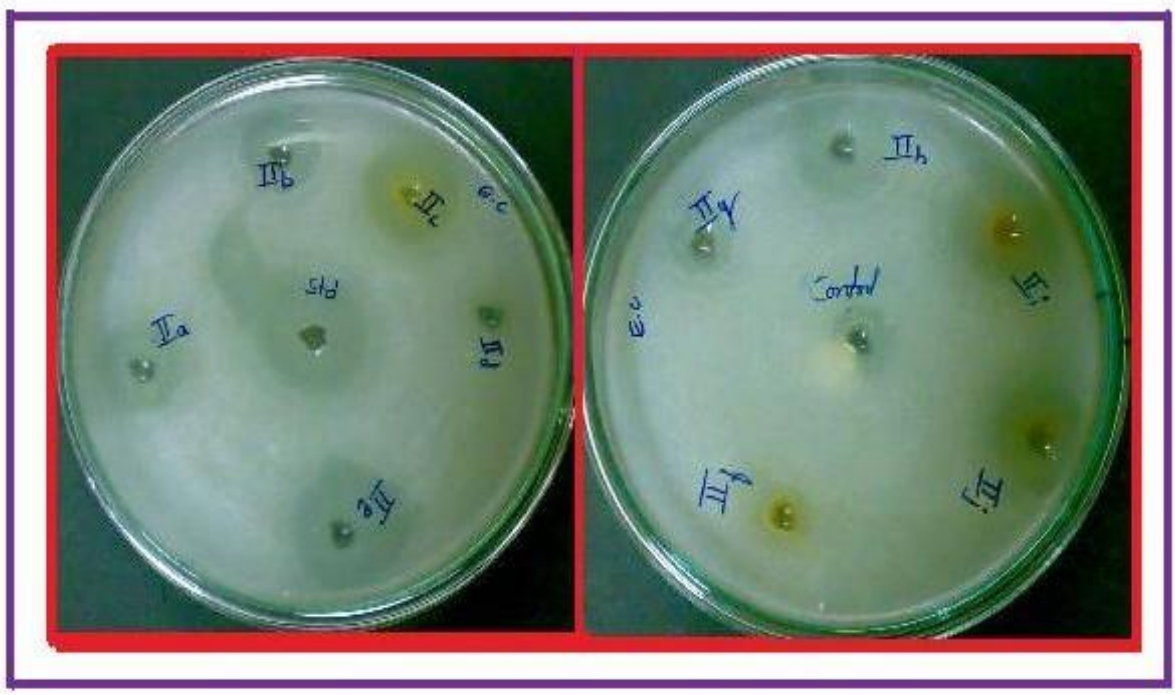


Figure 15: Zone of inhibition of derivatives against *E. coli*.

Table 11: Antifungal activity of synthesized pyrazole moiety based derivatives.

Compound	Zone of Inhibition in mm			
	Saceharomyces Cerevisiae		*Aspergillus niger*	
	50µg	100µg	50µg	100µg
P_1	5	9	5	9
P_2	4	8	4	8
P_3	4	7	4	7
P_4	7	10	6	8
P_5	5	9	7	10
P_6	9	14	8	11
P_7	6	11	9	11
P_8	8	12	8	12
P_9	6	10	5	10
P_{10}	4	7	4	9
P_{11}	5	8	5	8
P_{12}	6	8	4	6
P_{13}	4	9	6	9
P_{14}	8	12	5	7
P_{15}	5	8	6	9
DMSO	-	-	-	-
Ketoconazole	10	15	12	17

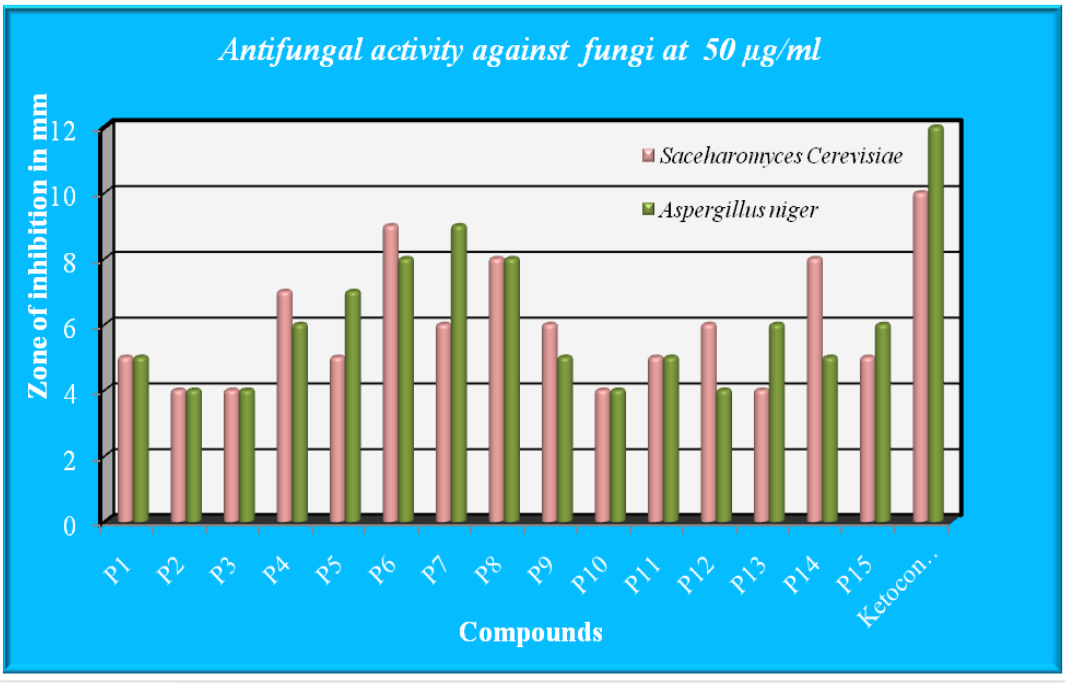


Figure 16: Showing zone of inhibition of the synthesized derivatives against fungi.

Results and Discussion

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.

We have synthesized derivatives (P_1-P_{15}) by cycloaddition reaction of substituted hydrazine with

fluorinated chalcones using conventional method and characterization of compounds through IR, H^1NMR and Mass spectroscopy.

Biological Activity

Antibacterial activity

According to data found from antibacterial evaluation 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.

Conclusion

Cycloaddition reaction of chalcones reacts with hydrides 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 .Evaluation of antifungal activity, of the synthesized derivatives P_7 and P_8 showed effective results against fungi.

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The authors did not receive any financial sponsorship for the project.

Conflict of Interest

The author declares no conflict of interest.

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