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Synthesis, Characterization and Evaluation of Biological Activities of Novel Pyrazoline Derivatives

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ABSTRACT:

This research focuses on the design, synthesis, and comprehensive characterization of a series of novel pyrazoline derivatives with potential biological activities. The study employs a multi-step synthetic approach, combining various chemical reactions to introduce diverse functional groups and optimize the pharmacological properties of the target compounds. The structural elucidation of the synthesized derivatives is carried out using advanced spectroscopic techniques, such as nuclear magnetic resonance (NMR) and mass spectrometry, ensuring the confirmation of molecular composition and purity. Antimicrobial screening is conducted to assess the compound's efficacy against a panel of bacterial and fungal strains, providing insights into their potential as antimicrobial agents. Furthermore, the investigation extends to the assessment of the derivatives' antioxidant properties, shedding light on their ability to combat oxidative stress. The findings from this research contribute to the understanding of the structure-activity relationship of pyrazoline derivatives and offer valuable insights into their potential pharmacological applications. The novel compounds synthesized in this study may serve as promising candidates for further development as therapeutic agents, addressing the growing demand for innovative and effective drugs in various medical fields.

Key words: Heterocyclic compounds, Pyrazoline, Anti-oxidant, Anti-bacterial and Characterization.

INTRODUCTION:

A chemical that either eliminates or prevents the development of germs is known as an antimicrobial. Based on the bacteria they are most effective against; antimicrobial medications may be categorized [1-3]. Examples of treatments for bacteria and fungus include antibiotics and antifungals. Microbial infection, which is brought on by many kinds of bacteria, viruses, and fungi, is one of the most prevalent illnesses that kill millions of individuals worldwide [4-6]. Humans are significantly impacted by the activity of bacteria. Microorganisms are more crucial to our everyday lives than most of us realize. Their activities have had a huge influence on our environment now and will continue to do so in the future. Microorganisms fundamentally benefit us and shouldn't be considered apart from us [7,8]. They are used in the manufacturing of a wide range of goods, including dairy products, certain cuisines, medicines, medicinal agents, and chemicals.

The antibiotic penicillin was first used in medicinal settings in the 1940s. Penicillin, a remarkable medication in terms of safety and effectiveness, helped many disabled troops survive World War II and helped usher in the age of antimicrobial chemotherapy. New types of antibacterial drugs were sequentially developed throughout the next two decades. In 1944, the aminoglycoside antibiotic streptomycin was created using the soil bacterium Streptomyces griseus. Later, substances like chloramphenicol, tetracycline, macrolide, and glycopeptide (like vancomycin) were produced from soil bacteria. In 1962, a quinolone antibacterial drug known as nalidixic acid was synthesized. The antibacterial spectrum remained larger and the antimicrobial activity was greater thanks to improvements in each type of antibacterial agent. We'll talk about beta lactam antibiotics as an example. Penicillin, monobactams, carbapenems, and cephems are some of the beta-lactam antibiotics. Initially, S. aureus and other Gram-positive bacteria were resistant to penicillin. Methicillin, which produces the penicillin-hydrolysing enzyme penicillinase, was later developed to treat S. aureus that was resistant to penicillin [9-11]. Ampicillin and piperacillin were created as a result of attempts to widen the antibacterial range. Cephems were developed in the 1960s and are now extensively utilized.

INTRODUCTION OF HETEROCYCLIC GROUPS:

Each week, organic chemists produce hundreds of unique heterocyclic molecules. In most cases, a chemist has a specific reason for developing a certain substance, which is often motivated by theoretical considerations, biological processes, medicinal chemistry, or a combination of all three. The heterocyclic compounds are widely distributed in nature and are essential to all living things. They exhibit an essential role in the metabolism of all living cells [12]. Due to their widespread distribution in nucleic acid strands and their participation in almost every physiological activity of plants and animals, nitrogen heterocycles are the most prevalent among the huge number of heterocycles found in nature, particularly those incorporating oxygen or sulphur.

Heterocyclic compounds have several applications in both business and daily life [13-15]. For instance, the majority of sugars and their derivatives, with the exception of vitamin C, have a five- or six-membered ring with one oxygen atom in the formula (Furanosied structure or Pyranose structure, respectively) [16]. Additionally, the majority of vitamin B group members have heterocyclic rings containing nitrogen.

For instance, vitamin B6 (pyridoxine), which is generated from the pyridine, is necessary for the metabolism of amino acids [17-18]. The heterocyclic compounds play a significant role in the medicine and pharmaceutical industries as well. Due to their unique chemical reactivity, about 80% of medicines now in use have a heterocyclic foundation. The majority of medications entering pharmacopeias are heterocyclic substances, including the tranquilizer chlordiazepoxide, the antidepressant imipramine, the hypertension guanethidine, the diuretic and antihypertensive indapamide, etc. Numerous antibiotics include heterocyclic rings, including penicillin, cephalosporin, norfloxacin, streptomycin, and others [19-21]. As wide spectrum anthelmintics, several veterinary medicines, such as pyrantel and morantel, are the preferred medication. Atrazine and Simazine, two well-known herbicides, are examples of heterocyclic agricultural compounds. Plant pigments with heterocyclic rings, including indigo, haemoglobin, anthocyanins, and chlorophyll, have made significant contributions to colour chemistry. Numerous additional heterocyclic coloring substances have also been in use since ancient times. Additionally, the ionic molecular crystal of heterocyclic tetra Selena fulvalene was the first to exhibit superconductivity. One of the most active areas of organic chemistry study is heterocyclic chemistry, which is expanding quickly. In 1998, published research on the creation of heterocyclic compounds made up about 60% of all available organic chemistry literature. Today, however, this percentage is much higher due to the availability of novel heterocyclic compounds in a variety of fields, including materials science, biochemistry, pharmaceuticals, and others [17].

Heterocyclic molecules are cyclic mixtures of carbon with other atoms including oxygen, nitrogen, and sulphur. Pyrrole, furan, and thiophene are examples of heterocyclic compounds with a single heteroatom, whereas azole, pyrrole, thiazole, thiadiazole, oxadiazole, triazene, and other heterocyclic molecules have several heteroatoms. Heterocyclic compounds come in a wide variety, and many of them have therapeutic value [22].

EXPERIMENTAL METHODOLOGY

4.1 Chemicals and Reagents

Chemicals and reagents used in the research work were of AR and LR grade and procured from Astron Chemicals, Ahmedabad Lobachemie Private Limited, Mumbai Krishna chemical Industry, Vadodara Merck specialities Private Limited, Mumbai

The chemicals were used as obtained.

4.2 Analytical Techniques

4.2.1 Thin Layer Chromatography (TLC):

Compounds purity was checked by using Silica gel G coated aluminium plates/Merck Silica gel as stationary phase and various combinations of ethyl acetate, n-hexane, methanol, toluene, benzene as mobile phase. The spots on TLC plates were visualized under ultraviolet lamp and/or by using iodine chamber.

4.2.2 Physical data:

Open capillary method was used to determine the melting point of synthesized compounds and were uncorrected.

4.3 Instruments Characterization of synthesized compounds were carried out by IR spectra, Mass spectra, NMR spectra.

4.3.1 Infra-Red spectra: FTIR DRS 8400, Shimadzu were used to record IR spectra of synthesized compounds as KBr pellets in the range of 4000 – 500 cm-1.

4.3.2 1H Nuclear Magnetic Resonance Spectra: 1H NMR spectra were recorded on Varian 400 MHz spectrometer in DMSO solvent.

4.3.3 Mass Spectra: Mass spectra were obtained using 2010EV LCMS Shimadzu instrument. Mass spectra were recorded on Agilent MS ion trap system.

EXPERIMENTAL METHODOLOGY

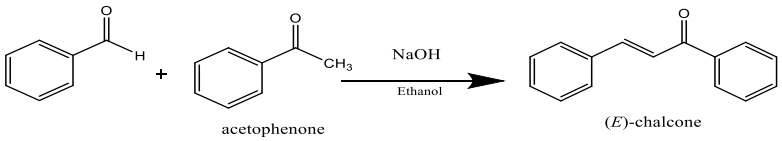
Scheme:

Step-1:

Synthesis of Chalcone with acetophenone:

In a round bottom flask placed equimolar quantity of substituted **benzaldehyde**, acetophenone and add 30 ml ethanol and few grams of NaOH and stirred on magnetic stirrer, maintained the temperature below 90ºC and stirring continued for 1-2hrs then reflux the reaction mixture for 1-2 hrs. After completion of the reaction monitored by TLC. The reaction mixture was poured into ice cold water then precipitation was formed. The product was collected by filtration, dried and purified with ethanol.

Step-1

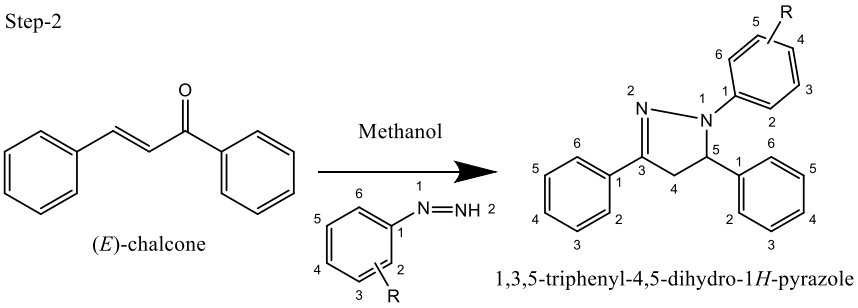


Step-2:

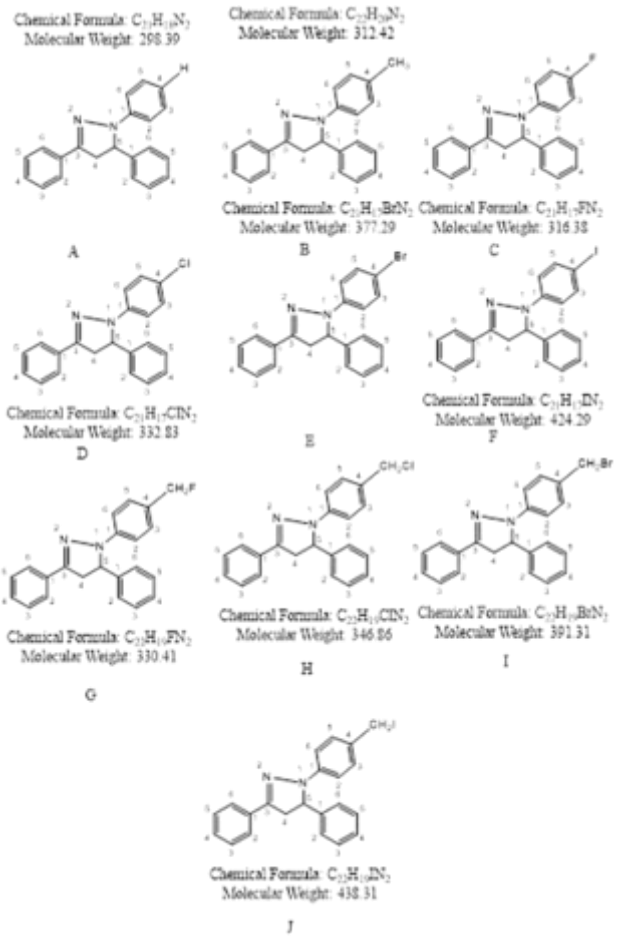
Synthesis of 1,3,5-triphenyl-4,5-dihydro-1H-pyrazole with acetophenone:

In a round bottom flask placed **acetophenone** and **Phenyl diazine** (0.01mole) and 20ml of methanol were refluxed for 1 hr at 95ºC. Completion of the reaction was monitored by TLC in solvent system 6:4 (Chloroform: Hexane). Then, the reaction mixture was cooled to room temperature and poured in to crushed ice. The product was separated by filtration and dried. It was purified recrystallize from methanol; crystalline product was obtained.

Step-2



Synthesis of compounds



RESULTS AND DISCUSSION:

Methods of identification:

1. Melting point
2. TLC
3. Infrared Spectroscopy
4. Nuclear Magnetic Resonance
5. Mass Spectroscopy.

The synthesized compounds were identified by using following method

Melting point:

The melting point of the compounds is determined by the capillary tube method. The synthesized compounds were start losing their crystallinity at a particular temperature.

Thin layer chromatography: -

Pre-coated TLC plates with silica gel GF 250 are used. Samples of reactants and products are prepared with suitable solvents.

The characterization was carried out using sophisticated methods like Infra-red spectroscopy, Nuclear magnetic resonance spectroscopy and Mass spectroscopy.

Infrared Spectroscopy:

One of the most potent analytical methods, infrared spectroscopy allows for the potential of chemical identification. The most significant benefit of using infrared spectroscopy over other common methods of structural research is how rapidly it may provide vital details about the functional groups present in the molecule. The approach is based on the straightforward observation that a chemical compound exhibits pronounced, selective infrared absorption. A chemical compound's molecules absorb infrared radiation and then vibrate slightly, creating tightly packed absorption bands known as the IR absorption spectrum, which can span a large wavelength range. The distinctive functional groups and bonds that are present in a chemical material correspond to a number of bands that are present in the IR spectra. A chemical compound's IR spectrum can be used as a fingerprint to identify it. By employing a potassium bromide pellet method and an FT-IR spectrometer, the infrared spectra of the produced derivatives were recorded.

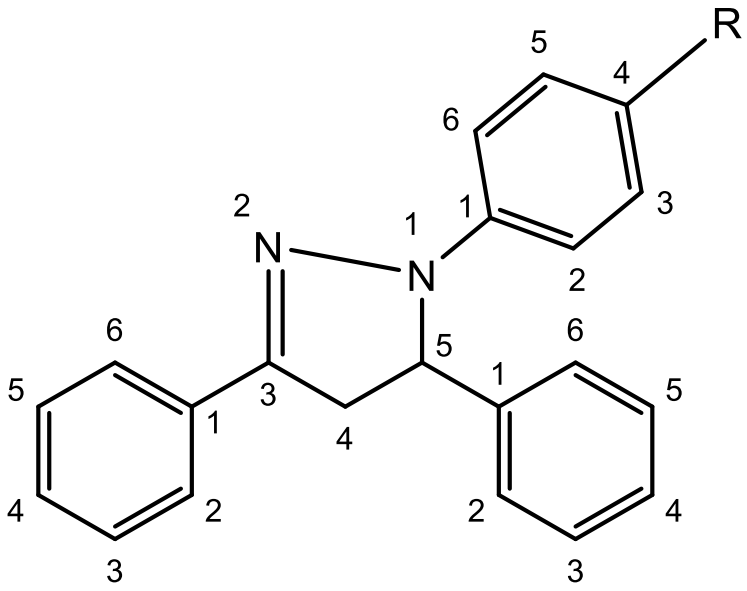
Nuclear Magnetic Resonance Spectroscopy:

This area of spectroscopy involves the use of radiofrequency waves to cause changes in the magnetic energy levels of molecule nuclei. Nuclei kept in a magnetic field produce the magnetic energy levels. The energy level shift is impossible without the magnetic field because the spin states of nuclei are degenerated, or have the same energy. The use of an external magnetic field, which necessitates various types of Rf radiation to bring them into resonance, allows for the energy level change. This phenomenon can be measured. It is an effective instrument for looking at the structure of nuclei. Tetra methyl silane was used as the internal standard in 1HNMR spectra of the synthesised derivatives, which were performed using Bruker spectrometers operating at 400-MHz and 500-MHz. Dimethyl sulphoxide (DMSO) was used as the solvent for a 1H NMR spectrum, and the chemical shift data were shown as delta values linked to trimethyl silane (TM) in ppm.

Mass spectroscopy:

There are three key tasks that a mass spectrometer must do. A stream of highly energetic electrons is used to first bombard molecules, turning some of them into ions that are subsequently accelerated by an electric field. Second, under an electric or magnetic field, the accelerated ions are split based on their mass to charge ratios. Finally, a device that can count the number of ions impacting it is used to identify the ions that have a specific mass-to-charge ratio. The output of the detector is enhanced and supplied to a recorder. The trace from the recorder is a graph of observed particles as a function of mass-to-charge ratio, or mass spectrum. Using an MSD spectrometer, the mass of the synthesised chemical was measured.

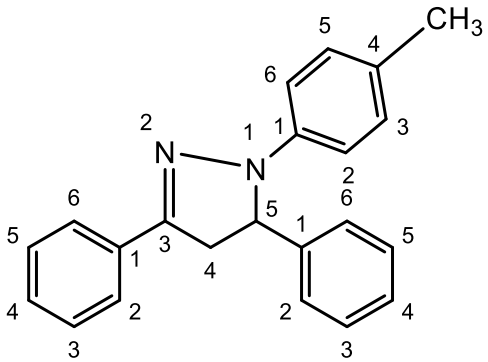
PHYSICAL DATA OF SYNTHESISED COMPOUNDS:



S.no	R	Mol. Formula	Mol. Weight	Melting point	% Yield
1	H	C21H18N2	298.39	102 ºC	57%
2	CH3	C22H20N2	312.42	85ºC	70%
3	F	C21H17FN2	316.38	91ºC	60.0%
4	Cl	C21H17ClN2	332.83	80ºC	69.60%
5	Br	C21H17BrN2	377.29	102ºC	62%
6	I	C21H17IN2	424.29	82ºC	56%
7	CH2F	C22H19FN2	330.41	80ºC	51%
8	CH2Cl	C22H19ClN2	346.86	102 ºC	67.8%
9	CH2Br	C22H19BrN2	391.31	98ºC	59%
10	CH2I	C22H19IN2	438.31	91ºC	68%

SPECTRAL DATA OF SYNTHESISED COMPOUNDS:

Spectral data of 3**,5-diphenyl-1-(p-tolyl)-4,5-dihydro-1H-pyrazole:**



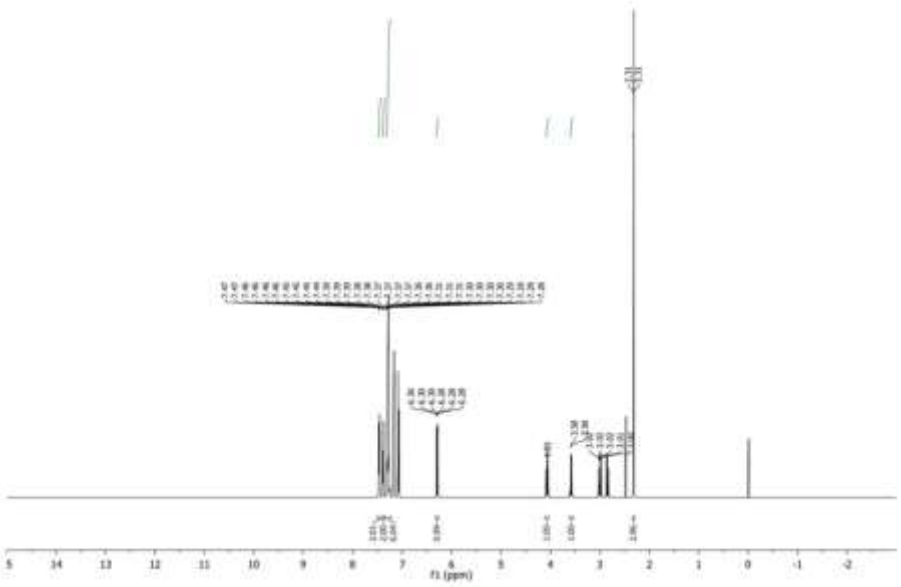
Mass Spectrum (EI-MS): M+1 peak observed at 311.26

IR (KBr) cm¯¹: 3226 (-NH), 2986 (Aromatic C-H), 1620 and 1504(C=C), 1072 (C-N), 1602 (C=N),

¹H-NMR (500 MHZ, DMSO) δ ppm:

Peaks

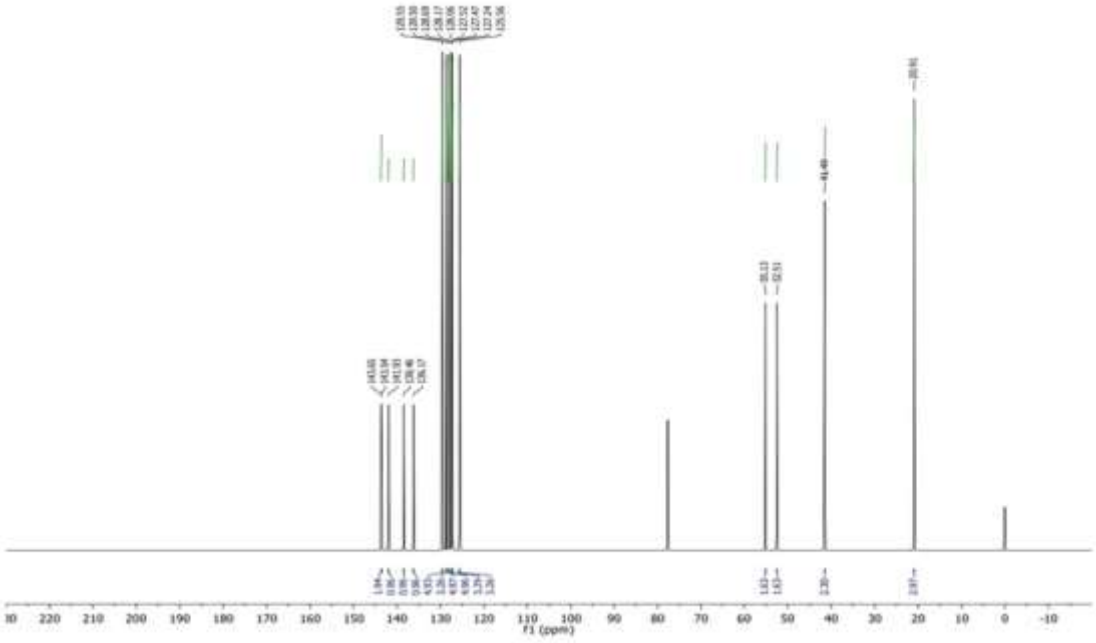
	ppm	Hz	Type
1	7.49	3744.0	-Ar-CH
2	7.48	3738.7	Ar-CH
3	7.46	3733.3	Ar-CH
4	7.45	3725.8	Ar-CH
5	7.44	3722.5	Ar-CH
6	7.42	3713.3	Ar-CH
7	7.39	3695.8	Ar-CH
8	7.37	3687.9	Ar-CH
9	7.36	3681.8	Ar-CH
10	7.29	3648.0	Ar-CH
11	6.30	3151.3	=CH-C
12	4.05	2024.7	-CH
13	3.58	1792.3	-CH
14	3.02	1509.0	-CH2
15	3.00	1502.2	-CH2
16	2.31	1157.6	-CH3
17	2.31	1156.5	-CH3



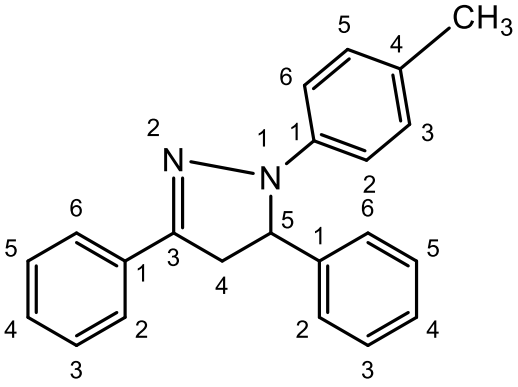
13C NMR:

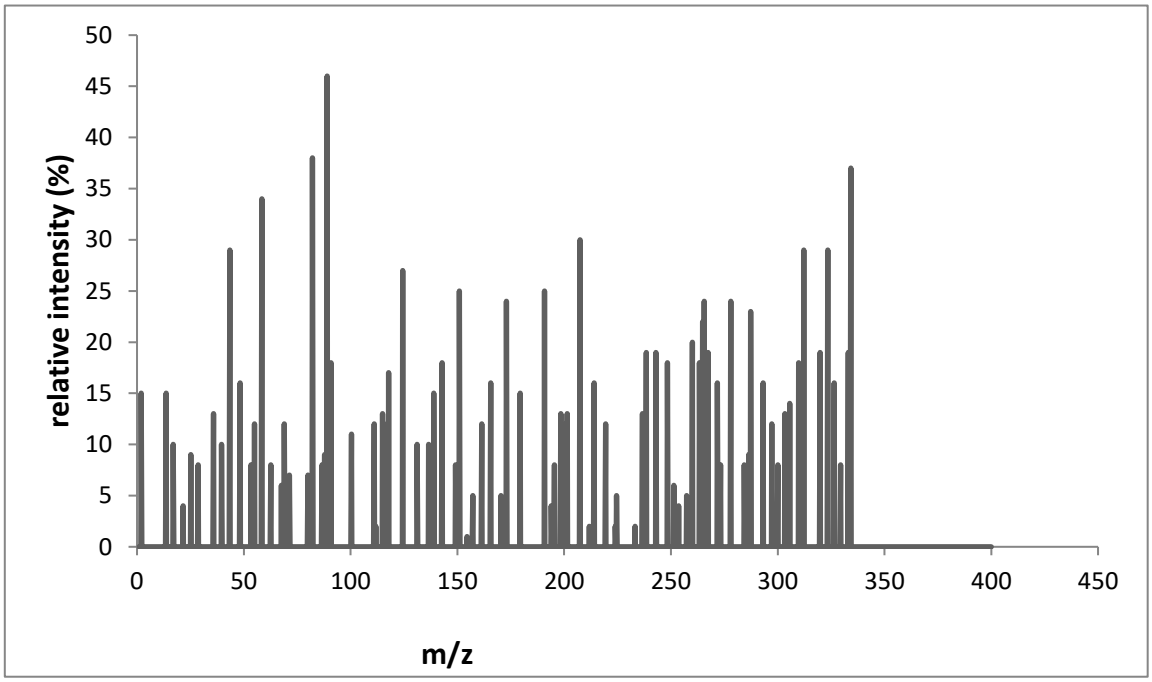
Peaks

	ppm	Hz	Type
1	**143.65**	**17961.5**	**Ar-C=C**
2	**143.54**	**17947.6**	**Ar-C=C**
3	**141.93**	**17746.4**	**Ar-C=C**
4	**138.46**	**17312.3**	**-C-CH3**
5	**136.17**	**17025.9**	**Ar-C**
6	**129.55**	**16197.9**	**Ar-C**
7	**129.50**	**16191.5**	**Ar-C**
8	**128.69**	**16090.1**	**Ar-C**
9	**128.17**	**16025.7**	**Ar-C**
10	**128.06**	**16011.9**	**Ar-C**
11	**127.52**	**15944.6**	**Ar-C**
12	**127.47**	**15938.0**	**Ar-C**
13	**127.24**	**15909.6**	**Ar-C**
14	**125.56**	**15699.0**	**Ar-C**
15	**55.13**	**6893.6**	**-CH**
16	**52.51**	**6565.2**	**-CH**
17	**41.49**	**5187.0**	**-CH2**
18	**20.91**	**2615.0**	**-CH3**

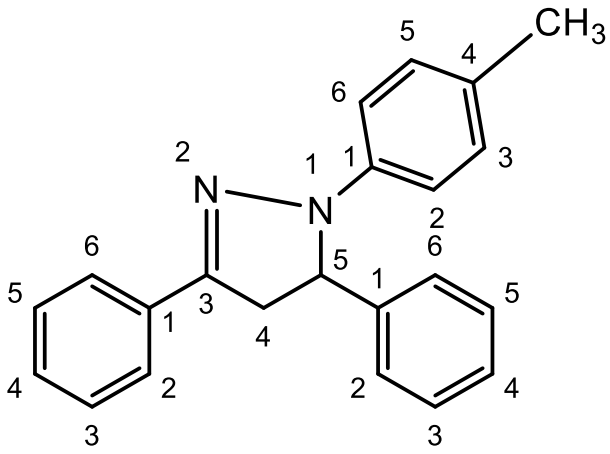


Mass spectru of 3,5-diphenyl-1-(p-tolyl)-4,5-dihydro-1H-pyrazole:

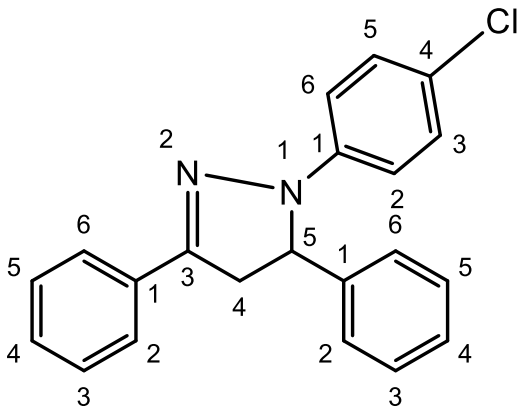




IR Spectrum of 3,5-diphenyl-1-(p-tolyl)-4,5-dihydro-1H-pyrazole:



Spectral data of 1-(4-chlorophenyl)-3,5-diphenyl-4,5-dihydro-1H-pyrazole:



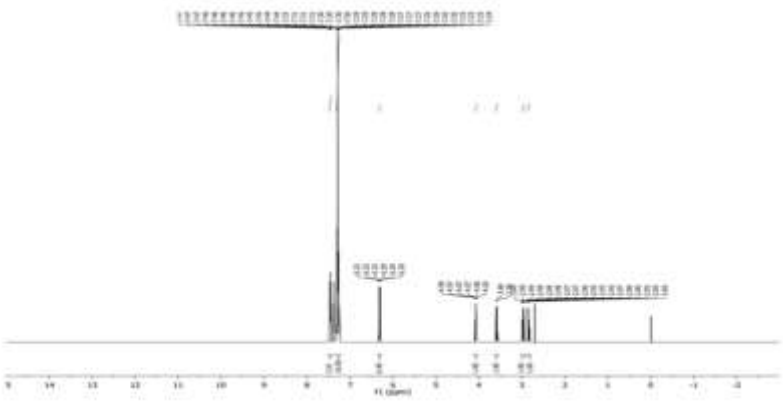
Mass Spectrum (EI-MS): M+1 peak observed at 332.67

IR (KBr) cm^{-1}: 3228 (-NH), 2961(Aromatic C-H), 1662 and 1592(C=C), 1014(C-N), 1620(C=N), 729(C-Cl).

^{1}H-NMR (500 MHZ, DMSO) δ ppm:

Peaks

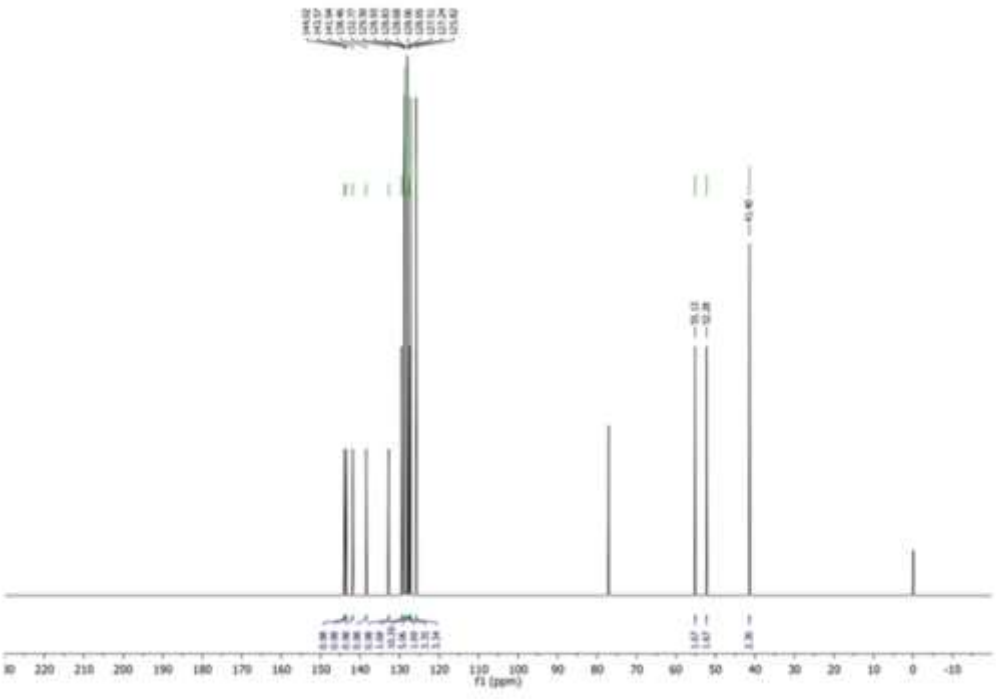
	ppm	Hz	Type
1	7.49	3745.4	Ar-CH
2	7.48	3741.5	Ar-CH
3	7.47	3735.9	Ar-CH
4	7.46	3731.0	Ar-CH
5	7.44	3719.8	Ar-CH
6	7.33	3667.2	Ar-CH
7	7.31	3658.4	Ar-CH
8	7.29	3648.3	Ar-CH
9	7.28	3642.8	Ar-CH
10	7.27	3635.9	Ar-CH
11	7.25	3627.4	Ar-CH
12	7.25	3626.3	Ar-CH
13	6.32	3161.0	-CH=
14	4.09	2045.3	-CH
15	3.61	1807.1	-CH
16	2.99	1497.7	-CH2
17	2.85	1423.8	-CH2



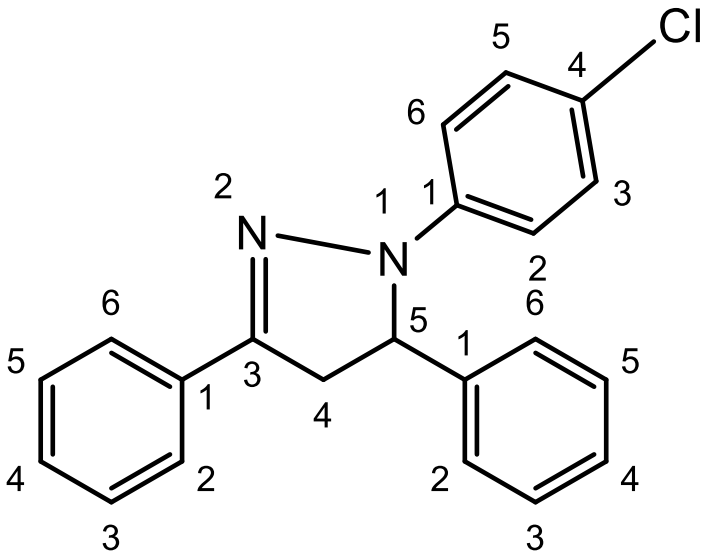
13C-NMR (500 MHZ, chloroform) δ ppm:

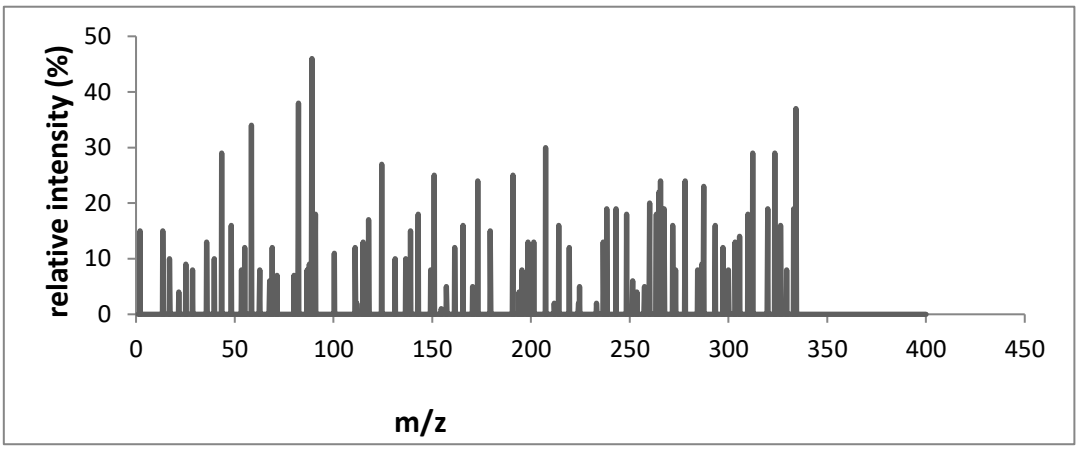
Peaks

	ppm	Hz	Type
1	144.02	18007.0	Ar-C
2	143.57	17950.5	Ar-C
3	141.94	17746.8	Ar-C
4	138.46	17312.3	Ar-C
5	132.77	16600.5	C=C-Cl
6	129.50	16191.5	Ar-C
7	128.93	16119.9	Ar-C
8	128.83	16107.5	Ar-C
9	128.68	16089.6	Ar-C
10	128.06	16011.9	Ar-C
11	128.05	16010.2	Ar-C
12	127.51	15942.5	Ar-C
13	127.24	15909.3	Ar-C
14	125.82	15731.2	Ar-C
15	55.13	6893.6	-CH
16	52.28	6536.6	-CH
17	41.40	5176.5	-CH2

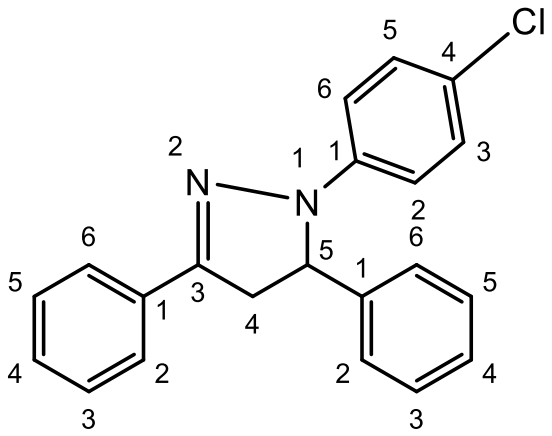


IR Spectrum of 1-(4-chlorophenyl)-3,5-diphenyl-4,5-dihydro-1H-pyrazole:





Mass spectrum of 1-(4-chlorophenyl)-3,5-diphenyl-4,5-dihydro-1H-pyrazole:



PHARMACOLOGICAL EVALUATIONS

Antibacterial activity of synthesised compounds:

Antibacterial activity by Agar Well Diffusion method by measuring the zone of inhibition in mm.

Materials:

- Nutrient Broth Media
- Nutrient Agar media
- Dimethyl sulfoxide
- Ciprofloxacin
- Distilled water

Test Organisms:

Gram positive

1. *Staphylococcus aureus*
2. *Micrococcus luteus*

Gram negative

1. *Escherichia coli*
2. *Klebsiella pneumoniae*

Nutrient media Composition:

Beef extract: 3 grams

Peptone: 5 grams

Sodium chloride: 5 grams

Agar agar: 15 grams

Distilled Water: 1000 liters

pH: 7.4±0.2

Preparation of bacterial cultures for assay:

The test organisms were sub cultured using nutrient broth medium. The tubes containing sterilized media were inoculated with respective bacterial strains. After incubation at 37±1ºC for 24 hours, they were stored in refrigerator. The stock cultures were maintained. Bacterial inoculums were prepared by transferring a loop full of culture to nutrient broth in conical flasks. The flasks were incubated at 37±1ºC for 48 hours before the experiment.

Test sample preparation:

The test compounds were prepared for assay by dissolving them in dimethyl sulfoxide in required concentrations making 50µg/ml, 100µg/ml, 500µg/ml, and 1000µg/ml respectively for evaluation. A reference standard for both Gram-positive and Gram-negative bacteria Ciprofloxacin was made in same concentrations as test compounds where dimethyl sulfoxide as control.

Assay by Agar well diffusion method:

By using the well diffusion method, test compounds' antibacterial susceptibility was evaluated. About 25ml of the sterilized (autoclaved at 120oC for 20min) nutrient agar medium was added to sterile Petri dishes, and the plates were set aside for the agar to solidify. By applying the bacterial suspension (1ml/100ml, or cfu/ml) to the surface of the agar plates with a sterile L-shaped glass rod, bacterial lawns were created. On the agar plates, wells were made using a sterile borer (6mm). The prepared test sample concentrations (100 g/ml, 500 g/ml, and 1000 g/ml), together with the control, were aseptically put into wells measuring about 50 l. To ensure that the solution diffused effectively into the nutrient agar medium, the plates were left in the refrigerator undisturbed for at least two hours. The plates were then incubated for a further 24 hours at 37°C. After the incubation period, the diameter of the inhibition zone in each well was manually measured using a millimeter scale, and the results were tabulated. The entire experiment was run in duplicate. To investigate the impact of the solvent on bacteria, dimethyl sulfoxide was kept constant as a control.

Antibacterial activity on Klebsiella pneumoniae:

COMPOUNDS	100µg/ml	500µg/ml	1000µg/ml
A	2mm	5mm	9mm
B	3mm	9mm	15mm
C	2mm	4mm	8mm
D	4mm	9mm	16mm
E	1mm	2mm	5mm
F	3mm	6mm	8mm
G	2mm	4mm	9mm
H	1mm	5mm	9mm
I	1mm	4mm	6mm
J	2mm	4mm	7mm
Standard (Ciprofloxacin)	4mm	9mm	16mm

Antibacterial activity on Micrococcus luteus:

COMPOUNDS	100µg/ml	500µg/ml	1000µg/ml
A	2mm	5mm	7mm
B	5mm	9mm	15mm
C	3mm	5mm	8mm
D	4mm	9mm	15mm
E	1mm	1mm	2mm
F	2mm	5mm	8mm
G	1mm	2mm	3mm
H	2mm	5mm	7mm
I	1mm	5mm	8mm
J	2mm	2mm	3mm
Standard (Ciprofloxacin)	5mm	8mm	16mm

Antibacterial activity on Staphylococcus aureus:

COMPOUNDS	100µg/ml	500µg/ml	1000µg/ml
A	2mm	5mm	9mm
B	2mm	5mm	14mm
C	2mm	5mm	8mm
D	4mm	7mm	15mm
E	1mm	1mm	2mm
F	1mm	2mm	4mm
G	1mm	2mm	4mm
H	2mm	5mm	9mm
I	1mm	5mm	6mm
J	2mm	5mm	8mm
Standard (Ciprofloxacin)	6mm	9mm	15 mm

Antibacterial activity on Escherichia coli:

COMPOUNDS	100µg/ml	500µg/ml	1000µg/ml
A	2mm	7mm	8mm
B	2mm	9mm	15mm
C	3mm	6mm	8mm
D	1mm	2mm	14mm
E	1mm	1mm	2mm
F	2mm	5mm	8mm
G	1mm	2mm	5mm
H	1mm	6mm	8mm
I	1mm	6mm	10mm
J	2mm	2mm	5mm
Standard (Ciprofloxacin)	5mm	9mm	16 mm

***IN-VITRO* ANTI-OXIDANT ACTIVITY**

Evaluation of anti-oxidant activity of new 1,3,4-Thiadiazole derivatives

Materials:

Ascorbic acid: (Analytical grade, Merck India)

Methanol: (HPLC grade, Merck India)

DPPH: (α, α-diphenyl, β-picryl hydrazyl) (Sigma Aldrich)

Test compounds and Double Distilled water.

Preparation of Standard Solution of Ascorbic Acid:

Required amount of Ascorbic acid was accurately weighed and dissolved in distilled water to prepare 1mM stock solution. Solutions of different concentrations (1nM, 3nM, 10nM, 30nM, 1µM, 3µM, 10µM, 30µM, 100µM, 300µM, 1mM) were prepared.

Preparation of DPPH Solution:

0.5mM solution of DPPH was prepared by dissolving the19.71mg of DPPH in 100ml of methanol. The solution was protected from sunlight to prevent the oxidation of DPPH.

Preparation of Test Compounds:

Required amount of test compounds was dissolved in methanol and 1mM stock solution was prepared. Solutions of concentration ranging from 1nM to 1mM were prepared.

Principle:

The method is based on principle described by Blois (1958) method. The model of scavenging the DPPH radical is most widely used method to evaluate the anti-oxidant activity in relatively shorter time compared with other methods. The effect of anti-oxidant on DPPH radical scavenging was thought to be their hydrogen donating ability (Baumann *et al., 2002*).

DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radical was determined by the decrease in its absorbance at 517nm induced by anti-oxidants. The absorption maximum of stable DPPH radical in methanol was at 517nm. The decrease in absorbance of DPPH radical caused by anti-oxidants, because of the reaction between anti-oxidant molecules and radical, progresses, which result in the scavenging of the radical by hydrogen donation. Hence, DPPH is used as a substrate to evaluate the anti-oxidant activity.

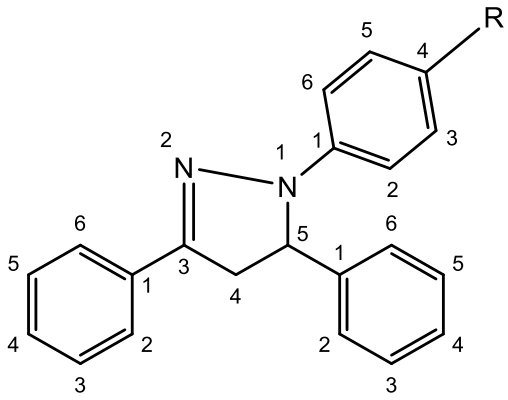
Procedure:

To 2.8ml of test sample/ascorbic acid, 0.2ml of DPPH solution was added, mixed thoroughly and absorbance was measured at 517nm against blank, prepared in an identical way but without the test compound. The results were plotted on a graph and IC50 value was calculated.

Absorbance of blank - Absorbance of % inhibition= ----------X 100

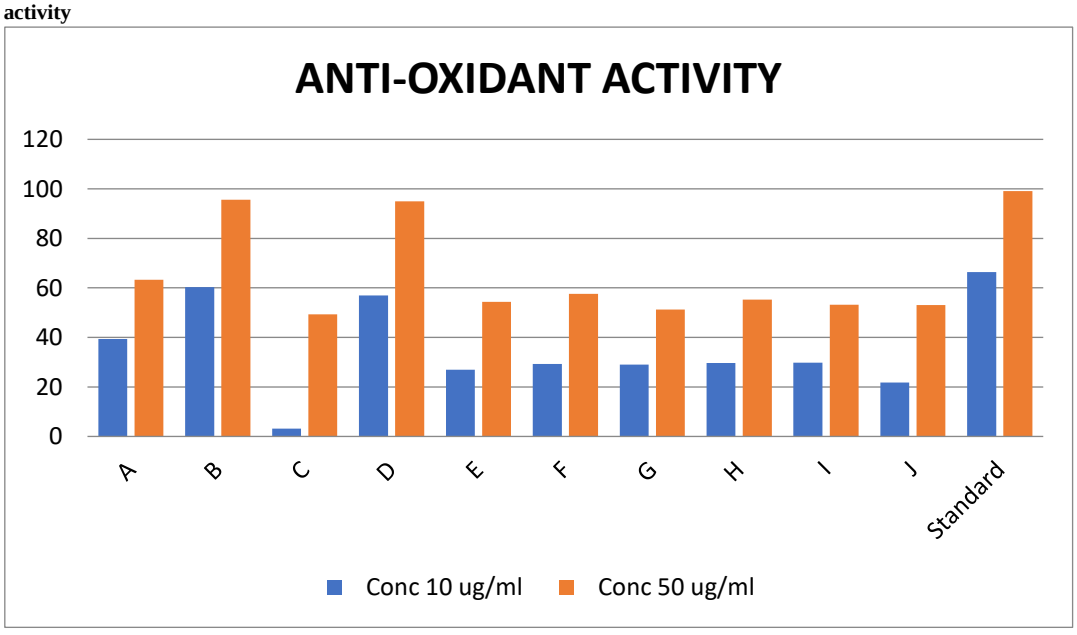
Absorbance of blank

IC_{50} values of standard and synthesised molecules:



S.NO.	COMPOUNDS	Conc 10 ug/ml	Conc 50 ug/ml
1	A	39.37	63.28
2	B	60.31	95.63
3	C	3.18	49.35
4	D	56.93	94.96
5	E	26.91	54.30
6	F	29.32	57.63
7	G	29.06	51.29
8	H	29.67	55.27
9	I	29.74	53.20
10	J	21.79	53.08
11	Standard Ascorbic acid	66.37	99.07

Antioxidant activity



Series one Concentration 10ug/ml and series 2 is Concentration 50 ug/ml

RESULTS AND DISCUSSION

The preliminary studies on antimicrobial activity of the new Pyrazole derivatives have generated some interesting data. An attempt has been made to infer the ultimate outcome of the present studies basing on this data.

The compounds were confirmed by TLC, melting point and spectral studies such as FT-IR, MS, and ^{1}H NMR.

All the synthesized new Pyrazole derivatives were evaluated for antibacterial activity by using standard ciprofloxacin, antifungal activity by using standard fluconazole and antioxidant activity by using ascorbic acid.

ANTIMICROBIAL ACTIVITY:

All the new Pyrazole derivatives employed in the antimicrobial activity. All the test compounds were prepared for assay by dissolving them in DMSO in required quantity in concentrations making 100µg/ml, 500 µg/ml and 1000µg/ml respectively for evaluation of antibacterial activity against gram positive and gram-negative bacteria. Ciprofloxacin used as standard.

Bacteria:

Gram positive

Staphylococcus aureus

Micrococcus luteus

Gram negative

Escherichia coli

Klebsiella pneumonia

Among all the synthesized compounds the following shown high activity for **antibacterial activity** when comparing with standard ciprofloxacin **Chlorine And methyl derivatives are found to have promising antibacterial activity.**

ANTIOXIDANT ACTIVITY

1. The IC**50** values of all synthesized test compounds were found between for compounds and standard with different concentrations
2. All the compounds were tested at 1nM to 1mM concentrations and results were compared with standard drug (Ascorbic acid) at the same concentrations.
3. Among these compounds, compound Cl & CH3 showed (**Chlorine and methyl)** effective antioxidant activity.

CONCLUSION

Broadly the following conclusion could be drawn from the results of these investigations.

Synthetic work of the studies could go positively as per the planning and as such in all the reactions carried out. The expected compounds alone could be obtained.

Newly synthesized compounds were characterized by TLC, IR, ^{1}H-NMR and Mass spectral analysis.

New Pyrazoline derivatives showed promising antimicrobial activity. Compounds **Methyl and Chlorine** Pyrazole derivatives were found to be the more potent antibacterial activity respectively towards gram positive (*Micrococcus luteus*) and gram negative (*klebsiella pneumonia*)

New Pyrazole derivatives showed promising antioxidant activity. Compounds (**R=Cl)** & **(R= CH3)** were found to be more potent antioxidant compounds among the all-test compounds.

Compliance with ethical standards

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Disclosure of conflict of interest:

the authors declare that there are no conflicts of interest

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