



DESIGN, SYNTHESIS, CHARACTERIZATION AND BACTERIOLOGICAL EVALUATION OF NEWER 1,3,5 TRIARYL PYRAZOLE DERIVATIVES

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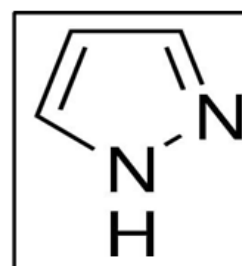
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<p>Article Info</p> <p>Article Received: 08 January 2026, Article Revised: 27 February 2026, Article Accepted: 18 March 2026.</p> <p>DOI: https://doi.org/10.5281/zenodo.19307631</p>	<p>ABSTRACT</p> <p>This research has been successfully synthesized six compounds of 1,3,5-triaryl pyrazole derivatives by two steps reaction. Firstly, pyrazoline compound was obtained by one-pot reaction of aromatic ketones, aldehyde and hydrazine in basic condition. Then, pyrazole compound was obtained by oxidative aromatization of compound in the presence of acetic acid. Chemical structure of predicted molecules was confirmed by FTIR spectroscopy data analysis. Antibacterial activity of compounds was evaluated by Agar well diffusion Method. Biological evaluation result showed that pyrazole compounds had weak Antibacterial activity against Bacillus subtilis compare to standard drug Metronidazole.</p> <p>KEYWORD: Pyrazole, Antibacterial, Metronidazole, Bacillus subtilis.</p>
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1. INTRODUCTION

Heterocyclic compound is the class of cyclic organic compounds those having at least one hetero atom (i.e. atom other than carbon) in the cyclic ring system. The most common heteroatoms are nitrogen (N), oxygen (O) and Sulphur (S). Heterocyclic compounds are frequently abundant in plants and animal products; and they are one of the important constituent of almost one-half of the natural organic compounds known. The unstrained unsaturated heterocyclic compounds include Pyridine, Thiophene, Pyrrole, Furan and their benzo fused derivatives. Quinoline, Isoquinoline, Indole, Benzothiophene, and Benzofuran are some important example of benzo-fused heterocycles. Heterocyclic compounds have a wide application in pharmaceuticals, agrochemicals and veterinary products. Many heterocyclic compounds are very useful and essential for human life. Various compounds such as hormones, alkaloids antibiotic, essential amino acids, hemoglobin, vitamins, dyestuffs and pigments have heterocyclic structure.

Pyrazole is a heterocyclic organic compound characterized by a ring structure composed of three carbon atoms and two nitrogen atoms in adjacent positions, with the molecular formula C₃H₄N₂. Nevertheless, substitutions on the ring can modulate these properties, as, for instance, electron donating groups were shown to increase the acidity of the pyrrole-like NH group. Pyrazoles are aromatic molecules because of their planar conjugated ring configurations with six delocalized π-electrons.



Structure of pyrazole

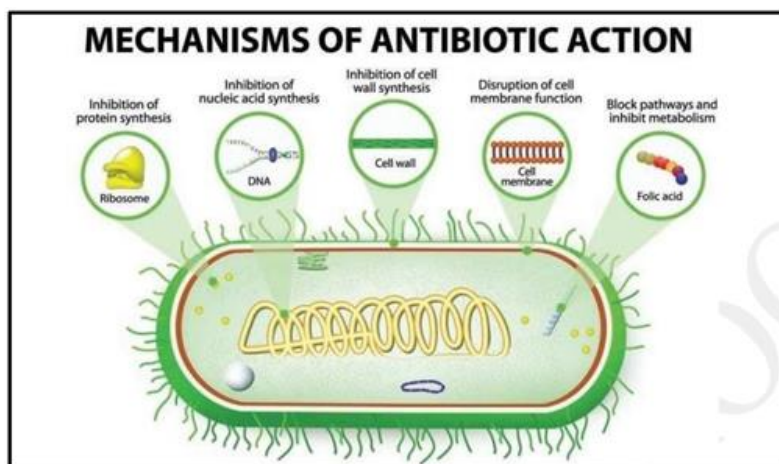
Mechanism of Action of pyrazole

There are different types of antibiotics, which work in their unique way. However, the two main they work include:

1. A bactericidal antibiotic, such as penicillin, kills the

bacteria. These drugs usually interfere with either the formation of the bacterial cell wall or its cell contents.

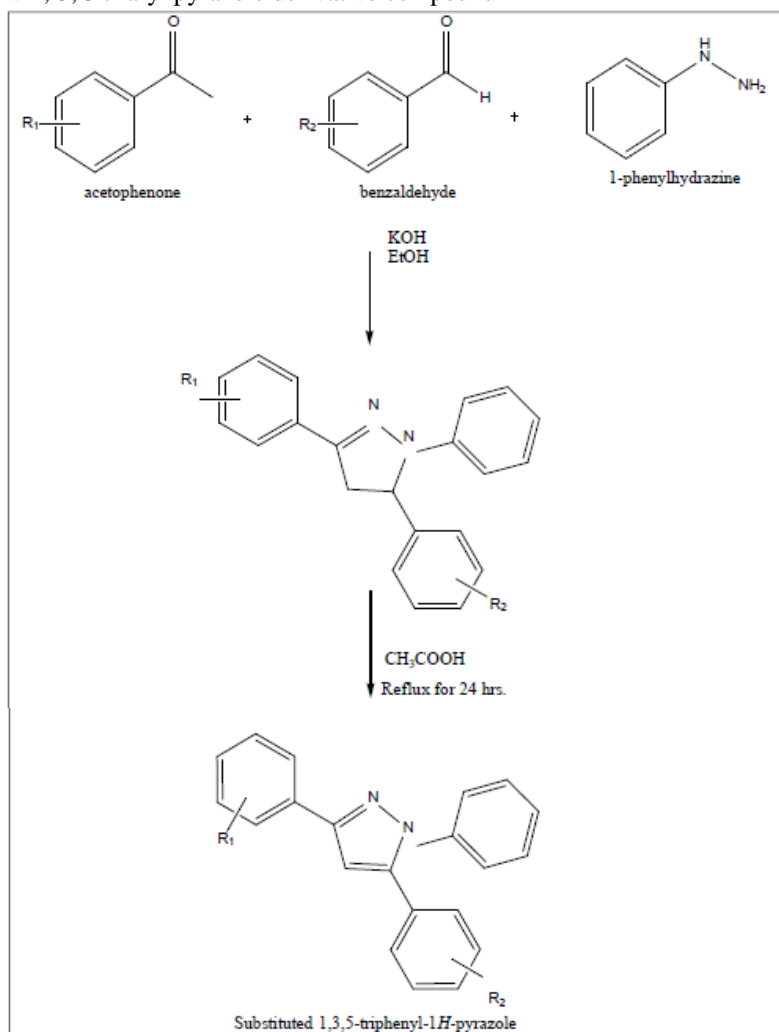
2. A bacteriostatic stops bacteria from multiplying.



Mechanism of Action of Antibiotics

2. EXPERIMENTAL**Synthetic scheme**

Synthetic scheme of new 1, 3, 5 triaryl pyrazole derivative compound



R1 (A)	NAME OF FUNCTIONAL GROUP	R2 (B)	NAME OF FUNCTIONAL GROUP
1	3- nitro Benzaldehyde	1	2-Hydroxy Acetophenone
2	2- nitro Benzaldehyde	2	3-Hydroxy Acetophenone
3	2-Hydroxy Benzaldehyde	3	4-Fluoro Acetophenone
4	4-methoxy Benzaldehyde	4	3-methoxy Acetophenone

Procedure

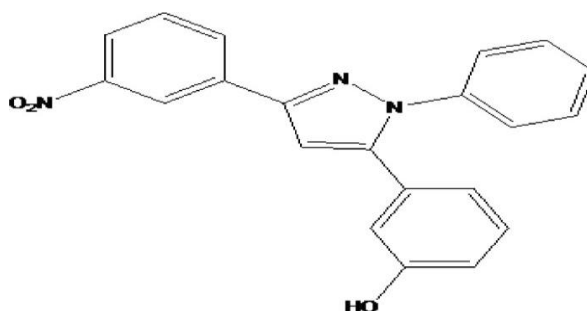
Step: 1

Mixture of aromatic acetophenone 1 (3 mmol), aromatic benzaldehyde 2 (3 mmol), potassium hydroxide (30%, 5 mL) and phenylhydrazine 3 (6mmol) in ethanol (10 mL) was reacted in pressure tube with a stirbar using sealed-vessel reactor at 80°C for 10 minutes. Reaction progress was controlled using TLC plates per 5 minutes reaction time. After the completion of reaction, the mixture was poured to crushed ice to yield solid product. The solid compound was filtered and washed and then recrystallized using methanol.

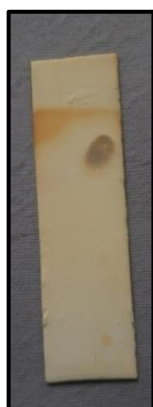
Step: 2

Intermediate product (1 mmol) was dissolved in glacial acetic acid (5 mL), then the mixture was refluxed at 85°C for 24 hours. Reaction progress was controlled using TLC plates per 6 hours reaction time. After the completion of reaction, the mixture was poured to crushed ice to yield solid product. The solid compound was filtered off dried. The crude product was purified by thin layer chromatography using n-hexane:ethylacetate (9:1) isocratic system to yield compound.

Compound 1



Structure of compound 1



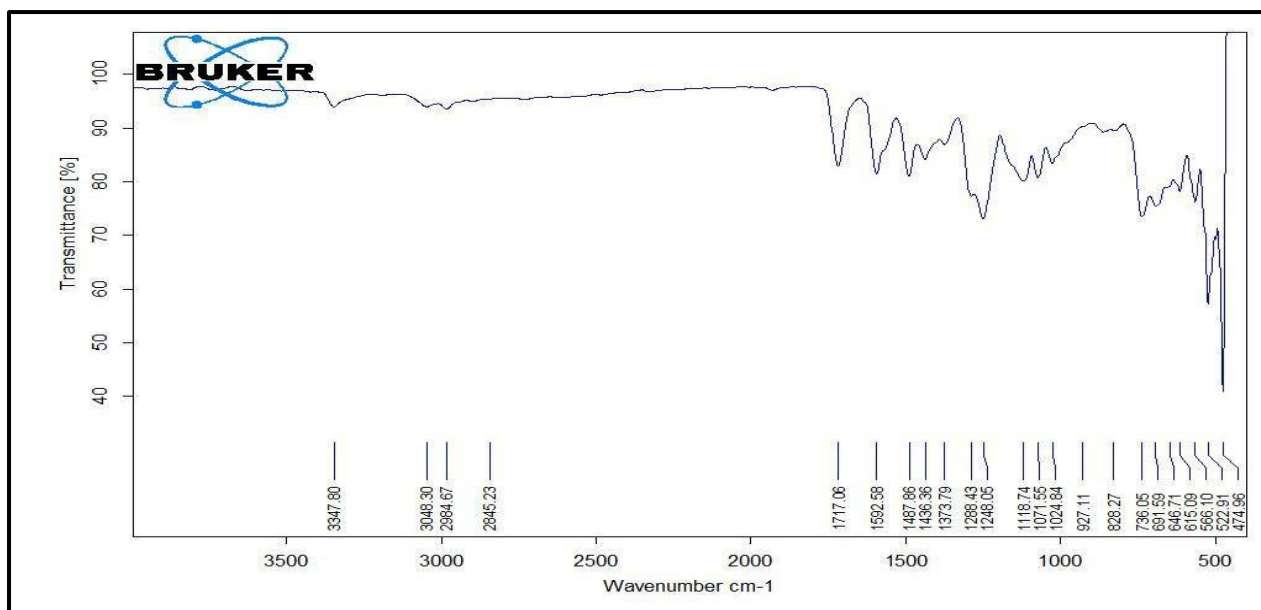
Step 1 TLC Plate



Step 2 TLC Plate Physical data of compound 1

IUPAC Name	2-(5-(2-hydroxyphenyl)-1-phenyl-1H-pyrazole-3-yl)phenol
Molecular Formula	C ₂₁ H ₁₅ N ₃ O ₃
Molecular Weight	357g/mol
Melting Point	99°C
TLC	R _f value of step 1 TLC Plate: 0.7
	R _f value of step 2 TLC Plate: 0.8
	Solvent System: n-hexane:ethylacetate (9:1)
	Detecting Agent: Iodine Chamber

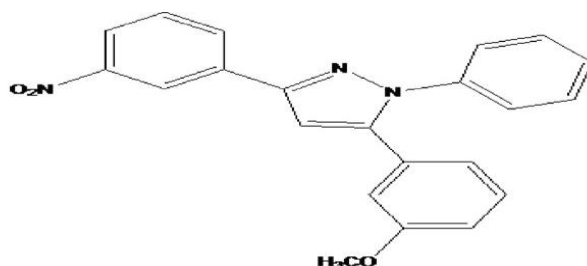
IR Graph of compound 1



Interpretation of compound 1 IR data

Functional Group	Standard Peak (cm ⁻¹)	Observed Peak (cm ⁻¹)
NO ₂	1550-1500	1592
OH	3550-3200	3347
Aromatic Nitrogen	1342-1266	1288

Compound 2



Structure of compound No 2



Step 1 TLC Plate



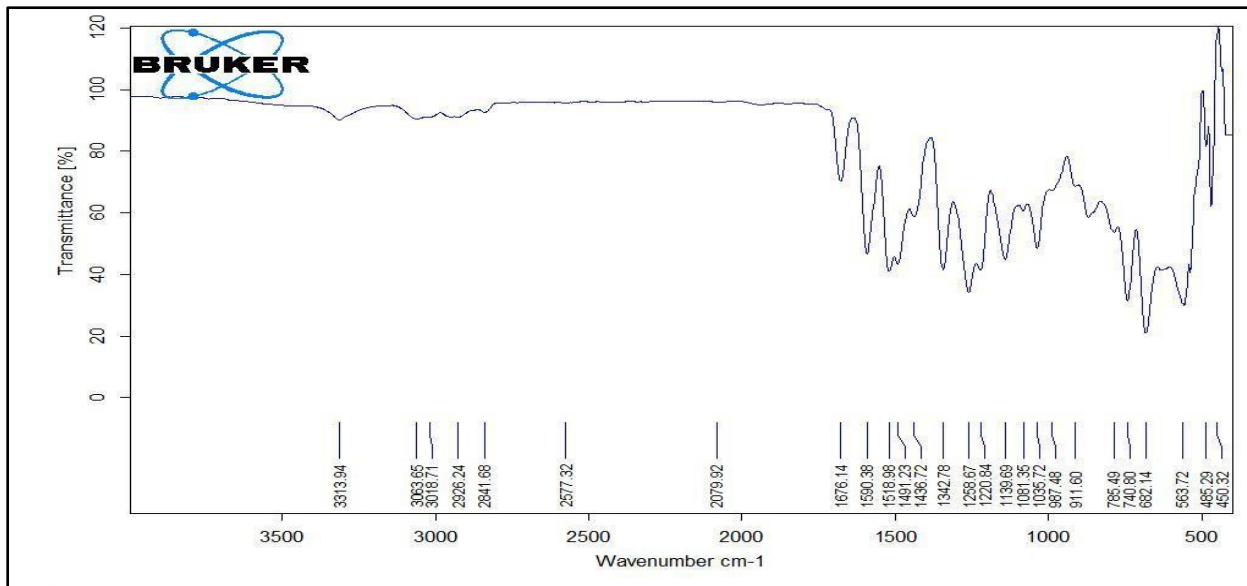
Step 2 TLC Plate

Physical data of compound 2

IUPAC Name	5-(3-methoxyphenyl)-3-(3-nitrophenyl)-1H-pyrazole
Molecular Formula	C ₂₁ H ₁₇ N ₃ O ₃
Molecular Weight	359g/mol
Melting Point	122 °C

TLC	Rf value of step 1 TLC Plate:0.7
	Rf value of step 2 TLC Plate: 0.8
	Solvent system : n-hexane:ethylacetate (9:1)
	Detecting Agent: Iodine Chamber

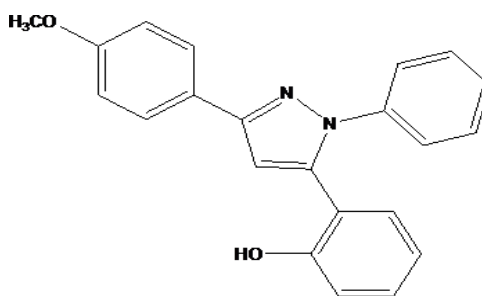
IR Graph of compound 2



Interpretation of Compound 2 IR data

Functional Group	Standard Peak (cm ⁻¹)	Observed Peak (cm ⁻¹)
NO ₂	1550-1500	1590
OCH ₃	2951-2834	2926
Aromatic Nitrogen	1342-1266	1258

Compound 3



Structure of compound 3



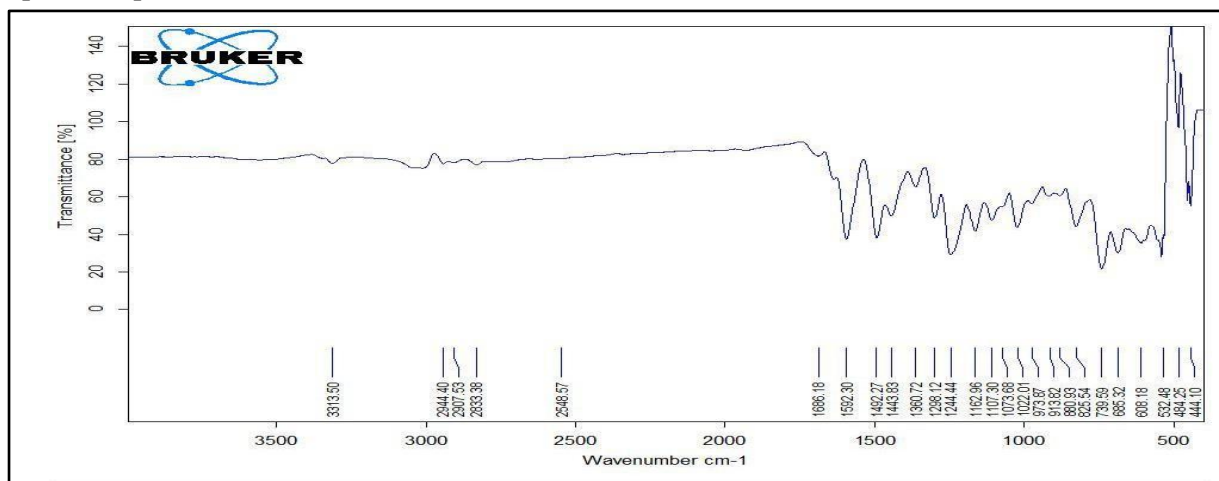
Step 1 TLC Plate



Step 2 TLC Plate Physical data of compound 3

IUPAC Name	2-(3-(4-methoxyphenyl)-1-phenyl-1H-pyrazole-5-yl)phenol
Molecular Formula	C ₂₁ H ₁₈ N ₂ O ₂
Molecular Weight	330g/mol
Melting Point	80 °C
TLC	R _f Value of step 1 TLC Plate:0.6
	R _f value of step 2 TLC Plate:0.8
	Solvent System: n-hexane:ethylacetate (9:1)
	Detecting Agent: Iodine Chamber

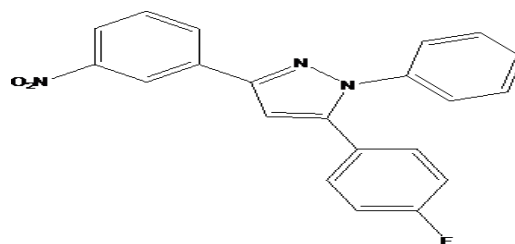
IR Graph of compound 3



Interpretation of Compound 3 IR data

Functional Group	Standard Peak (cm ⁻¹)	Observed Peak (cm ⁻¹)
OH	3550-3200	3313
OCH ₃	2951-2834	2944
Aromatic Nitrogen	1342-1266	1592

Compound 4



Structure of compound 4



Step 1 TLC plate

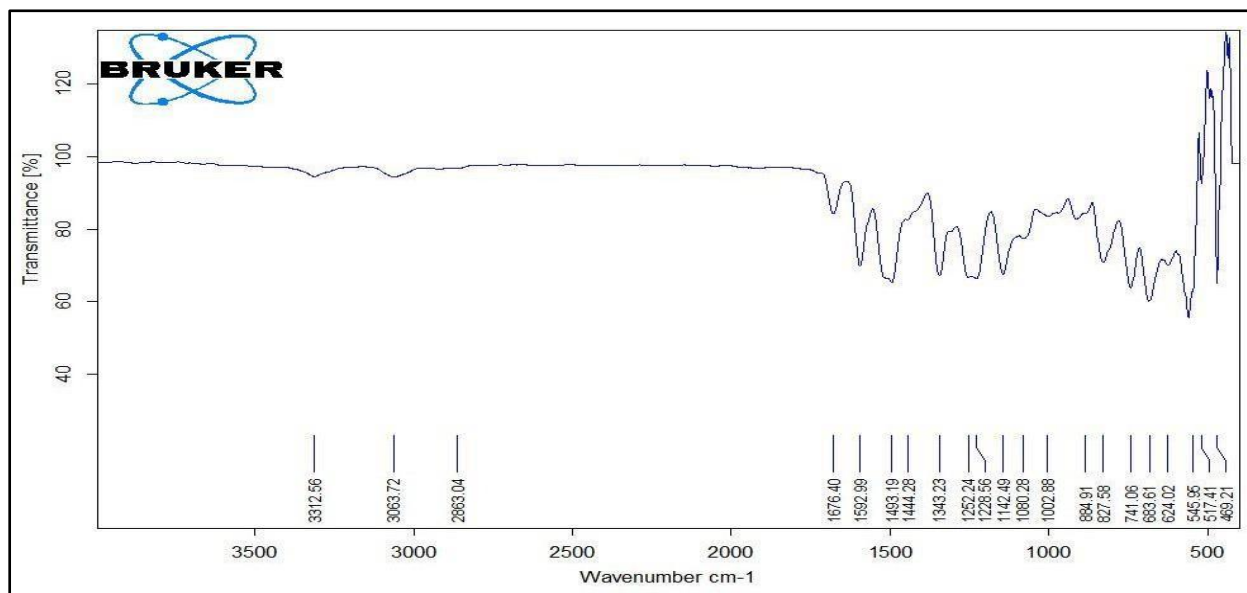


step 2 TLC plate

Physical data of compound 4

IUPAC Name	5-(4-fluorophenyl)-3-(3nitrophenyl)-1-phenyl-1H-pyrazole
Molecular Formula	C ₂₁ FH ₁₄ N ₃ O ₂
Molecular Weight	359 g/mol
Melting Point	115 °C
TLC	R _f value of step 1 TLC Plate:0.8
	R _f value of step 2 TLC Plate:0.7
	Solvent System: n-hexane:ethylacetate (9:1)
	Detecting Agent: Iodine Chamber

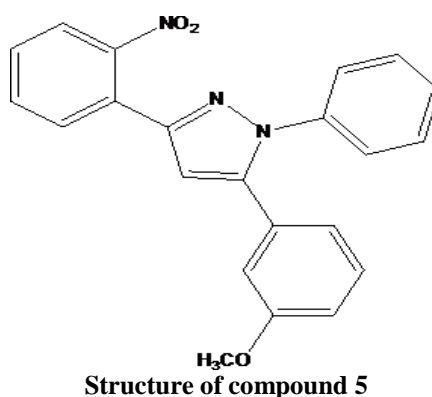
IR Graph of compound 4



Interpretation of Compound 5 IR data

Functional Group	Standard Peak (cm ⁻¹)	Observed Peak (cm ⁻¹)
F	1400-1000	1142
Aromatic Nitrogen	1342-1266	1343

Compound 5





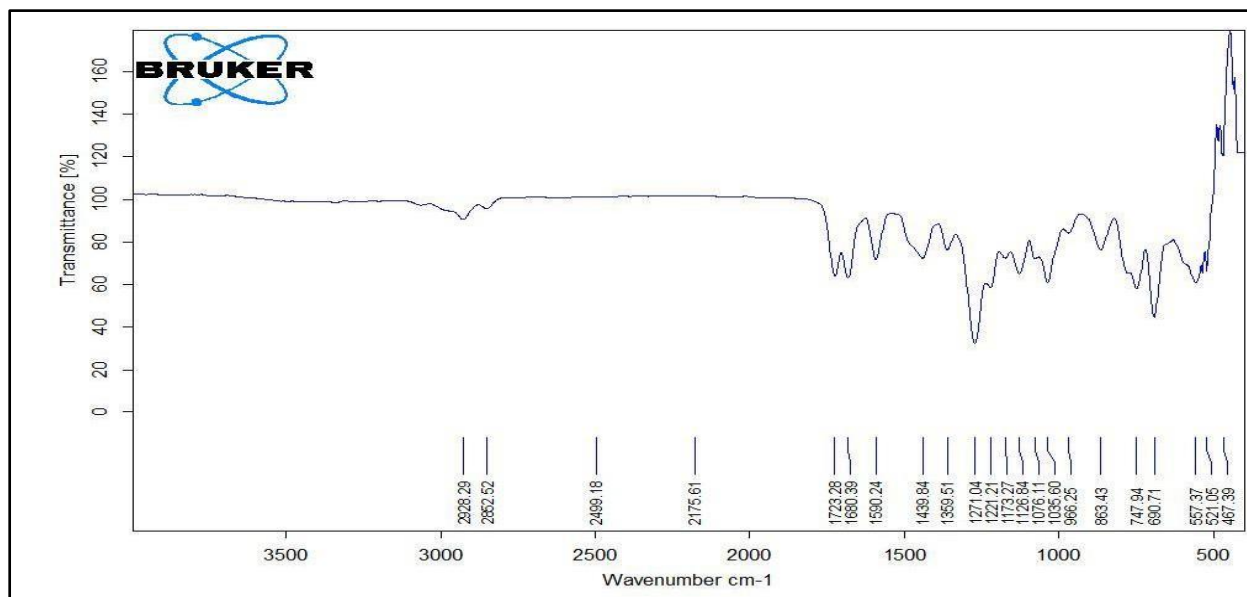
Step 1 TLC plate



Step 2 TLC plate Physical data of compound 5

IUPAC Name	5-(3-methoxyphenyl)-3(2-nitrophenyl)-1-phenyl-1H-pyrazole
Molecular Formula	C ₂₂ H ₁₇ N ₃ O ₃
Molecular Weight	371g/mol
Boiling Point	80°C
TLC	R _f value of step 1 TLC Plate:0.7
	R _f value of step 2 TLC Plate:0.9
	Solvent System: n-hexane:ethylacetate (9:1)
	Detecting Agent: Iodine Chamber

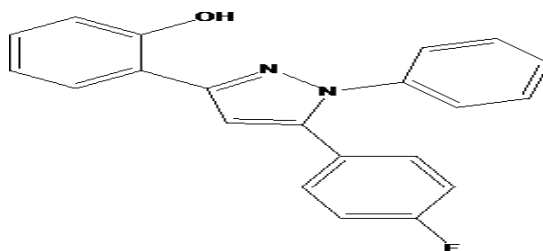
IR Graph of compound 5



Interpretation of Compound 5 IR data

Functional Group	Standard peak (cm ⁻¹)	Observed Peak (cm ⁻¹)
NO ₂	1550-1500	1590
OCH ₃	2951-2834	2928
Aromatic Nitrogen	1342-1266	1271

Compound 6



Structure of compound 6



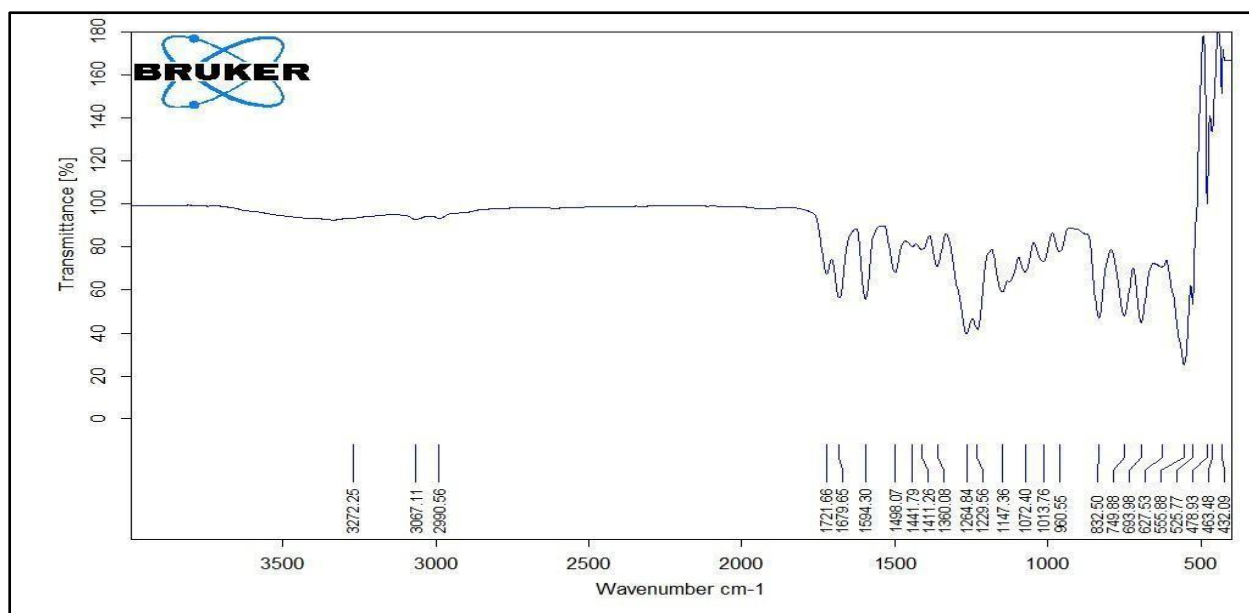
Step 1 TLC Plate



Step 2 TLC Plate Physical data of compound 6

IUPAC Name	2-(5-(4-fluorophenyl)-1-phenyl-1H-pyrazol-3-yl)phenol
Molecular Formula	C ₂₁ H ₁₅ N ₂ O
Molecular Weight	330g/mol
Boiling Point	68°C
TLC	R _f value of step 1 TLC Plate:0.8
	R _f value of step 2 TLC Plate:0.9
	Solvent System: n-hexane:ethylacetate (9:1)
	Detecting Agent: Iodine Chamber

IR Graph of compound 6



Interpretation of Compound 6 IR data

Functional Group	Standard Peak (cm ⁻¹)	Observed Peak (cm ⁻¹)
OH	3550-3200	3272
F	1400-1000	1264
Aromatic Nitrogen	1342-1266	1360

3.0 BIOLOGICAL ACTIVITY OF NEWLY SYNTHESIS COMPOUND MICROBIAL SCREENING

All above synthesized compounds were evaluated and tested for their antibacterial activity against *Bacillus subtilis* as Gram positive bacteria.

Agar disc diffusion method was widely used for determination of the preliminary antibacterial. Metronidazole used as a standard drug.

The results were recorded for each tested compound as the average diameter of zone of inhibition of bacterial growth around the discs in mm.

Agar well Diffusion Method Composition of medium

- Beef extract
- Peptone
- Agar
- Dist. Water- 250ml
- pH 7.0-7.2

Microorganism used

- *Bacillus subtilis*

Standard drug used

- Metronidazole

Procedure

Step: 1 Preparation of Agar medium

- Prepare MHA from the dehydrated medium according to the manufacturer's instructions. Media should be prepared using distilled water or deionized water.
- Heat with frequent agitation and boil to dissolve the medium completely. Sterilize by autoclaving at 121°C for 15 min.
- Check the pH of each preparation after it is sterilized, which should be between 7.2 and 7.4 at room temperature.
- Cool the agar medium to 40-50°C. Pour the agar into sterile glass or plastic petri dish on a flat surface to a uniform depth of 4 mm.
- Allow to solidify.
- Prior to use, dry plates at 30-37°C in an incubator, with lids partly ajar, for not more than 30 minutes or until excess surface moisture has evaporated. Media must be moist but free of water droplets on the

surface. Presence of water droplets may result to swarming bacterial growth, which could give inaccurate results. They are also easily contaminated.

Step: 2 Inoculum

- From a pure bacterial culture (not more than 48 hours, old except for slow growing organisms), take four or five colonies with a wire loop.
- Inoculate the agar by streaking with the swab containing the inoculum.
- Rotate the plate by 60° and repeat the rubbing procedure. Repeat two times. This will ensure an even distribution of the inoculum.
- Allow the surface of the medium to dry for 3-5 minutes but not longer than 15 minutes to allow for absorption of excess moisture.

Step: 3 Agar well diffusion

- The inoculated agar was poured into the assay plate (9 cm in diameter), and allowed to cool down on a leveled surface.
- Once the medium had solidified, four wells, each 4 mm in diameter, were cut out of the agar, and 20 l of the anti-fungal agent were placed into each well. A total of four antibacterial were placed into each plate and incubated at 35 °C for 24 hours.
- Because of disagreement over the criteria to determine the end point of the diameters of the clear zone of inhibition of growth, it was measured both at 50% and 80%. [20,21]

Step: 4 Incubation

- Incubate plates in an inverted position at 30°C or at an optimum growth temperature.
- Observe for the zone of inhibition after 16 to 18 hours. Slow growing organisms may require longer incubation period.

Step: 5 Reading and Measurement of Zone of Inhibition

- The zone of inhibition is the point at which no growth is visible to the unaided eye.
- Record the presence of individual colonies within zones of inhibition.
- Read and record the diameter of the zones of inhibition using a ruler graduated to 0.5mm.

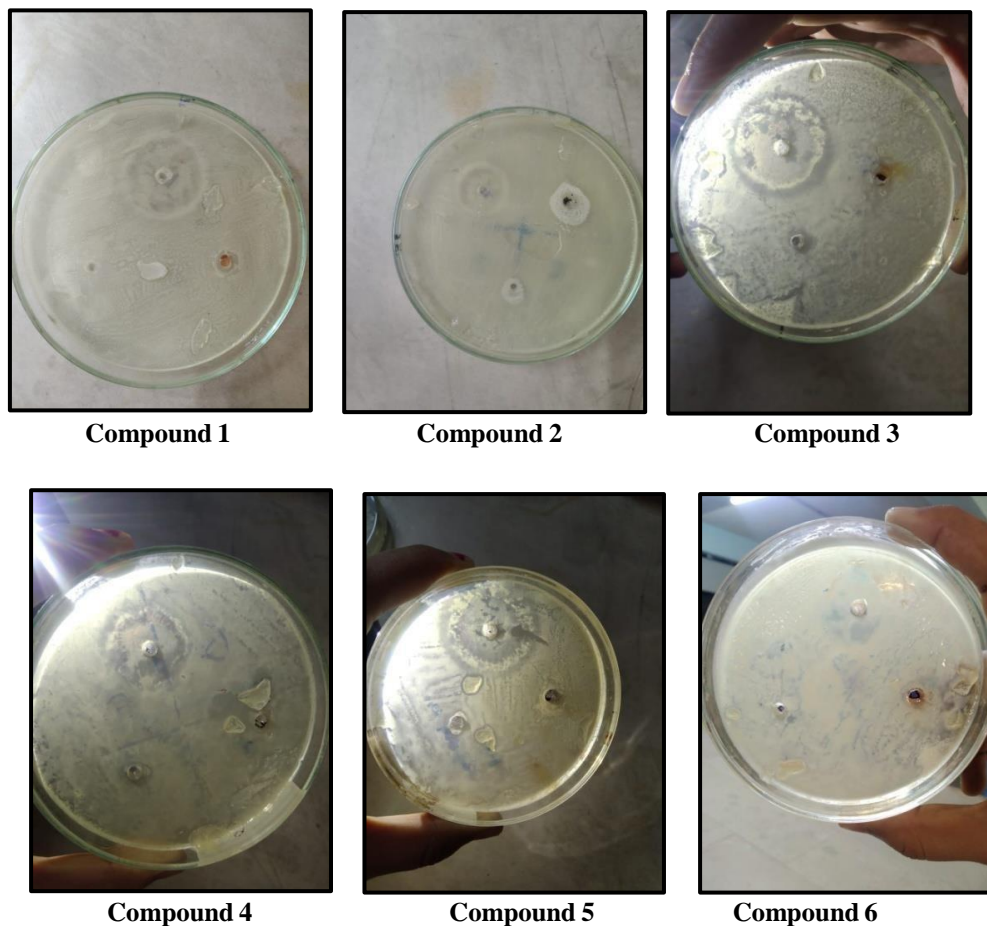


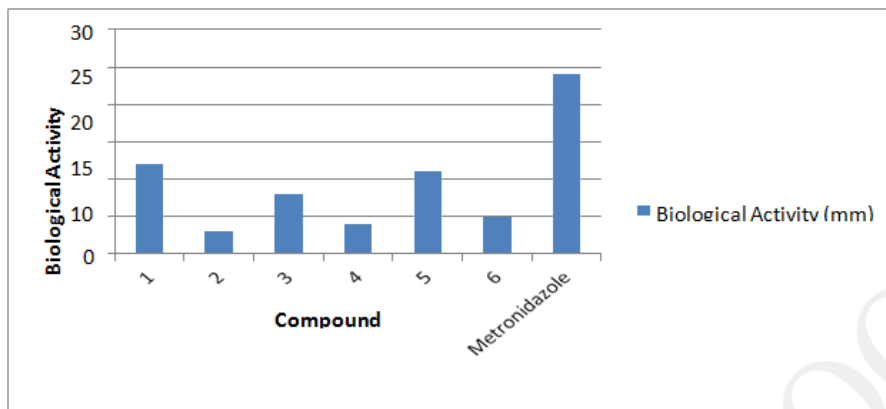
Figure: Biological Activity.

OBSERVATION

Observation table

Compound	Biological Activity (mm)
1	12
2	3
3	8
4	4
5	11
6	5
Metronidazole	24

Graph



Graph of Biological Activity

4.0 CONCLUSION

In this study 1,3,5-triaryl pyrazole derivative compound been successfully synthesized by oxidative aromatization of pyrazoline in presence of acetic acid. Chemical structures of all the synthesized compounds were confirmed by IR Spectroscopy analysis. Microbial Assay of Pyrazole compound showed Antibacterial Activity. Among all these compound, **compound no. 1** having.

Highest Antibacterial activity than other compounds.

5.0 REFERENCE

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