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Research Article

Design and Synthesis of Novel Triazole Derivative as Antimicrobial Agents

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ABSTRACT

The emergence of antimicrobial resistance has become a major global health concern, necessitating the development of new and effective antimicrobial agents. Heterocyclic compounds containing the 1,2,4-triazole nucleus have attracted significant attention in medicinal chemistry due to their diverse biological activities, including antibacterial, antifungal, anti-inflammatory, and anticancer properties. The present research work focuses on the design, synthesis, characterization, and antimicrobial evaluation of novel triazole derivatives as potential antimicrobial agents. A series of five triazole derivatives were synthesized using appropriate synthetic routes and reaction conditions. The synthesized compounds include 3-Phenyl-5-Amino-1,2,4-Triazole, 3,5-Diphenyl-1,2,4-Triazole. The structures of the obtained triazole derivatives were confirmed by various spectroscopic techniques including Infrared (IR) Spectroscopy, Proton Nuclear Magnetic Resonance (^1H NMR) Spectroscopy, Carbon-13 Nuclear Magnetic Resonance (^13C NMR) Spectroscopy, and Mass Spectrometry. IR spectral studies confirmed the presence of characteristic functional groups such as amino ($–NH_2$), aromatic C–H, C=N, C–N, and nitro ($–NO_2$) groups. The ^1H NMR and ^13C NMR spectra provided detailed information regarding the proton and carbon environments present in the synthesized molecules, confirming their proposed structures. Mass spectrometric analysis further established the molecular weights and fragmentation patterns of the compounds, supporting successful synthesis. (1)

INTRODUCTION

The fast rise in microbe resistance to current antibiotics has made the creation of novel antimicrobial drugs one of the most significant areas of pharmaceutical and medicinal chemistry. Globally, infectious diseases brought on by bacteria, fungus, and other microbes continue to cause major health issues. Due to overuse and misuse, many traditional antibiotics have progressively lost their effectiveness, resulting in antimicrobial resistance (AMR). As a result,

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scientists are always looking for new heterocyclic compounds with potent antibacterial qualities and low toxicity. The 1,2,4-triazole nucleus has become one of the most important heterocyclic compounds due to its diverse biological and pharmacological properties. Five-membered aromatic heterocyclic compounds with three nitrogen atoms are known as triazole derivatives. Excellent biological effects, including antibacterial, antifungal, antiviral, anti-inflammatory, antioxidant, anticancer, anticonvulsant, and analgesic qualities, are displayed by these nitrogen-rich compounds. Triazole derivatives are widely used in medicinal chemistry and drug development because of their exceptional therapeutic potential. The triazole ring's nitrogen atoms increase electron density and strengthen binding interactions with biological targets such microorganism proteins and enzymes. Triazole compounds work by interfering with DNA synthesis, affecting the creation of cell membranes, blocking microbial enzyme systems, and stopping microbial growth. They are good prospects for the development of new antibacterial medications due to their potent aromatic character and great chemical stability. Antimicrobial agents are compounds that either stop germs from growing or kill them. These substances are primarily categorised as antiviral, antifungal, antibacterial, and antiparasitic medications. Antifungal agents prevent the growth of fungi, whereas antibacterial treatments combat bacteria. The capacity of antimicrobial drugs to specifically target microbial cells without harming human cells determines how successful they are. To combat the growing resistance that harmful microbes have gained, new antimicrobial chemicals must be discovered. Pharmaceutical products that include triazoles show how crucial this heterocyclic nucleus is to contemporary medicine. The following are a few commercially available triazole derivatives:

- Fluconazole – widely used antifungal drug for Candida infections.
- Itraconazole – broad-spectrum antifungal agent effective against systemic fungal infections.
- Voriconazole – used in severe invasive fungal infections.
- Ribavirin – antiviral drug containing triazole-related heterocyclic features.
- Alprazolam – triazole fused benzodiazepine used for anxiety disorders. [1, 2]

These commercially available medications unequivocally show that triazole derivatives have great therapeutic promise and can be further altered to produce molecules with increased antibacterial activity. The design and synthesis of new triazole derivatives as antibacterial agents is the main focus of the current research project. By adding phenyl, amino, and nitro substituents to the triazole nucleus' structure, antibacterial efficacy, lipophilicity, and contact with microbial targets may all be enhanced. Because of the combined actions of electron-withdrawing functional groups and aromatic substitution, the produced compounds are anticipated to exhibit excellent antibacterial and antifungal characteristics. [3,4]

EXPERIMENTAL METHODOLOGY

1) Synthesis of 4-Amino-3-Phenyl-5-(2,4-dinitrophenyl)-1,2,4-Triazole

1. Take a clean, dry 100 mL round bottom flask.
2. Add 1.98 g of 2,4-dinitrophenylhydrazine to the flask.
3. Add 1.0 mL benzaldehyde (≈1.06 g) slowly with continuous stirring.

4. Add 1.54 g ammonium acetate to the mixture.
5. Add 25 mL ethanol as solvent and mix thoroughly.
6. Add 2–3 drops of glacial acetic acid to catalyze the reaction.
7. Attach a reflux condenser to the flask.
8. Heat the reaction mixture under reflux at 78–80°C for 5–6 hours.
9. Monitor the progress using TLC (ethyl acetate: toluene = 3:7). 2–3 drops
10. After completion, allow the reaction mixture to cool to room temperature.
11. Pour the mixture into 50–60 mL ice-cold water with stirring.
12. A colored precipitate (orange-yellow) will form.
13. Filter the solid using vacuum filtration.
14. Wash the product with cold water followed by a small amount of cold ethanol.
15. Dry the crude product and recrystallize from ethanol to obtain pure compound. [5,6]

2) Synthesis of 4-Amino-3-Phenyl-5-Phenyl-1,2,4-Triazole

1. Take a clean, dry 100 mL round bottom flask.
2. Add 1.45 g phenylhydrazine hydrochloride.
3. Add 1.0 mL benzaldehyde (≈1.06 g) slowly with stirring.
4. Add 0.60 g urea. 5. Add 25 mL ethanol as solvent.
5. Add 2–3 drops glacial acetic acid.
6. Attach a reflux condenser.
7. Heat under reflux at 78–80°C for 4–5 hours.
8. Monitor reaction by TLC.
9. After completion, cool to room temperature.
10. Pour into 50 mL ice-cold water.
11. Collect precipitated solid by filtration.
12. Wash with cold water and ethanol.
13. Recrystallize from ethanol. [7,8]

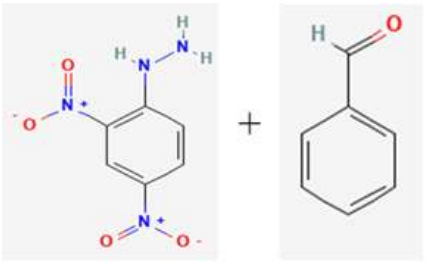
SCHEMES

1) Reacton scheme for 4-Amino-3-Phenyl-5-(2,4-dinitrophenyl)-1,2,4-Triazole

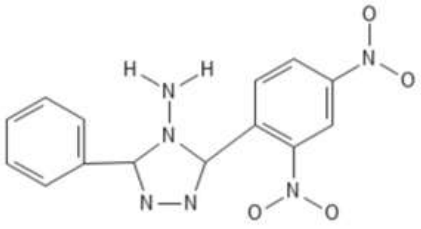
2,4-Dinitrophenylhydrazine + Benzaldehyde

↓ (Ethanol, Ammonium acetate)

→ 4-Amino-3-Phenyl-5-(2,4-dinitrophenyl)-1,2,4-Triazole



(Ethanol, Ammonium acetate)→

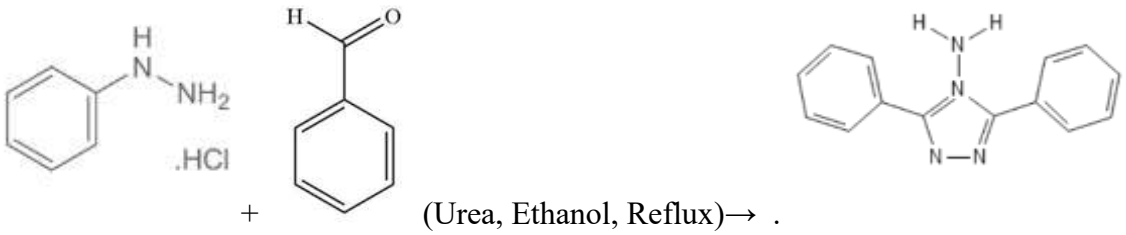


2) Reaction Scheme for 4-Amino-3-Phenyl-5-Phenyl-1,2,4-Triazole

↓ (Urea ,Ethanol, Reflux)

→ 4-Amino-3,5-Diphenyl-1,2,4-triazole

Phenylhydrazine·HCl + Benzaldehyde



+ (Urea, Ethanol, Reflux)→ .

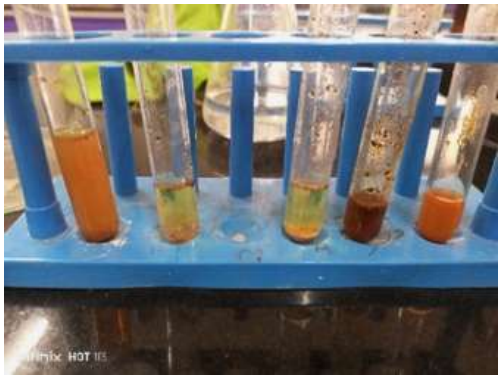
IDENTIFICATION TEST WITH RESULT

Table No. 1: List Of Parameters

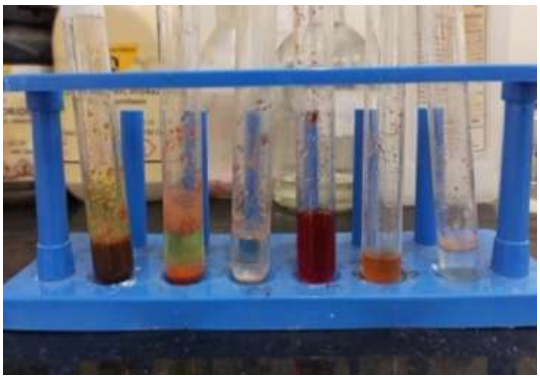
Sr. no.	Name of Parameter	COMP A	COMP B
1	Practical Yield	2.2 gm	1.80 gm
2	Theoretical Yield	3.2 g/mol	2.52 g/mol
3	% Practical Yield	68 % w/w	71% w/w
4	Appearance	Crystalline Solid	Light yellow crystalline solid
5	Color	– Yellow to orange	Brown to greenish
6	Odour	Aromatic odour	Odour less or characteristic aromatic odour
7	Solubility	Soluble in ethanol, chloroform, DMSO and DMF; sparingly soluble in water.	Soluble in ethanol, methanol and DMSO; sparingly soluble in water.
8	Melting Point	260–270 °C	182–184 °C

Table No. 2: List Of Chemical Tests [9,10]

Sr. No.	Test	Procedure	Observation	
			Compound A	Compound B
1	**Ferric Chloride Test**	Dissolve a small amount of compound in ethanol. Add 2–3 drops of neutral $FeCl_3$ solution.	No color change	**Decolorization**
2	**Bromine Water Test**	Asmall quantity of the compound was dissolved In ethanol and treated with bromine water.	Decolorization of bromine water.	Decolorization of bromine water.
3	**Ninhydrin test**	Add 2–3 drops of ninhydrin solution to the sample solution. Heat gently.	Purple/blue color formation.	Purple/blue color formation.
4	**Sodium Bicarbonate Test**	Add compound to $NaHCO_3$ solution	No effervescence	No effervescence



Compound A



Compound B

Fig.1: Physical test and Chemical test of Compound A and B

TLC of chemical compound

Compound A TLC

Compound B TLC

Fig.2:TLC of Compound A and B

Table No. 3: TLC of Compound A and B

Compound		Distance travel (in CM)	R.F Value
Compound A	Solvent	6.5	---
	Benzaldehyde	5.9	0.90
	End product	6.2	0.95
Compound B	Solvent	6.5	---
	Benzaldehyde	6	0.9
	End product	5.7	0.8

Biological test

1. Disc Diffusion Method

- Purpose: Simple screening of antimicrobial activity
- **Procedure:** Soak sterile filter paper discs in compound solution. Place on inoculated agar plates. Incubate for 24 hours.

Compound A **Compound B**

Fig.3: Disc Diffusion Method of Compound A and B

Table No. 4: Disc Diffusion Method for Compound A and B

Test Organism	Type	Zone of Inhibition	
		Compound A	Compound B
Escherichia coli	Gram-negative	14 mm	11–14 mm
Staphylococcus aureus	Gram-positive	18 mm	14–18 mm

2. Antifungal Activity (Poisoned Food Technique)

- **Purpose:** Test against fungi (e.g., *Aspergillus*, *Candida*)
- **Procedure:** Mix compound with agar medium. Inoculate fungus at center. Incubate for 2–3 days.

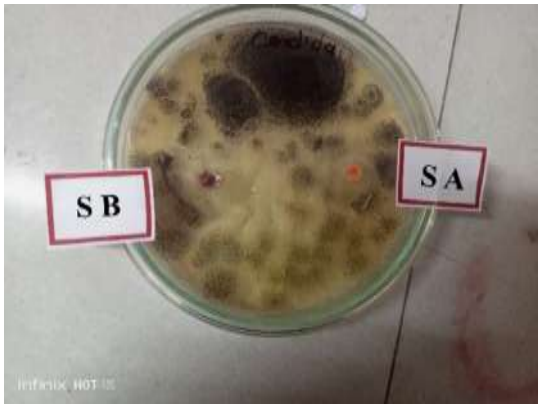


Fig.4: Antifungal Activity test Compound A and B

Table No. 5: Antifungal Activity test for Compound A and B

Test Fungus		Activity Level	Zone of Inhibition	
			Compound A	Compound B
Aspergillus niger		Moderate	8 mm	9 mm

CHARACTERIZATION OF COMPOUND

1. IR spectroscopy for Compound A and B

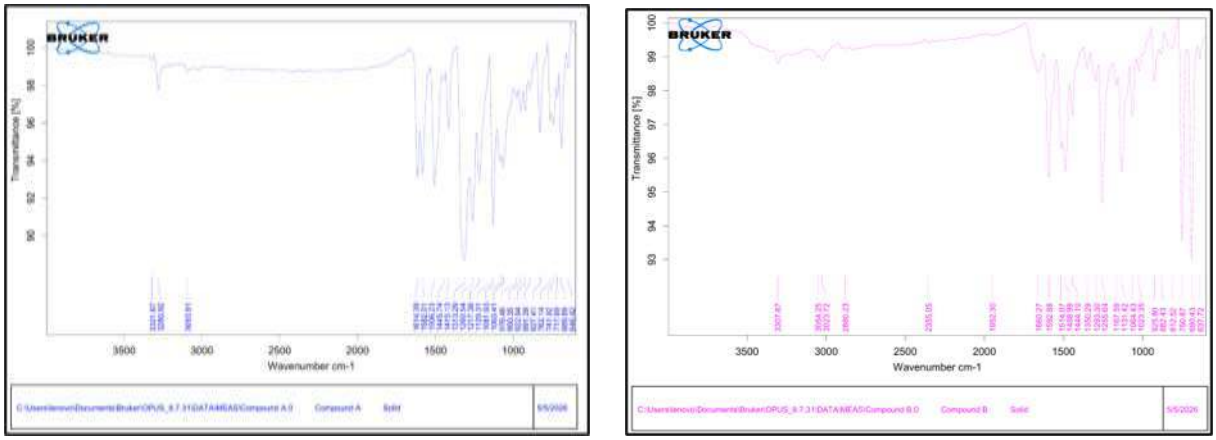


Fig. 5: IR Spectroscopy of Compound A and B

Table No. 5: Interpretation of Compound A and B. (IR)

Compound A		Compound B	
IR Peak (cm^{-1})	**Functional Group**	**IR Peak (cm^{-1})**	**Functional Group**
3321, 3280	$–NH_2$ stretching	3307	N–H stretching (amino group)
3093	Aromatic C–H	3054, 3023	Aromatic C–H stretching
1614	C=N (triazole ring)	1660	C=N stretching of triazole ring
1582, 1506	Aromatic C=C	1592, 1514	Aromatic C=C stretching
1313–1217	C–N stretching	1350–1167	C–N stretching
762–689	Monosubstituted phenyl ring	750, 690	Monosubstituted phenyl ring

2. Mass spectroscopy

2.1 Mass Spectroscopy of Compound A

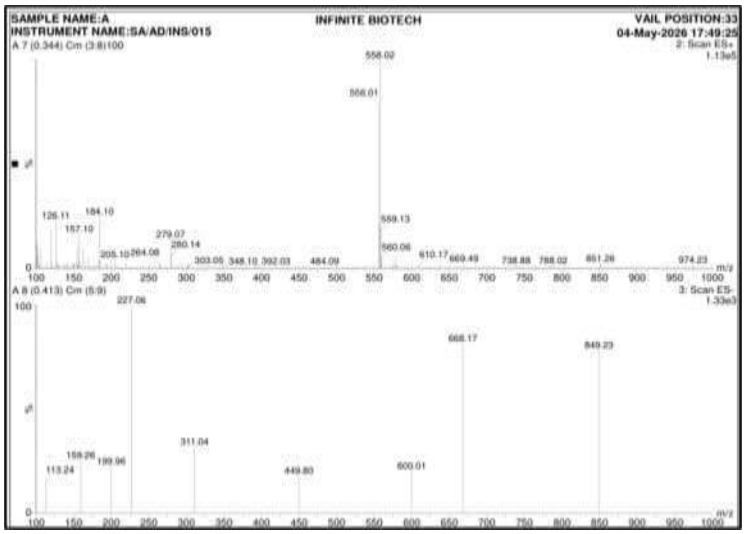


Fig. 6: Mass Spectroscopy of Compound A

Table No. 6: Mass Spectroscopy of Compound A

M/z (observed)	Relative Intensity (%)	Possible Assignment	M/z (observed)	Relative Intensity (%)	Possible Assignment
126.11	15	Fragment ion	449.80	18	Fragment ion
157.10	18	Fragment ion	558.02	100	$[M+H]^+$
184.10	22	Fragment ion	668.17	60	Adduct /Dimer ion

227.06	100	$[M+H-NO_2]^+$	849.23	65	Adduct /Dimer ion
279.07	25	$[M+H-NH_2]^+$	974.23	12	High mass fragment
311.04	30	Fragment ion			

2.2 Mass Spectroscopy of Compound B

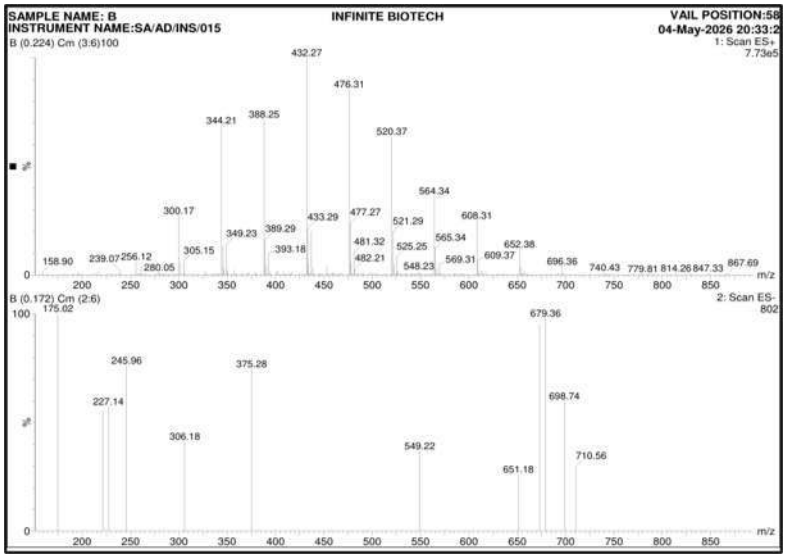


Fig. 7: Mass Spectroscopy of Compound B

Table No.7: Mass Spectroscopy of Compound B

M/z (observed)	Relative Intensity (%)	Possible Assignment	M/z (observed)	Relative Intensity (%)	Possible Assignment
158.90	8.2	Fragment ion	476.31	78.4	Fragment ion
175.02	22.4	Aromatic fragment	520.37	60.6	Fragment ion
227.14	18.6	Fragment ion	564.34	42.7	Fragment ion
245.96	28.7	Phenyl fragment	608.31	30.5	Fragment ion
256.12	15.3	Fragment ion	652.38	24.8	Fragment ion
300.17	35.1	Fragment ion	679.36	85.2	High mass fragment
305.15	20.5	Fragment ion	740.43	16.7	High mass fragment
344.21	48.6	Fragment ion	779.81	12.3	High mass fragment
375.28	32.9	Fragment ion	814.26	10.5	High mass fragment
388.25	62.3	Fragment ion	867.69	9.4	High mass fragment
432.27	**100.0**	**Base peak $(M+H)^+$**	975.83	6.2	High mass fragment

3. NMR Spectroscopy

3.1 ^{1}H NMR Spectroscopy of Compound A

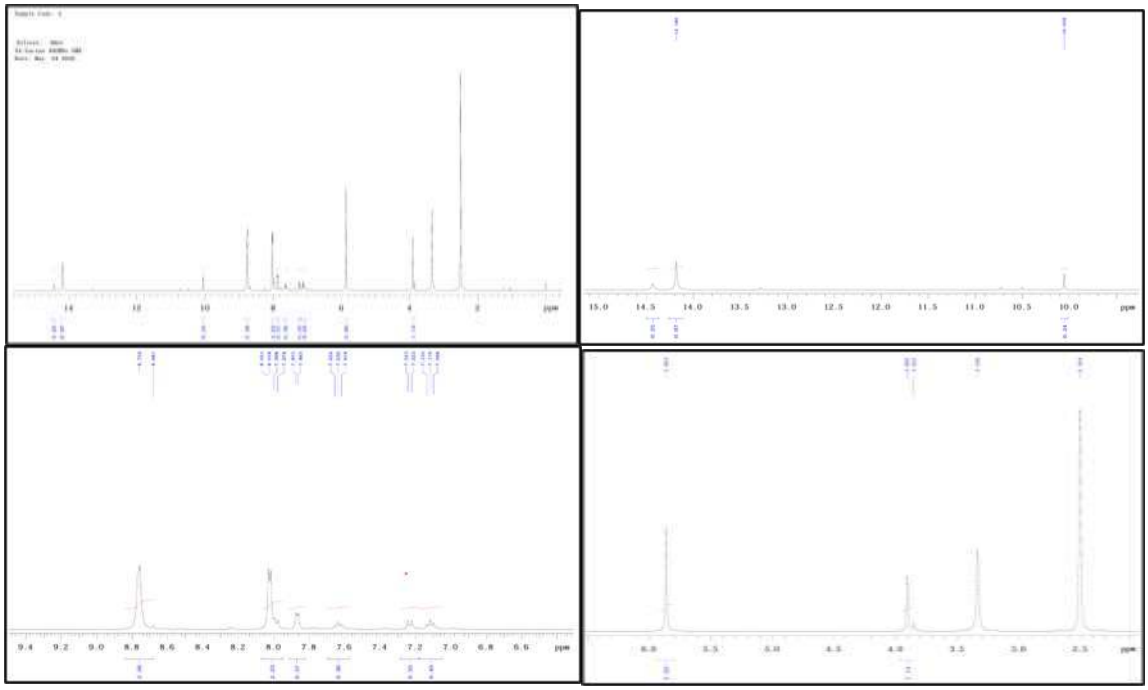


Fig. 8: ^{1}H NMR Spectroscopy of Compound A

Table no. 8: ^{1}H NMR Spectroscopy of Compound A

δ (ppm)	Assignment	δ (ppm)	Assignment
14.18	N–H proton of triazole ring (strongly deshielded)	7.66–7.62	Aromatic protons
10.06	Amino proton (–NH_2) / exchangeable proton	7.24–7.10	Phenyl ring protons
8.76–8.68	Aromatic proton adjacent to electron-withdrawing group	3.34	Residual water in DMSO
8.03–7.98	Aromatic protons	2.50	Residual DMSO-d_6 solvent peak
7.88–7.86	Aromatic protons	7.66–7.62	Aromatic protons

Interpretation

The spectrum contains: One highly deshielded N–H signal at δ 14.18 ppm, One exchangeable –NH_2 signal around δ 10.06 ppm, Multiple aromatic proton signals between δ 7.1–8.8 ppm, No aliphatic proton signals

This pattern is characteristic of a 1,2,4-triazole derivative containing an amino group and a nitro-substituted aromatic ring.

Based on the NMR data together with the IR spectrum you previously uploaded (showing NH_2 and strong aromatic/nitro absorptions), the compound is most consistent with: 4-Amino-3-Phenyl-5-(2,4-dinitrophenyl)-1,2,4-Triazole

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ 14.18 (s, 1H, N–H), 10.06 (s, 2H, NH_2), 8.76–8.68 (m, aromatic H adjacent to NO_2), 8.03–7.98 (m, aromatic H), 7.88–7.86 (m, aromatic H), 7.66–7.62 (m, aromatic H), 7.24–7.10 (m, phenyl H).

3.2 ^{1}H NMR Spectroscopy of compound B

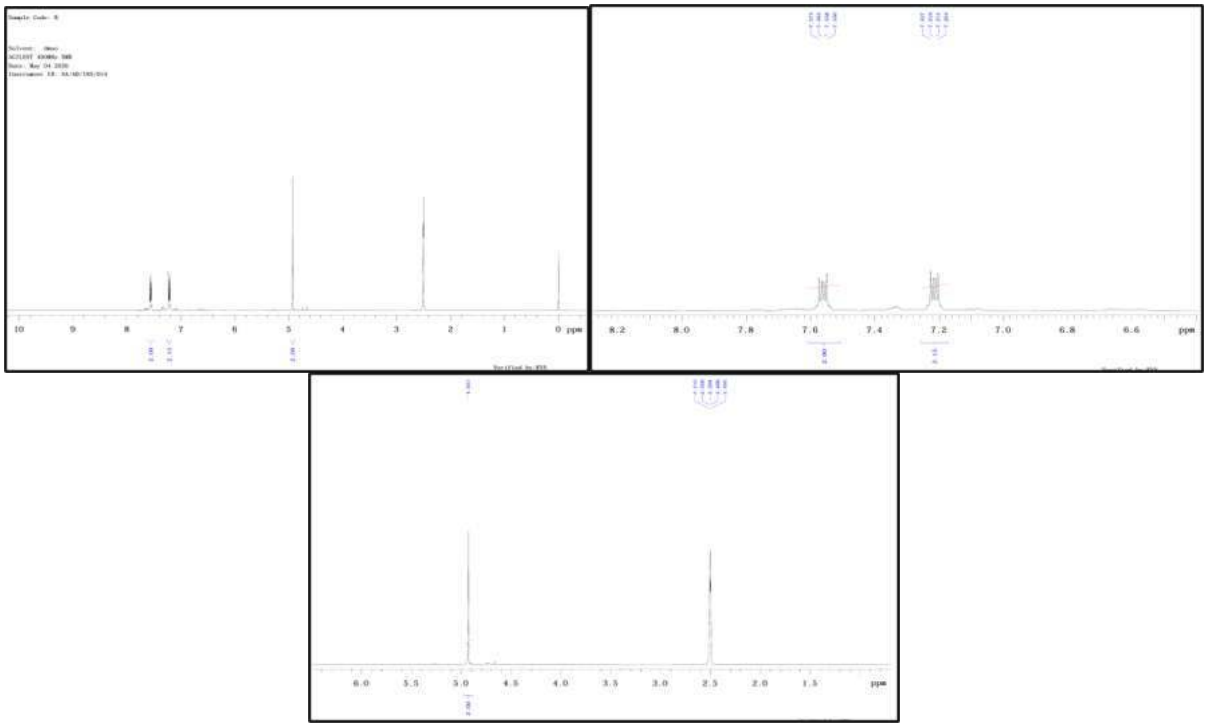


Fig. 9: ^{1}H NMR Spectroscopy of Compound B

Table no. 9: ^{1}H NMR Spectroscopy of Compound B

δ (ppm)	Multiplicity	Assignment
7.57–7.55	Multiplet	Aromatic protons (Phenyl ring)
7.23–7.20	Multiplet	Aromatic protons (Phenyl ring)
4.93	Broad singlet	–NH_2 protons
2.50	Residual DMSO-d_6 solvent peak	

Interpretation

The spectrum shows: A broad signal at δ 4.93 ppm corresponding to an amino group (–NH_2). Aromatic proton signals between δ 7.20–7.57 ppm, indicating the presence of phenyl rings. No aliphatic proton signals are observed. The total aromatic integration is consistent with two phenyl rings attached to a heterocyclic nucleus.

Most Probable Compound

The NMR pattern is most consistent with: 4-Amino-3,5-Diphenyl-1,2,4-Triazole

Expected Structure ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ 7.57–7.55 (m, aromatic H, phenyl rings), 7.23–7.20 (m, aromatic H, phenyl rings), 4.93 (br s, 2H, NH_2). These data support the structure of 4-Amino-3,5-Diphenyl-1,2,4-Triazole ($C_{14}H_{12}N_4$).

3.3 ^{13}C NMR Spectroscopy of Compound A

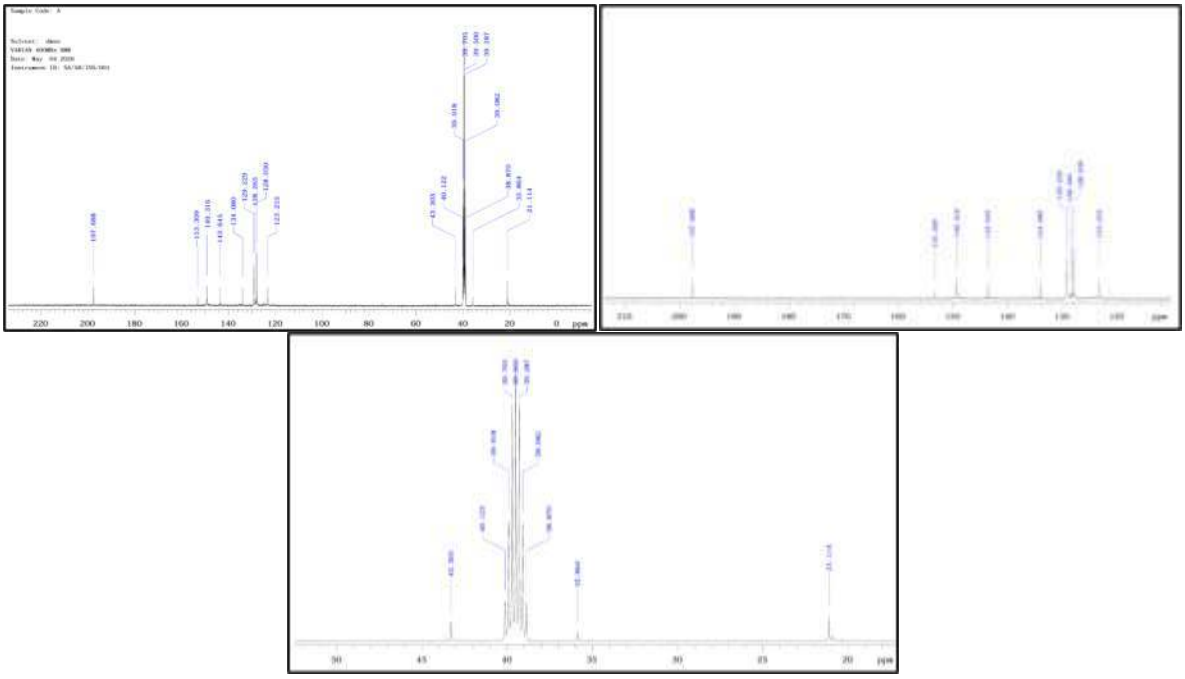


Fig. 10: ^{13}C NMR Spectroscopy of Compound A

Table no. 10: ^{13}C NMR Spectroscopy of Compound A

δ (ppm)	Assignment
197.69	Highly deshielded carbon (likely C=N/C=S region or impurity)
153.31	Triazole ring carbon attached to N
149.32	Triazole/aromatic carbon attached to electron-withdrawing group
143.65	Aromatic carbon bonded to NO_2
134.08	Quaternary aromatic carbon
129.23	Aromatic CH carbon
128.27	Aromatic CH carbon
128.03	Aromatic CH carbon
123.26	Aromatic carbon adjacent to NO_2 group

Additional peaks at 39–40 ppm are due to DMSO-d_6 solvent and should not be assigned to the compound.

Interpretation

The spectrum contains: Multiple aromatic carbons between 123–134 ppm, Deshielded carbons at 143–153 ppm, characteristic of carbons attached to nitrogen and nitro groups, A pattern consistent with a 1,2,4-triazole ring, A nitro-substituted aromatic ring

Most Probable Compound

Combining this ^{13}C NMR data with the previously supplied IR and ^{1}H NMR data, the compound is most consistent with: 4-Amino-3-Phenyl-5-(2,4-dinitrophenyl)-1,2,4-Triazole

Carbon Assignments ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 153.31, 149.32, 143.65, 134.08, 129.23, 128.27, 128.03, 123.26. Signals at ~39.5 ppm correspond to DMSO-d_6 solvent.

Therefore, the spectral data support the structure of 4-Amino-3-Phenyl-5-(2,4-dinitrophenyl)-1,2,4-Triazole.

3.4 ^{13}C NMR Spectroscopy of Compound B

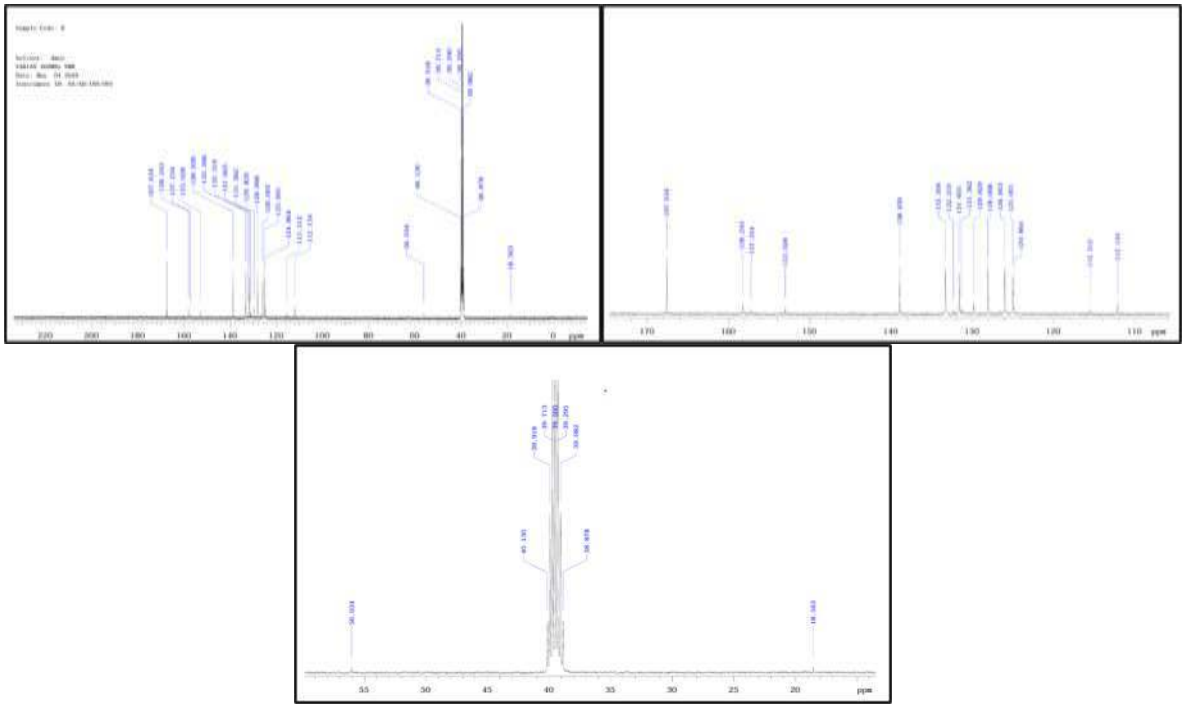


Fig. 11: ^{13}C NMR Spectroscopy of Compound B

Based on the ^{13}C NMR spectrum (DMSO-d_6) of Sample B, the significant carbon signals are:

Table no. 11: ^{13}C NMR Spectroscopy of Compound B

δ (ppm)	Assignment	δ (ppm)	Assignment
167.63	Triazole ring C=N carbon	131.36	Aromatic carbon
158.24	Triazole/aromatic carbon attached to heteroatom	129.83	Aromatic CH carbon
157.23	Triazole C=N carbon	128.07	Aromatic CH carbon
153.03	Aromatic carbon bonded to electron-withdrawing substituent	126.00	Aromatic CH carbon
138.94	Quaternary aromatic carbon	125.00	Aromatic CH carbon
133.31	Ipso phenyl carbon	124.86	Aromatic CH carbon
132.32	Aromatic carbon	115.51	Aromatic carbon
131.61	Aromatic carbon	112.13	Aromatic carbon

Additional peaks:

- 39–40 ppm → DMSO-d_6 solvent peaks (ignore for structure assignment)
- 56.03 ppm → trace solvent/impurity or methoxy-containing impurity
- 18.56 ppm → minor impurity peak

Interpretation

The spectrum shows: Multiple aromatic carbons (112–139 ppm), Three highly deshielded carbons at 167.63, 158.24, and 157.23 ppm, characteristic of a 1,2,4-triazole ring, No aliphatic carbon signals attributable to the main compound, Carbon count and aromatic pattern consistent with a diphenyl-substituted triazole

When combined with the previously provided ^{1}H NMR spectrum, which showed: NH_2 signal at ~4.93 ppm, Aromatic protons only in the 7.2–7.6

ppm region the data are most consistent with: 4-Amino-3,5-Diphenyl-1,2,4-Triazole (Molecular Formula: $C_{14}H_{12}N_4$)

^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 167.63, 158.24, 157.23, 153.03, 138.94, 133.31, 132.32, 131.61, 131.36, 129.83, 128.07, 126.00, 125.00, 124.86, 115.51, 112.13. Signals at δ 39–40 ppm correspond to DMSO-d_6 solvent.

Therefore, the spectral data support the identification of 4-Amino-3,5-Diphenyl-1,2,4-Triazole.

CONCLUSION

Two new 1,2,4-triazole derivatives were effectively synthesized and described in this study: 4-Amino-3-Phenyl-5-(2,4-dinitrophenyl)-1,2,4-Triazole and 4-Amino-3-Phenyl-5-Phenyl-1,2,4-Triazole. To verify their structure and determine their antibacterial ability, the produced compounds underwent a variety of identification and biological evaluation assays.[13,14]

The successful production of the intended triazole derivatives was demonstrated by the preliminary identification tests, which included chemical tests like the Ninhydrin test for the amino group and physical characterization (appearance, color, melting point, and solubility investigations). Spectral analyses, such as IR, Mass Spectrometry, and NMR spectroscopy, provided additional structural support by revealing distinctive absorption bands and signals that corresponded to the triazole ring, amino group, aromatic protons, and substituted phenyl moieties.

Using biological techniques as the Antifungal Activity and Disc Diffusion Method against certain bacteria strains, the antibiotic activity of the produced compounds was assessed. Effective antibacterial activity was demonstrated by both drugs' discernible zones of inhibition. The nitro-substituted compound, 4-Amino-3-Phenyl-5-(2,4-dinitrophenyl)-1,2,4-Triazole, exhibited relatively higher antimicrobial and antifungal activity among the synthesized derivatives. This could be explained by the presence of electron-withdrawing nitro groups that improve interaction with microbial targets. The significance of the 1,2,4-triazole scaffold in antibacterial drug design was confirmed by the molecule 4-Amino-3-Phenyl-5-Phenyl-1,2,4-Triazole, which also showed notable activity. [15,16,17]

Overall, the findings indicate that the produced triazole derivatives have encouraging antibacterial qualities and could be useful lead compounds for the creation of novel antimicrobial drugs. To determine their medicinal potential, more research involving thorough pharmacological analysis, toxicity assessment, and structure–activity connection studies is advised. [18,19]

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