

## Research Article

### *Design and Synthesis of Novel Tetrazole Derivatives with Potential Antimicrobial Activity*

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#### **Abstract**

Tetrazole five membered heterocyclic ring contain four nitrogen and one carbon atom. Tetrazole shows diverse pharmacological activity. A series of tetrazole derivatives A(1-4) were synthesized by reaction between tetrazole aldehyde and aromatic hydrazine by Mannich base reaction mechanism. The Synthesized compounds A(1-4) were confirmed by IR, <sup>1</sup>H NMR, mass spectral, and elemental analysis. Synthesized compounds A(1-3) and were screened for antibacterial activity. The compound 2 was highly active against *S. aureus* in antibacterial screening. It was show that synthesized derivatives show microbial activity against both gram-negative and gram-positive bacterial species. New tetrazole derivatives show significant potential and provide important directions for future antibacterial drug development.

**Keywords:** *Heterocyclic compound, Tetrazole derivatives, Bacterial strain*

#### **Introduction**

The worldwide rise of antibiotic-resistant organisms is causing infectious diseases to re-emerge as a major threat, undermining the significant advances achieved in modern medicine. [1]. When bacteria are treated with antimicrobial substances, their growth, reproduction, or even death can be inhibited. They come in a variety of types, such as antibiotics [2]. The long-term and widespread use of antibiotics has contributed significantly to the emergence of antimicrobial resistance (AMR) in microorganisms [3,4]. Although microbial infections are commonly treated with antibacterial and antifungal agents, the rapid rise of antibiotic resistance has created a major barrier to effective therapy. This growing resistance has become a critical global concern, posing a serious threat to human health and directly impacting quality of life. Addressing AMR has therefore become an urgent priority, requiring the development of timely and effective solutions.

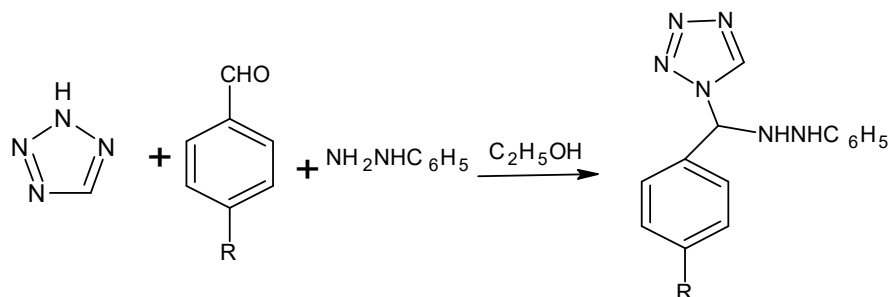
Given this alarming situation, the discovery and development of new antimicrobial agents is essential for managing microbial infections [5–7]. Experts agree that innovative therapeutic strategies are necessary to combat antibiotic-resistant pathogens and prevent their further spread, a task that will demand significant scientific effort and collaboration.

Meanwhile, fungal infections are also increasing worldwide, especially in developing countries where access to appropriate healthcare is limited, placing vulnerable populations at heightened risk. Notably, invasive candidiasis remains a major health challenge, with mortality rates reaching up to 40% [9], and it has become the third most common cause of bloodstream infections [8].

Azoles—including imidazoles, triazoles, and tetrazoles—exhibit a wide range of potent antibacterial properties and are widely used to treat both systemic and topical mycoses, particularly those associated with AIDS-related fungal infections [10]. Tetrazoles, in particular, show a strong affinity for benzodiazepine receptors [2], and various tetrazole derivatives have been identified as effective  $\alpha$ -mannosidase inhibitors due to their mannose-mimicking behavior [3,4]. Among them, **1,5-disubstituted-1H-tetrazoles** serve as valuable peptide bioisosteres [11], and numerous tetrazole derivatives possess notable medicinal significance, having demonstrated antifungal [12,13], antinociceptive [14,15], antimycobacterial [16], anti-inflammatory [17], antiproliferative [18], anticonvulsant [19], and antibacterial [12] activities. The **Mannich reaction** also plays a crucial role in the synthesis of biologically active compounds [20,21]. Mannich bases are well known for their diverse biological activities, including cytotoxic [22,23], anticonvulsant [24], and antibacterial properties [25]. In light of these findings, we set out to synthesize a new series of tetrazole derivatives and evaluate their **antibacterial activity against dental-plaque-related pathogens** as well as their **cytotoxic potential**.

## Material and method

Synthesis of 1-[phenyl(2-phenylhydrazinyl)methyl]-1H-tetrazole A(1-4): A mixture of tetrazole aldehyde and hydrazine of 0.1 mole of each compound were taken in rbf, the mixture was refluxed in ethanol for 3 hours. The obtained product was poured in crushed ice. The product was recrystallised using ethanol solvent. [26-27]



**Scheme-1**

**Antimicrobial Activity-** A newly synthesized compound were screened for anti bacterial activity by using different bacteria strain. The bacterial strain for the study of antimicrobial activity were E.coli, Pseudomonas aeruginosa and Bacillus subtilis.

**Agar Cup Method:** Ciprofloxacin was used as the reference standard for evaluating antibacterial activity. The assay was performed using a modified agar cup method. Briefly, 0.2 mL of the test culture suspension was mixed with sterile 1.5% melted top agar cooled to room temperature. The mixture was poured onto sterile base agar medium (autoclaved at 121 °C for 15 minutes) in Petri dishes and allowed to solidify. Three wells were then made in each agar plate using a sterile cup borer. Into these wells, 0.1 ml of the test solution, 0.1 mL of the reference solution, and 0.1 mL of the DMSO solvent (control) were added, respectively. The plates were kept at 4–5 °C for 20 minutes to allow diffusion, followed by incubation in an upright position at 37 °C for 24 hours. After incubation, the presence of a clear zone of inhibition around each well indicated the organism's susceptibility to the antimicrobial agent. Antibacterial activity was quantified by measuring the diameter of the inhibition zones, with larger zones corresponding to higher activity. [28, 29]

**Determination of the minimal inhibitory concentration (MIC):** Dimethylsulfoxide was used to dissolve the compound at a concentration of 64 µg/mL. The solution was produced in two dilutions (64, 32,..., 0.5 µg/mL). The appropriate wells were infected with the microbe suspensions at doses of 10<sup>6</sup>CFU/mL (colony forming unit/mL). For 24 hours, the plates were incubated at 36° C. [30]

## Result and Discussion

A series of compound 1-3 were synthesized from condensation method and reaction is given in Scheme-1 and physical property in Table-1

**Table-1: Physical Characterization of synthesized drug**

Derivative	R	M.F	M.W	Yield (%)
1	H	C <sub>14</sub> H <sub>14</sub> N <sub>6</sub>	266.30	60
2	Cl	C <sub>14</sub> H <sub>13</sub> ClN <sub>6</sub>	300.74	70
3	OH	C <sub>14</sub> H <sub>14</sub> N <sub>6</sub> O	282.30	52

The structure of synthesized is characterized by IR, 1H-NMR and Mass Spectroscopy. IR 3408, 1660, 1315, and 1512 cm respectively. <sup>1</sup>H NMR spectrum of the compound (1a) shows that signals obtained at 6.9.60, 6.32, and 2.22 corresponding to NH<sub>2</sub>, CH, and NH respectively.

**1-[phenyl(2-phenylhydrazinyl)methyl]-1H-tetrazole 1:** IR (KBr, cm): 3326 (NH<sub>2</sub>), 2826 (CH=Str) 3100 (NH), 1530 (N=N), 1364 (C-N), 967 (NH), 822 (ArH). NMR (DMSO-d<sub>6</sub>), H (ppm) 9.40 (2H, s, NH<sub>2</sub>), 8.67 (1H, s, 5CH-tetrazole), 7.40-7.36 (4H, m, phenyl), 5.42 (1H, s, -CH-), 2.22 (1H, s, NH) (Relative intensity % m/z = 265.82 (M, 10%)

**1-[(4chlorophenyl)(2-phenylhydrazinyl)methyl]-1H-tetrazole 2:** IR (KBr, cm): 3326 (NH<sub>2</sub>), 2826 (CH=Str) 3100 (NH), 1530 (N=N), 1364 (C-N), 816 (Ar-Cl) 967 (NH), 822 (ArH). NMR (DMSO-d<sub>6</sub>) H (ppm) - 9.40 (2H, s, NH<sub>2</sub>), 8.67 (1H, s, 5CH-tetrazole), 7.40-7.36 (4H, m, ph), 5.42 (1H, s, -CH-), 2.22 (1H, s, NH) (Relative intensity % m/z = 299.82 (M, 10%)

**1-[(4hydroxyphenyl)(2-phenylhydrazinyl)methyl]-1H-tetrazole 3 :** 3326 (NH<sub>2</sub>), 2826 (CH=Str) 3100 (NH), 1530 (N=N), 1364 (C-N), 726 (Ar-OH) 967 (NH), 822 (ArH). NMR (DMSO-d<sub>6</sub>), H (ppm) 9.40 (2H, s, NH<sub>2</sub>), 8.67 (1H, s, 5CH-tetrazole), 7.40-7.36 (4H, m, ph), 5.42 (1H, s, -CH-), 2.22 (1H, s, NH), 9.45 (1H, s, Ph-OH) Ms (Relative intensity % m/z = 281.82 (M, 10%)

**Antimicrobial activity:** The recrystallized product was examined for antibacterial activity in vitro. Out of the three tetrazole compounds, 1, 3 and 2 shown moderate inhibition against gram-negative bacterial species, particularly Escherichia coli, according to the findings in Table 2, whereas 1 and 3 demonstrated maximum action against the majority of gram-negative organisms. Nearly every molecule in the series demonstrated maximal inhibition against gram-positive organisms.

**Table-2 Antimicrobial activity of compound by Zone of Inhibition**

Derivative	Zone of Inhibition		
	Bacterial Strain		
	E. coli	Pseudomonas aeruginosa	Bacillus subtilis
1	12	13	15
2	18	20	23

3	14	12	17
Std	30	30	30

Antimicrobial activity of compound in 10% DMSO Standard Drug- Ciprofloxacin the antibacterial properties of three synthesized compounds (1-3) when compared to standard drug like ciprofloxacin. Compound 1 was highly effective against *Pseudomonas aeruginosa* (MIC: 8  $\mu\text{g/mL}$ ). Compound 3 showed high activity against *Escherichia coli* when combined with conventional antibiotics (MIC: 4  $\mu\text{g/mL}$ ).

**Determination of the minimal inhibitory concentration (MIC):** Dimethyl sulfoxide (DMSO) was used to dissolve the compound at a concentration of 64  $\mu\text{g/mL}$ . The solution was then prepared in two additional dilutions (32 and 0.5  $\mu\text{g/mL}$ ). The corresponding wells were inoculated with microbial suspensions at a concentration of  $10^8$  CFU/mL (colony-forming units per milliliter). The plates were incubated at 36 °C for 24 hours. [31]

**Table 3 Provides a summary of the data and minimum inhibitory concentrations.**

Derivative	MIC		
	Bacterial Strain		
	E.coli	Pseudomonas aeruginosa	Bacillus subtilis
1	60	65	46
2	40	45	30
3	68	12	17
Std	0.5	0.5	32

Antimicrobial activity of compound in 10% DMSO

Standard Drug- Ciprofloxacin

**Conclusion-** The text is a conclusion from a research paper on the creation and testing of tetrazole derivatives, which are a class of organic compounds used in medicinal chemistry.

- A synthetic method for develop novel tetrazole derivatives was outlined.
- Compound (1) demonstrated excellent activity against *S. aureus* (a type of bacteria).
- The presence of a chlorine substituent on the phenyl rings made most compounds active in antibacterial screenings.

- The research indicates the potential for these novel derivatives to have antibacterial and cytotoxic (toxic to living cells) activity, guiding future drug development.

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