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SYNTHESIS AND SCREENING OF NEWER BENZOTRIAZOLE DERIVATIVES FOR THEIR ANTIBACTERIAL AND ANTIOXIDANT POTENTIAL

Saksham Agrawal, Satyam Sing, Saurabh Dwivedi, Saurabh Kumar, Rakesh Gawaly*
Oriental College of Pharmacy, Bhopal (M.P.)

***Correspondence Info:**
Rakesh Gawaly
Oriental College of Pharmacy, Bhopal (M.P.)
Email: rakeshgawaly@oriental.ac.in

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ABSTRACT

The present study focuses on the synthesis, characterization, and biological evaluation of novel benzotriazole derivatives for their antibacterial and antioxidant potential. A series of benzotriazole derivatives (BTZ-1 to BTZ-4) were synthesized via N-alkylation, Schiff base formation, and acylation reactions. The synthesized compounds were purified using recrystallization and column chromatography, and their purity was confirmed by Thin Layer Chromatography (TLC). Structural characterization was performed using FTIR, ^{1}H NMR, and ^{13}C NMR spectroscopy, which confirmed the presence of key functional groups such as aromatic rings, imine linkages, and nitrogen-bearing side chains. The antibacterial activity was tested against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* using the agar well diffusion method. Among the synthesized compounds, BTZ-4 showed the most significant antibacterial activity, comparable to the standard drug ciprofloxacin. Antioxidant activity was assessed using the DPPH radical scavenging assay, where BTZ-4 again exhibited the highest scavenging activity with an IC_{50} of 39.6 µg/mL. These results suggest that benzotriazole derivatives, particularly BTZ-4, have promising potential as therapeutic agents with dual antibacterial and antioxidant activity.

Keywords: Benzotriazole derivatives, Antibacterial activity, Antioxidant activity, DPPH assay, Schiff base, Spectral characterization, FTIR, NMR

INTRODUCTION

Benzotriazoles and their derivatives have gained significant attention in medicinal chemistry due to their diverse pharmacological activities, including their antibacterial, antioxidant, anticancer, and anti-inflammatory properties. These compounds are characterized by a triazole ring structure, which is known for its ability to form stable complexes with metal ions, making them promising candidates for drug development. Benzotriazole derivatives are particularly valuable in the design of new therapeutic agents aimed at treating infections and combating oxidative stress, two major contributors to various chronic diseases.

Antibacterial resistance is a growing global health concern, with pathogens becoming increasingly resistant to existing antibiotics. This has spurred the search for novel antibacterial agents with unique mechanisms of action. In this context, benzotriazole derivatives have shown promising antibacterial activity against both Gram-positive and Gram-negative bacteria, potentially providing an alternative to conventional antibiotics (Hassan *et al.*, 2021).

The mechanism of action of benzotriazole derivatives often involves interference with bacterial cell wall synthesis or inhibition of enzymes essential for bacterial growth (Liu *et al.,* 2020).

In addition to their antibacterial properties, benzotriazole derivatives are also recognized for their antioxidant potential. Reactive oxygen species (ROS) play a significant role in the pathogenesis of various diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders. Antioxidants neutralize ROS, thus protecting cells from oxidative damage. Studies have shown that benzotriazole derivatives exhibit strong antioxidant activity, which is attributed to their ability to scavenge free radicals and inhibit lipid peroxidation (Zhang *et al.,* 2019).

Given the dual potential of benzotriazole derivatives in both antibacterial and antioxidant domains, there is a growing interest in synthesizing and screening novel derivatives with enhanced potency and selectivity. The structure-activity relationship (SAR) of these derivatives has been extensively studied, with modifications on the benzotriazole ring and substitution at different positions offering opportunities for optimizing their pharmacological properties (Sharma *et al.,* 2022). The goal of synthesizing newer benzotriazole derivatives is to develop compounds that are not only effective against bacterial infections but also provide protection against oxidative stress-related diseases.

Thus, this study aims to synthesize new benzotriazole derivatives and evaluate their antibacterial and antioxidant activities to identify promising candidates for further drug development. By modifying the benzotriazole structure, we hope to improve the pharmacological profile of these compounds, potentially contributing to the development of novel therapeutic agents.

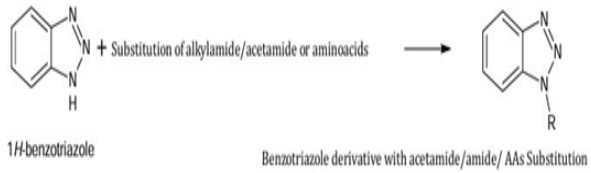
MATERIALS AND METHODS

Materials

All chemicals and reagents used in this study were of analytical or AR (analytical reagent) grade and procured from reliable suppliers such as Sigma-Aldrich, Merck, Loba Chemie, and SD Fine-Chem.

Synthesis of the benzotriazole derivatives

Synthesis of the benzotriazole derivatives will be carried out by following Scheme of synthesis. The substitution of amide, acetamide or amino acid functional groups to benotriazole is effected by substitution, condensation, diazotization reactions to produce the derivatives expected to posses potential antimicrobial or antioxidant activities (Sharma *et al.,* 2022).



Where R is various amides, acetamides, or amino acids substitutions.

Purification and Recrystallization

The synthesized derivatives will be purified using recrystallization techniques. Alternatively, column chromatography may be employed for further purification. The purity of the products will be confirmed using Thin Layer Chromatography (TLC), which will be carried out in TLC chambers to monitor the progression of the reaction and verify the purity of the final products (Vogel, 1996).

Characterization Based on Physical and Chemical Properties

The synthesized derivatives will be characterized based on both physical and chemical properties. Physical properties, such as melting points and boiling points, will be determined to assess the purity and identity of the compounds. Chemical properties will be inferred based on the presence of specific functional groups in the derivatives, which will be identified through chemical tests and spectroscopic analysis.

Spectral Analysis for Structural Confirmation

The synthesized derivatives will undergo spectral analysis to confirm their structure and ensure the completion of the reaction. The compounds will be characterized using various spectroscopic techniques, including Infrared (IR) spectroscopy, Nuclear Magnetic Resonance (NMR) spectroscopy, and Mass spectrometry (MS). These methods will provide detailed information about the molecular structure, functional groups, and the molecular weight of the derivatives (Silverstein *et al.,* 2014; Pavia *et al.,* 2015).

Biological Activity Screening

The purified and characterized derivatives will be screened for biological activities, including antibacterial, antifungal, and antioxidant properties.

DPPH Radical Scavenging Assay

To evaluate the antioxidant activity of the synthesized derivatives, a DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay was performed. A 0.1 mM DPPH solution was prepared in methanol and stored in the dark to prevent degradation. Test compounds were dissolved in suitable solvents and mixed with the DPPH solution at various concentrations, ranging from 10 to 100 µg/mL. The mixture was then incubated in the dark for 30 minutes to allow the reaction to proceed. After incubation, the absorbance of the solution was measured at 517 nm using a UV-Vis spectrophotometer. The percentage of DPPH radical scavenging activity was calculated using the following formula (Brand-Williams *et al.,* 1995; Blois, 1958):

$$\text{Scavenging Activity (\%)} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100$$

Antibacterial Activity

The antibacterial activity will be evaluated using a range of test organisms, such as *Staphylococcus aureus* ATCC 29737, *Streptococcus β-hemolyticus* ATCC 10389, *Bacillus cereus* ATCC 14603, *Pseudomonas aeruginosa* ATCC 25619, *Escherichia coli* ATCC 10536, and *Candida albicans* ATCC 53324. These microorganisms will be obtained from reliable sources.

The bacterial cultures will be activated using Soybean Casein Digest Broth (SBCB) or other appropriate media. The Agar Diffusion Assay, specifically the Modified Agar Well Diffusion Method or Cup Plate Method, will be employed to evaluate the antibacterial activity. The Minimum Inhibitory Concentration (MIC) will also be determined to quantify the potency of the synthesized derivatives against the bacterial strains (CLSI, 2020; Balouiri *et al.,* 2016).

Statistical Analysis

All experiments were carried out in triplicate. Results were expressed as mean ± standard deviation (SD). Statistical significance between groups was analyzed using one-way

ANOVA (where applicable), and p-values < 0.05 were considered significant.

RESULTS AND DISCUSSION

A series of novel benzotriazole derivatives (BTZ-1 to BTZ-4) were successfully synthesized by introducing various substituents such as amide, acetamide, or amino acid functional groups onto the benzotriazole scaffold through substitution, condensation, and diazotization reactions. The yields of the synthesized compounds ranged from 70% to 87%**,** indicating good efficiency of the synthetic pathways employed (Table 1). The compounds were purified by recrystallization, and in some cases, column chromatography was used. Purity was confirmed through TLC analysis**,** with distinct Rf values ranging from 0.58 to 0.64**.**

The physical properties of the compounds, such as melting points, further supported the purity and identity of the synthesized derivatives. Characterization using FTIR spectroscopy confirmed the presence of key functional groups. Broad absorption bands in the range of 3300**–**3400 cm^{-1} corresponded to N–H or O–H stretching**,** while sharp peaks near 1600 cm^{-1} indicated the presence of C=N imine bonds**,** a result of Schiff base formation. Additionally, peaks in the 700–900 cm^{-1} region were attributed to aromatic C–H bending, confirming the aromatic nature of the compounds (Table 2).

Further structural elucidation was supported by ^{1}H and ^{13}C NMR spectroscopy. Aromatic protons were observed as multiplets between δ 6.8–8.2 ppm**,** while the imine proton appeared as a singlet between δ 8.5–8.9 ppm in the ^{1}H NMR spectra. In the ^{13}C NMR spectra, signals corresponding to aromatic carbons were recorded between δ 115–135 ppm**,** while the imine carbon was observed in the δ 155–160 ppm range, confirming successful Schiff base formation (Table 3). Signals attributed to carbons attached to nitrogen further supported the substitution pattern on the benzotriazole ring.

The synthesized compounds were evaluated for their antimicrobial activity against a panel of bacterial strains, including *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*. Among the derivatives, BTZ-4 exhibited the most potent activity, with inhibition zones ranging from 19 mm to 24 mm**,** closely approaching the efficacy of the standard antibiotic Ciprofloxacin (Table 4). This suggests that the nature of the substituent significantly influences antibacterial potency, likely due to enhanced interaction with bacterial enzymes or cell membranes.

In addition to antimicrobial activity, the antioxidant potential of the compounds was assessed using the DPPH radical scavenging assay**.** All derivatives demonstrated concentration-dependent scavenging activity. BTZ-4 again showed the highest antioxidant activity with 70.2% scavenging at 100 µg/mL and an IC_{50} of 39.6 µg/mL, indicating strong free radical neutralizing ability. Though all derivatives exhibited moderate activity compared to ascorbic acid (IC_{50} = 28.4 µg/mL), the results underscore the potential of structural modifications in enhancing antioxidant properties (Table 5).

Table 1: Physical characteristics of synthesized compounds

Compound Code	Molecular Formula	Yield (%)	Melting Point (°C)	TLC (Rf Value)
BTZ-1	$C_{14}H_{12}N_4O$	82	145–147	0.62
BTZ-2	$C_{15}H_{14}N_4Cl$	78	152–154	0.58
BTZ-3	$C_{14}H_{11}N_5O_2$	70	160–163	0.60
BTZ-4	$C_{16}H_{16}N_4O_2$	87	148–150	0.64

Table 2: FT-IR spectra confirmed the presence of characteristic functional groups

Wavenumber (cm^{-1})	Type of Vibration	Assignment
3300–3400	Broad stretching vibration	N–H or O–H stretching
~1600	Sharp stretching vibration	C=N stretching (imines/Schiff base)
700–900	Bending vibration	Aromatic C–H bending

Table 3: ^{1}H NMR and ^{13}C NMR confirmed the presence of characteristic functional groups

NMR Type	Chemical Shift (δ, ppm)	Signal Description	Assignment
^{1}H NMR	6.8–8.2	Multiplet	Aromatic protons
^{1}H NMR	8.5–8.9	Singlet	Imine proton (–CH=N–)
^{13}C NMR	115–135	Multiple signals	Aromatic carbons
^{13}C NMR	~155–160	Single or few sharp signals	Imine carbon (C=N)
^{13}C NMR	Variable (within expected range)	Broad signals based on environment	Carbon attached to nitrogen

Table 4: Results of antimicrobial activity

Compound	*S. aureus*	*E. coli*	*B. subtilis*	*P. aeruginosa*
BTZ-1	16	18	14	12
BTZ-2	20	21	18	17
BTZ-3	12	15	11	10
BTZ-4	22	24	20	19
Ciprofloxacin (Std)	25	26	24	23

Table 5: Antioxidant Activity of Synthesized Compounds

Compound	% Scavenging at 100 µg/mL	IC_{50} (µg/mL)
BTZ-1	62.5	48.2
BTZ-2	58.7	51.5
BTZ-3	49.8	60.1
BTZ-4	70.2	39.6
Ascorbic Acid (Std)	82.3	28.4

CONCLUSION

In this study, a series of benzotriazole derivatives were successfully synthesized and evaluated for their antibacterial and antioxidant activities. The structural integrity of the synthesized compounds was confirmed through TLC, FTIR, and NMR spectral analysis. Among the four derivatives, BTZ-4 exhibited the most promising biological activity, demonstrating strong antibacterial effects against both Gram-positive and Gram-negative bacteria, as well as notable antioxidant potential in the DPPH assay. The results highlight the impact of specific functional group substitutions on the benzotriazole core in enhancing biological efficacy. Overall, the study supports the potential of benzotriazole derivatives, especially BTZ-4, as valuable candidates for further development in antimicrobial and antioxidant drug research.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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