

**SYNTHESIS AND BIOLOGICAL SCREENING OF NOVEL 1,3,4-THIADIAZOLE  
DERIVATIVES WITH ANTIMICROBIAL AND ANTIOXIDANT PROPERTIES**

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**Abstract:** The antibacterial and antioxidant activities of six novel 1,3,4-thiadiazole derivatives were evaluated. The compounds exhibited significant antibacterial effects against *Listeria monocytogenes*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* and moderate antifungal activity against *Candida albicans*. Antiradical activity was determined using a DPPH assay, with compound 3 demonstrating the highest effectiveness (59.9%). The lipid peroxidation inhibition assay revealed compound 3 as the most potent antioxidant, reducing malondialdehyde (MDA) levels by 49.9%. These results highlight the potential of 1,3,4-thiadiazole derivatives for therapeutic applications.

**Keywords:** 1,3,4-thiadiazole derivatives, Antibacterial activity, Antioxidant activity

**INTRODUCTION**

The exploration of heterocyclic compounds as potential therapeutic agents has gained significant momentum in medicinal chemistry. Among these, 1,3,4-thiadiazole derivatives have emerged as promising candidates due to their unique structural properties and broad spectrum of biological activities, including antibacterial, antifungal, antiviral, anticancer, and antioxidant effects. The presence of a thiadiazole moiety in their structure facilitates interactions with biomolecular targets, contributing to their pharmacological relevance.

The increasing prevalence of antibiotic-resistant bacteria and the global threat posed by free radical-induced oxidative stress underscore the urgent need for novel compounds with dual antibacterial and antioxidant activities. Antibiotic resistance has rendered many conventional drugs ineffective, necessitating the development of innovative molecules to combat resistant strains. Con-currently, oxidative stress is implicated in the pathogenesis of numerous chronic diseases, including cancer, diabetes, and cardiovascular disorders. Compounds capable of mitigating these effects are of significant interest for both therapeutic and preventive applications.

This study focuses on the synthesis and biological evaluation of six novel 1,3,4-thia-diazole derivatives, aiming to investigate their antibacterial properties against both Gram-positive and Gram-negative bacterial strains, as well as their antifungal activity against *Candida albicans*. Additionally, the antioxidant potential of these compounds was assessed using the DPPH radical scavenging assay and lipid peroxidation inhibiting mitochondrial systems. Previous research has demonstrated effective termite management strategies using bait formulations

**The results reveal a structure-activity – relationship** that highlights the potential of these derivatives as candidates for further development in the treatment of bacterial infections and oxidative stress-related conditions. The study provides valuable in-sights into their mechanisms of action and sets the stage for future research into their therapeutic applications.

The problem of free radicals has revolutionized the understanding of many processes occurring in the human body in the last decade. Today, free radicals are considered as a source of numerous disorders leading to the development of a number of diseases.

**MATERIALS AND METHODS**



Chemicals and Reagents - All reagents and solvents used in the synthesis and biological evaluation of the compounds were of analytical grade. The synthesized 1,3,4-thiadiazole derivatives (1–6)

1. 5-ethyl-1,3,4-thiadiazol-2-amine; 2. N-(5-ethyl-1,3,4-thiadiazol-2-yl)benzamide; 3. N-(5-ethyl[1,3,4]thiadiazol-2-yl)-toluenesulfonamide; 4. N-(5-ethyl-1,3,4-thiadiazol-2-yl)monochloroacetamide; 5. N-(5-ethyl-1,3,4-thiadiazol-2-yl)acetamide 6. N-(5-ethyl-1,3,4-thiadiazol-2-yl)propionamide were prepared using standard synthetic procedures, and their purity was confirmed by chromatographic and spectroscopic methods. DPPH (2,2-diphenyl-1-picrylhydrazyl) was purchased from Sigma-Aldrich, and other reagents, including Mueller-Hinton agar (MHA), Thio barbituric acid (TBA), and trichloroacetic acid (TCA), were obtained from reliable suppliers.

Synthesis of 5-ethyl-1,3,4-thiadiazole Derivatives.

The six compounds were synthesized through [insert synthetic procedure, e.g., cyclization or condensation reactions]. Detailed protocols, reaction conditions, and yields are provided in the supplementary section. The structural confirmation was achieved using mass spectrometry.

**Antibacterial Activity** - The antibacterial activity of the compounds was evaluated in vitro using the agar diffusion method (glass cylinder technique) as per the guidelines outlined in "MUK 4.2.1890–04."

#### Microorganisms Tested:

**Gram-positive bacteria:** *Listeria monocytogenes* 2, *Staphylococcus aureus* ATCC, *Staphylococcus aureus* D-8, *Staphylococcus aureus* D-5, *Enterococcus faecalis*, *Bacillus subtilis*;

**Gram-negative bacteria:** *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* UM 477, *Proteus mirabilis*;

**Fungi:** *Candida albicans*;

**Preparation of Microbial Suspensions:** The inoculum was prepared by suspending colonies from 18–24-hour cultures in sterile isotonic solution and adjusting turbidity to 0.5 McFarland standard (approximately  $1-2 \times 10^8$  CFU/mL);

**Agar Plate Preparation:** Mueller-Hinton agar was poured into Petri dishes and inoculated with microbial suspensions;

**Test Procedure:** Glass cylinders (10 mm outer diameter, 8.5 mm inner diameter) were placed on the agar surface, and 0.1 mL of test solutions (concentrations: 0.5%, 1.0%, 2.0%, and 5.0%) were added. Ethyl alcohol (96%) served as the solvent control, and antibiotic discs were included as a reference.

**Incubation:** Plates were incubated at  $37 \pm 1$  °C for 18–24 hours. The diameter of growth inhibition zones was measured using a micro ruler to the nearest millimeter.

#### Antiradical Activity

The antiradical activity of the substances was measured using a modified method by Zhang et al., based on their interaction with the stable free radical DPPH. The DPPH molecule is a stable radical characterized by stability in various media and over a wide temperature range. This is due to the maximum delocalization of the free electron throughout the molecule, spatial screening of atoms with the highest spin density, and the absence of hydrogen atoms in positions where isomerization or disproportionation can occur. It is delocalization that is the cause of the intense violet color of this radical in aqueous-alcoholic media ( $\lambda_{\max} = 520$  nm,  $\epsilon_{520} = 6.5 \times 10^3$



cm<sup>2</sup>/mol). When interacting with an antioxidant capable of donating a proton, the radical is reduced, which leads to decolorization of the DPPH solution.

### **Preparation of DPPH Solution:**

A 0.2 mM DPPH solution was prepared in 96% ethanol.

**Reaction Setup:** The reaction mixture contained 1 mL of DPPH solution and test compounds at a final concentration of 0.5 mg/mL.

**Incubation:** Samples were incubated in the dark at room temperature for 60 minutes, followed by centrifugation at 3000 rpm for 10 minutes.

**Spectrophotometric Analysis:** Absorbance was measured at 517 nm. The anti-radical activity (ARA) was calculated.

**Lipid Peroxidation Inhibition Assay** Induction of non-enzymatic Fe<sup>2+</sup>/ascorbate-dependent lipid peroxidation was performed by adding a mitochondrial suspension at a rate of 0.5 mg protein per 1 ml of MI, 10<sup>-5</sup> M FeSO<sub>4</sub> and 2 × 10<sup>-4</sup> M ascorbate to an incubation medium (IM) containing 125 mM KCl, 10 mM Tris-HCl (pH 7.4) (Zhang L., Liu C., Li D., Zhao Y., Zhang X., Zeng X., Yang Z., Li S., 2013). The substances were dissolved in 50% ethyl alcohol and stored in a dark place. Samples were added after adding the mitochondrial suspension to the reaction mixture. Incubation was performed at 37 °C in a water bath with constant stirring for 30 minutes. The reaction was stopped by adding 200 μl of 70% trichloroacetic acid (TCA). The precipitated protein was removed by centrifugation at 3000 rpm for 15 minutes.

For analysis, 2 ml of supernatant were collected, 1 ml of warm thiobarbituric acid (TBA) solution was added and the tubes were boiled for 15 minutes. After cooling, the volume was brought to 3 ml and the color intensity was measured on a spectrophotometer (Cary-60) at a wavelength of 535 nm.

The amount of formed MDA was determined using the molar extinction coefficient equal to 1.56 × 10<sup>5</sup> M<sup>-1</sup> cm<sup>-1</sup>. The concentration of MDA was expressed as nmol MDA/mg protein.

The ability of the compounds to inhibit lipid peroxidation (LPO) was evaluated in a mitochondrial suspension model.

**Isolation of Mitochondria:** Mitochondria were isolated from the liver of rats (150–200 g) using differential centrifugation. The liver was homogenized in isolation medium (125 mM KCl, 10 mM Tris-HCl, pH 7.4), and centrifugation steps were performed at 600 g (7 minutes) and 6000 g (15 minutes).

**Induction of LPO:** Lipid peroxidation was induced using Fe<sup>2+</sup>/ascorbate-dependent systems. The reaction mixture contained mitochondrial suspension (0.5 mg protein/mL), 10<sup>-5</sup> M FeSO<sub>4</sub>, and 2 × 10<sup>-4</sup> M ascorbate.

**Measurement of Malondialdehyde (MDA):** After 30 minutes of incubation at 37 °C, the reaction was stopped with 70% TCA, and the supernatant was reacted with TBA at 535 nm. MDA levels were calculated using the molar extinction coefficient of 1.56 ×

× 10<sup>5</sup> M<sup>-1</sup> cm<sup>-1</sup>.



**Table 1.** Antimicrobial activity of the compounds

<b>Microorganisms</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>
<i>Staphylococcus aureus</i> ATCC	+	-	-	-	-	-
<i>Staphylacoccus aureus</i> D-8	-	-	-	-	-	-
<i>Staphylacoccus aureus</i> D-5	-	-	-	-	-	-
<i>Enterococcus faecalis</i>	-	-	-	-	-	-
<i>Bacillus subtilis</i>	-	-	-	-	-	-
Gram-negative bacteria						
<i>Klebsiella pneumoniae</i>	-	-	-	-	-	-
<i>Eshericha coli</i> 477						
<i>Pseudomonas aerugino- sa</i> UM	+	+	+	+	+	+
<i>Proteus mirabilis</i>	+	+	+	-	-	-
Fungi's						
<i>Candida albicans</i>	+	-	+	-	-	-

**RESULTS AND DISCUSSIONS:**

As can be seen from Table 1, the synthesized thiadiazole derivatives have a bactericidal effect on both bacteria (gram-positive and gram-negative) and fungi. For example, all synthesized thiadiazole derivatives have a negative effect on the growth of the bacterium *Pseudomonas aeruginosa* UM. Sub-stances 1, 2, and 3 effectively suppressed the growth of the food pathogen *Listeria monocytogenes* 2 and the bacterial strain *Proteus mirabilis*. However, none of the studied substances affected the growth of the microorganism strains *Klebsiella pneumoniae*, *Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus* D-5, *Staphylococcus aureus* D-8, and their isolates. Antiradical activity results obtained indicate the presence of antiradical properties in all of the studied compounds, which is confirmed by a decrease in the absorption level of the reaction solutions in the following order: DPPH: 1: 2: 3: 4: 5: 6: 1.1. In percentage terms, this looks like: 100: 64.7: 70.0: 59.9: 67.9: 66.9: 92.4: 68.5: 70.8, respectively.

Thus, it was shown that all of the studied substances have antiradical activity (ARA) with varying degrees of effectiveness. The highest ARA was found in substance 3, whose antiradical effect was 40.5% of the control level. Compounds 1, 5, 4, 1.1 and 2 also showed antiradical activity, and their efficiency was distributed in the following descending order: 35.29%; 33.05%; 32.07%; 31.5% and 29.97%, respectively.



Table 2.

No	APA (DPPH (diphenylpicrylhydrazyl), %	LPO (malondialdehyde -MDA) nmol/mg protein
Control	100%	77.09
1.	64.7	59.79
2.	70.0	59.52
3.	59.9	38.48
4.	67.9	36.81
5.	66.9	47.23
6.	92.4	16.59

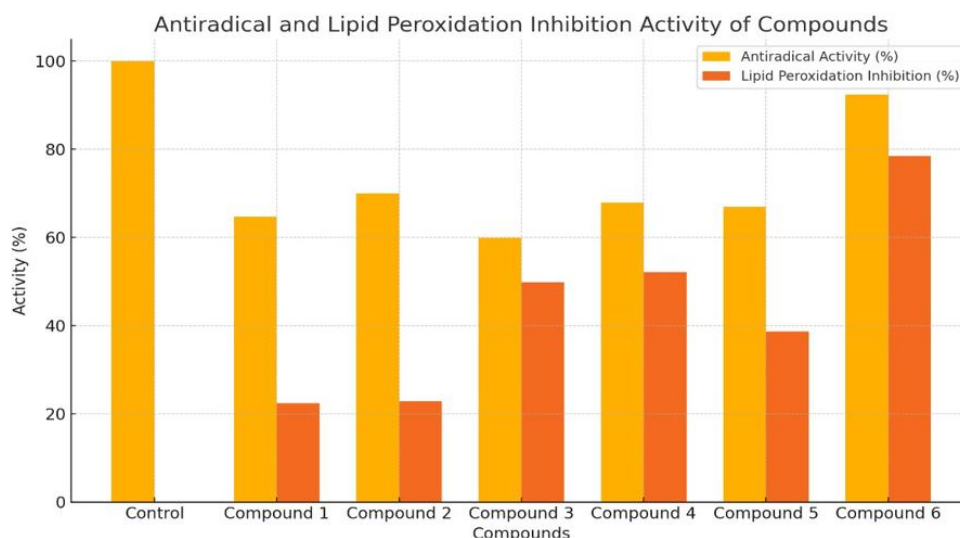
MDA accumulation under Fe/ascorbate-induced LPO in the control sample was 77.09 nmol/mg mitochondrial protein and was taken as 100% of the conventional units of the maximum level of MDA production under our experimental conditions.

Analysis of the obtained data showed that substances 3 and 4 possessed the highest antioxidant activity. In percentage terms, the decrease in the level of MDA accumulation in the presence of these substances was 49.9% and 47.7%, respectively. Substances 5 and

1.1 had a less pronounced antioxidant activity, the antioxidant effect of which was 61.2% and 55.3%, respectively. Substances 1 and 2 also showed an antioxidant effect, but among the studied compounds, their activity was the least pronounced and was about 77% relative to the control level.

Thus, all the studied substances demonstrated antioxidant activity. The obtained data correlate with the results previously obtained in the study of their antiradical effects. It should be noted that the most promising compound for further detailed study of its effect on oxidative processes in living systems is substance 3.

Figure 1. Antiradical and Lipid Peroxidation Inhibition Activity of Compounds



## CONCLUSION

In this study, six novel 1,3,4-thiadi-azole derivatives were synthesized and evaluated for their antibacterial and antioxidant activities. The results demonstrate that these compounds exhibit promising bio-logical activities, with potential applications in combating bacterial infections and oxidative stress-related disorders.

### Antibacterial Activity:

Compounds 1, 2, 3, and 4 effectively inhibited *Listeria monocytogenes* 2, while all six compounds showed notable activity against *Pseudomonas aeruginosa*.

The compounds displayed selective antibacterial properties, particularly against Gram-negative strains like *Proteus mirabilis*, indicating their potential as targeted antimicrobial agents.

### Antioxidant Activity:

The antiradical activity, assessed through the DPPH assay, revealed that all compounds exhibited free radical scavenging potential, with compound 3 showing the highest efficacy.

The lipid peroxidation inhibition assay further confirmed the antioxidant capabilities, with compounds 3 and 4 significantly reducing malondialdehyde (MDA) levels.

These findings underscore the importance of 1,3,4-thiadiazole derivatives as a versatile scaffold for developing multifunctional therapeutic agents. Among the tested compounds, **compound 3** emerged as the most promising candidate, demonstrating both potent antibacterial and antioxidant activities.

### Future research should focus on:

- Elucidating the mechanisms of action underlying the observed biological activities;
- Exploring structural modifications to enhance efficacy and selectivity;
- Investigating in vivo effects and potential applications in clinical settings.

The promising results reported here provide a solid foundation for further studies aimed at the development of **1,3,4-thiadi-azole derivatives** as effective agents for addressing global challenges in infectious diseases and oxidative stress management.

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