

Antimicrobial activity, minimum inhibitory concentration and cytotoxicity of thiadiazol compound

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Abstract

Aim: This study aims to investigate the biological activity and cytotoxicity of the prepared thiadiazole derivative compound.

Methods: A thiadiazol chemical, 4- [5-amino 1,3,4-thiadiazole-2-yl] phenol, was prepared by reacting 2.762 grams (0.01 moles) of 4-hydroxybenzoic acid with 1.822 grams (0.01 moles) of thiosemicarbazide. The prepared thiadiazole antimicrobial activity was tested at concentrations of 30, 50, 80, and 100 mg/mL against *Escherichia coli*, *Pseudomonas*, *Bacillus cereus*, and *Staphylococcus epidermidis*.

Results: The thiadiazol chemical compound was prepared and tested against several bacterial species. The minimum inhibitory concentration (MIC) for *Escherichia coli*, *Bacillus cereus*, and *Staphylococcus epidermidis* was 0.8 mg/mL. *Pseudomonas* was unaffected by this substance. Investigations were made into the cytotoxicity activities. When the concentration was less than 0.01 mg/mL, it was discovered that the produced thiadiazol also have no impact on the red blood cell.

Conclusion: The results revealed that the thiadiazole chemical compound had strong antibacterial activity against some of the pathogenic bacteria. This means that the chemical compound can be used as a narrow spectrum antibiotic.

Keywords: thiadiazole, antimicrobial activity, MIC

INTRODUCTION

The azole chemicals category includes thiadiazoles. These are heterocyclic compounds having sulfur and two nitrogen atoms that have five members. Two double bonds are present. Thiadiazole is a chemical compound that has an aromatic ring. Fischer first described thiadiazole in 1882, but Freud and Kuhn demonstrated the nature of the ring system in 1890. Thiadiazole (one sulfur and 2 nitrogen heteroatom in a cyclic five-membered ring) and related structurally chemical compounds are known as 1, 3, 4-thiadiazole (Sahu *et al.*, 2021) . A pharmacophore is a thiadiazole ring. It is also a bioisostere of the thiazole ring found in 3rd and 4th-generation

cephalosporin, which allows it to be used in the development of antimicrobial medicines(Georgeta *et al.*, 2018). Organic and pharmaceutical chemistry have been focusing on the design, preparation, and estimation of the bioactivity of these compounds with therapeutic possibilities. Furthermore, in the field of medicinal chemistry, heterocyclic molecules have attracted a lot of attention(Barbosa and de Aguiar, 2019).

Thiadiazole compounds are a promising class of molecules with diverse biological activities, making them valuable for drug discovery and development. Researchers continue to explore their potential in various therapeutic areas. Thiadiazole derivatives have demonstrated antimicrobial activity against different microorganisms. Studies have showed wide range of antibacterial and antifungal activities for many thiadiazole compounds(Ghudhaib *et al.*, 2014). The ¹⁰ strong aromaticity of the thiadiazole ring system contributes to their in vivo stability, enhancing their antimicrobial properties (Sahu *et al.*, 2021). Thiadiazoles and their derivatives have exhibited various pharmacological activities, including anti-inflammatory, anti-tubercular, and antimicrobial properties (Mishra and Jyoti, 2011). These versatile compounds offer a wide range of potential applications in medicine. Researchers have designed and synthesized Schiff bases derived from 1, 3, 4-thiadiazole-2-amine to investigate their biological properties, including antiproliferative and antimicrobial activities (Gür *et al.*, 2020) . Bacterial and fungal infection is wide spreadly during past decads even in hospitalized pateints (Vlad et. al., 2023; Lazarescu et. al., 2023). This study aims to prepare a thiadiazole derivative and investigated its biological activity, the minimum inhibitory concentration, and study the its effect on the RBCs lysis.

MATERIAL AND METHODS

Materials and Reagents

4-hydroxybenzoic acid, thiosemicarbazide, and concentrated sulfuric acid were purchased from Hyper Chim. and used directly without further purification. This study's whole supply of solvents was obtained from the Fluka firm.

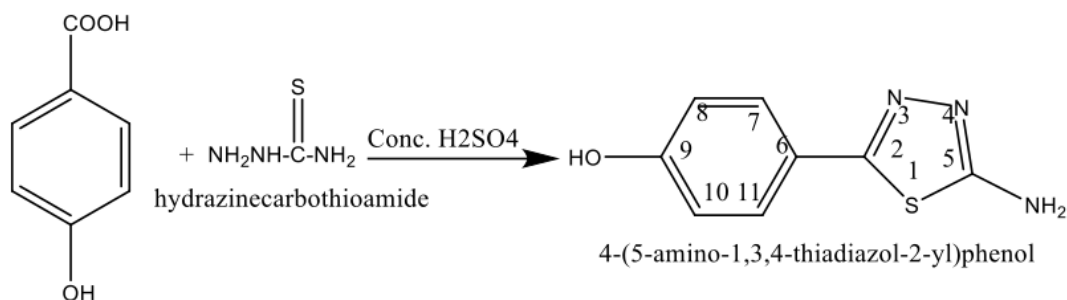
Physical measurements

The uncorrected melting point was established using an electrical instrument. The Euro vector EA-3000Ae Elemental analyzer is used to acquire CHNS elemental analysis results. The

Shimadzu FT-IR model 8400s sensor retrieved the IR spectrum as KBr pellets in the region of 400–4000 cm^{-1} . Using DMSO- d_6 as the solvent and TMS as the additional source, the ^1H NMR and ^{13}C -NMR spectra were analyzed on a Bruker 400 MHz.

Preparation of 4-[5-amino 1,3,4- thiadiazole-2-yl] phenol

The synthesized thiadiazole has already been prepared (Pawar *et al.*, 2013) method shown below in Figure (1) by mixing (2.762 grams, 0.01 mole) of 4-hydroxybenzoic acid and (1.822 grams, 0.01 mole) of hydrazinecarbathioamide in 250 mL round bottom flask with a magnetic bar, then 15ml of conc. The H_2SO_4 was added and continuously stirred in an ice bath for three hours before being cooled to room temperature and then poured onto broken ice. The mixture was neutralized by adding ammonium solution while mixing continuously, filtering the precipitate, and washing it with a concentrated sodium bicarbonate solution. It was then thrice rinsed with distilled water before being dried at 60°C . Yellow crystals were formed by recrystallization from absolute ethyl alcohol. Wt. 4.58 gm Yield (79%), with R_f value = 0.7 using (7:3 Ethyl acetate/ethyl alcohol). m.p. ($213\text{--}216^\circ\text{C}$), Elemental analysis for $\text{C}_8\text{H}_7\text{N}_3\text{OS}$; Found (calculated) = C: 49.54 (49.73), H: 3.60 (3.65), N: 21.93 (21.75), S: 16.38 (16.59). The FT-IR spectrum for prepared thiadiazole, KBr disk: ν_{OH} 3215cm^{-1} , $\nu_{\text{NH}_{\text{asym}}}$ 3174cm^{-1} , $\nu_{\text{NH}_{\text{sym}}}$ 3047cm^{-1} , $\nu_{\text{C-H}_{\text{arom}}}$ 3028cm^{-1} , $\nu_{\text{C=N}}$ 1668cm^{-1} , $\nu_{\text{NH bending}}$ 1598cm^{-1} , $\nu_{\text{C=C}_{\text{asym}}}$ 1516cm^{-1} , $\nu_{\text{C=C}_{\text{sym}}}$ 1454cm^{-1} , $\nu_{\text{C-N}}$ 1315cm^{-1} , $\nu_{\text{N-N}}$ 1165cm^{-1} , $\nu_{\text{C-S}}$ 702cm^{-1} . Using DMSO- d_6 as a solvent, the produced thiadiazole's ^1H -NMR spectra was captured (400Mz). The produced thiadiazole's ^1H -NMR spectrum generally reveals that the band at 6.94 ppm (d) is 2H (C10, C8), with a $J=8.75\text{ Hz}$, and the band at 7.76 ppm (d) is 2H (C11, C7), with a $J=9.19\text{ Hz}$. A band at 9.79ppm (s) may belong to 2H (NH_2 , and a broad band at 10.65ppm (s) attributed to 1H (OH). ^{13}C -NMR spectrum for the prepared thiadiazole was recorded by using DMSO $-d_6$: 116.31ppm (C10, C8), 128.87ppm (C11, C7), 132.58ppm (C6), 158.50 ppm (C9), 163.82ppm (C2) and 191.44ppm (C5).



4-hydroxybenzoic acid

Figure 1. Synthesis of 4-[5-amino-1,3,4-thiadiazol-2-yl] phenol 4-(5-amino 1,3,4-thiadiazol-2-yl) phenol bioactivity.

The antimicrobial activity of the prepared thiadiazole was done by using different concentrations (100, 80, 50, 30) mg/ml from 4-[5-amino 1,3,4- thiadiazole-2-yl] phenol (Thi-Hoa *et al.*, 2000). The antimicrobial susceptibility was evaluated using the Agar well diffusion method (Mounyr Balouiri *et al.*, 2016; Saeed *et al.*, 2019a ; Saeed *et al.*, 2019b ; Saeed *et al.*, 2020)

Minimum inhibitory concentration (MIC) of 4-[5-amino 1,3,4- thiadiazole-2-yl] phenol

The four bacterial strains were tested for susceptibility to the produced compound using varied doses of the compound (20, 10, 5, 4, 3, 2, 1, 0.8, 0.7) mg/ml. The MIC for produced compounds was determined using the good diffusion method. The produced compounds were dissolved in various concentrations of DMSO. McFarland standard 0.5 was used to modify the bacterial suspensions (Saeed *et al.*, 2019a ; Saeed *et al.*, 2019b ; Saeed *et al.*, 2020; Singh *et al.*, 2010).

Cytotoxicity test on blood cells

The method for assessing the cell toxicity of a produced chemical. One milliliter of blood was suspended in 20 milliliters of normal saline to make a physiological saline solution. In DMSO, various levels of the prepared chemical have been employed. A 2 mL of the erythrocyte suspension produced in the first stage was added to the sterile tubes, along with 0.1 mL of each concentration. The two controls were 2 mL of tab water and 0.1 mL of erythrocyte for the positive control and 2 mL of normal saline and 0.1 mL of erythrocyte for the negative control, respectively. Turbidity was assessed at 37 °C after 10, 30, and 60 minutes. The quantities that resulted after RBC lysis in a clear solution are an indicator of the test compound's toxicity to erythrocytes (Nair *et al.*, 1989 ; Saeed *et al.*, 2019a; Saeed *et al.*, 2019b; Saeed *et al.*, 2020).

RESULTS AND DISCUSSIONS

Biological activity of the prepared thiadiazole compound

The antibacterial activity of certain gram positive and some gram negative microorganisms was estimated using various concentrations of the produced thiadiazole as shown in Table (1) and Figure (2).

Table 1. Antibacterial activity against the chemical compound.

Kinds of bacteria	Inhibition zone (mm)			
	100 mg/mL	80 mg/mL	50 mg/mL	30 mg/mL
<i>Bacillus cereus</i>	15	14	14	14
<i>Staphylococcus epidermidis</i>	12	11	10	10
<i>Escherichia coli</i>	15	14	13	13
<i>Pseudomonas</i>	0	0	0	0

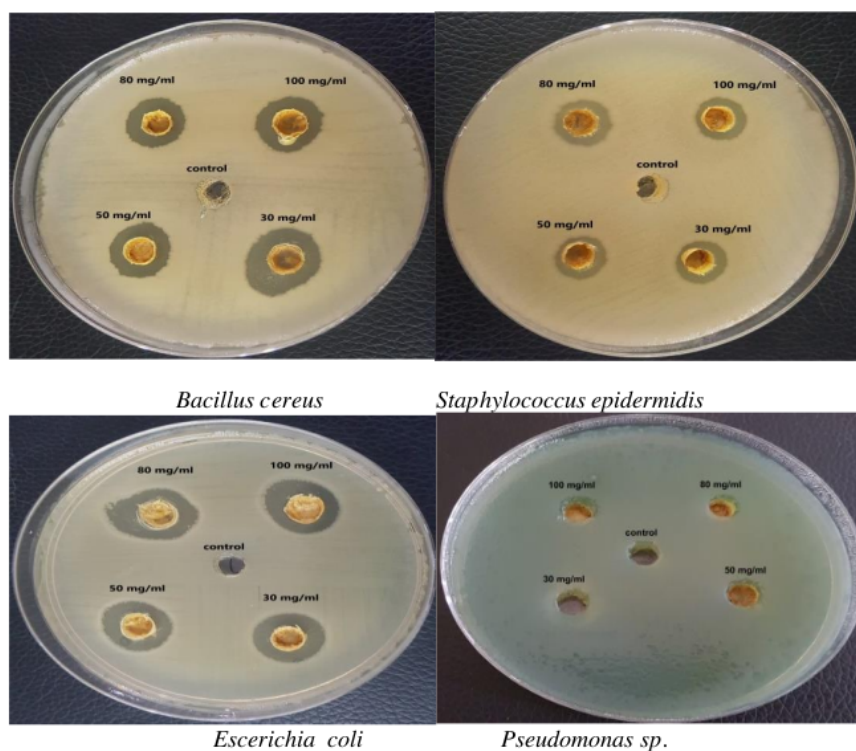


Figure 2. Antibacterial activity against tested bacteria

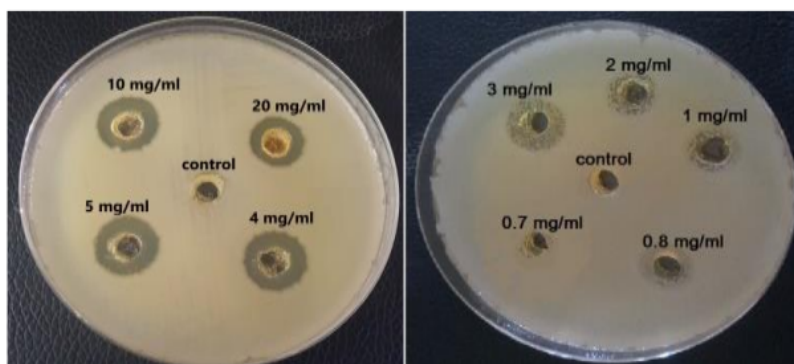
MIC of the prepared thiadiazole compound

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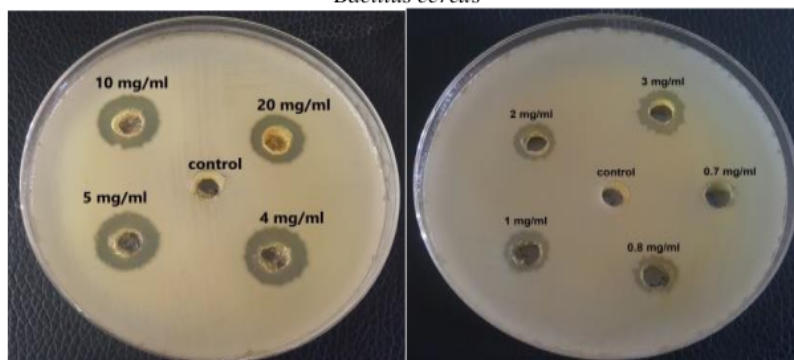
The minimum inhibitory concentrations of this chemical against different microorganisms were explained in the Table 2 and Figure 3.

Table 2. Minimal inhibitory concentration of the prepared thiadiazole toward bacteria.

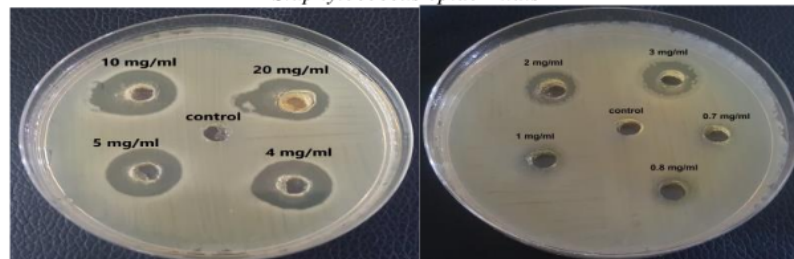
Conc. mg/mL	Diameter of the inhibition zone (mm)			
	<i>B. cereus</i>	<i>Staphylococcus epidermidis</i>	<i>E. coli</i>	<i>Pseudomonas sp.</i>
20	13	10	9	0
10	13	10	9	0
5	12	10	8	0
4	12	9	8	0
3	11	9	8	
2	9	8	7	
1	7	7	5	
0.8	5	5	5	
0.7	0	0	0	



Bacillus cereus



Staphylococcus epidermidis



Escherichia coli

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Figure 3. Minimum inhibitory concentration of the chemical compound.

Table 3. Effect of the chemical compound on blood RBC.

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0.1 mg	0.08 mg	0.05 mg	0.01 mg	0.005 mg	0.002 mg	0.0008 mg
High lysis	High lysis	Moderate lysis	Moderate lysis	Non lysis	Non lysis	Non lysis

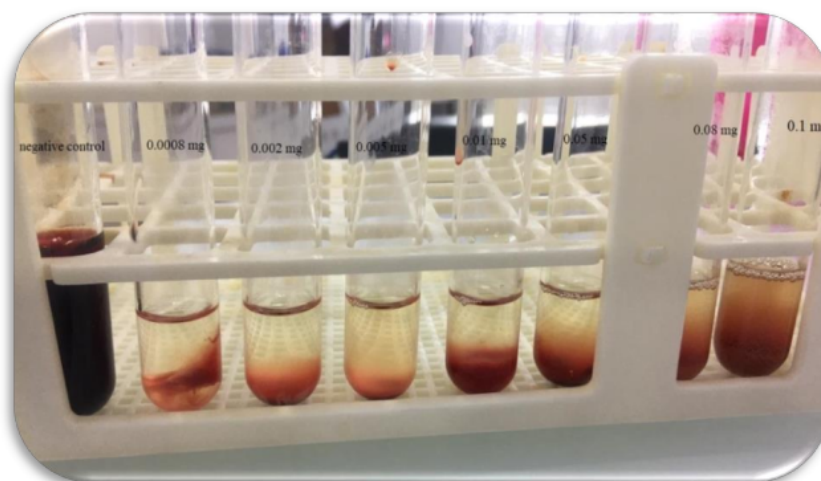


Figure 4. Effect of the compound on blood RBC.

Antimicrobial activity and MIC against *Staphylococcus epidermidis*, *Bacillus cereus* (MK468901.1), *Escherichia coli*, and *Pseudomonas*. were studied. The compound has antibacterial activity against *Staph. epidermidis* and *Bacillus cereus*, as well as *E. coli*. However, it has no effect against *Pseudomonas*. The antibacterial activity of the examined compound was found to be satisfactory. Within the range of 0.8mg/ml, the MIC value was computed against the three species of bacteria.

The findings revealed that the chemical had strong antibacterial activity against the majority of the pathogens tested, with MIC values of 0.8 mg/ml. This means that the chemical compound can be used as a narrow spectrum antibiotic (Ismail *et al.*, 2015). Antimicrobial resistance is

frequently used to describe antibiotic resistance, which occurs when microorganisms such as bacteria, viruses, fungi, and parasites are resistant to a treatment that was designed to cure the infection (Shirinazadeh *et al.*, 2018; Lazarescu *et. al.*, 2020).

The compound has not affect red blood cells at the concentrations of 0.002, 0.005 and 0.0008 mg/ml while decomposition was observed at high concentrations as shown in figure (4) and table (3). This means the compound is toxic in high concentrations and should be given in very low concentrations to be used safely as recommended by the World Health organization (WHO, 1996).

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