CASE REPORTS

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 thiadiazole 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 thiadiazole 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 had 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: MDR, Klebsiella pneumoniae, urinary tract infections, HtrA gene, MrkA gene

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 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 widespread during past decades even in hospitalized patients (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 its effect on the RBCs lysis.

MATERIAL AND METHODS

Materials and Reagents

4-hydroxybenzoic acid, thiosemicarbazide, and concentrated sulfuric acid were purchased from Hypex 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-3000 Ae 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-d6 as the solvent and TMS as the additional source, the 1H NMR and 13C-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 hydrazinecarbothioamide in 250 mL round bottom flask with a magnetic bar, then 15ml of conc. H₂SO₄ 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 Rf value = 0.7 using (7:3 Ethyl acetate/ethyl alcohol). m.p. (213-216°C), Elemental analysis for C₈H₇N₃O₃S; 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, ν NH asym. 3174 cm⁻¹, ν NH sym. 3047 cm⁻¹, ν C-H arom. 3028 cm⁻¹, ν C=N 1668 cm⁻¹, ν NH bending 1598 cm⁻¹, ν C=Sym. 1516 cm⁻¹, ν C=Sym. 1454 cm⁻¹, ν C-N 1315 cm⁻¹, ν N-N 1165 cm⁻¹, ν C-S 702 cm⁻¹. Using DMSO-d6 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 Hz, and the band at 7.76 ppm (d) is 2H (C11, C7), with a J=9.19 Hz. A band at 9.79ppm (s) may belong to 2H (NH2), and a broad band at 10.65ppm (s) attributed to 1H (OH). 13C-NMR spectrum for the prepared thiadiazole was recorded by using DMSO-d6: 116.31 ppm (C10, C8), 128.87 ppm (C11, C7), 132.58 ppm (C6), 158.50 ppm (C9), 163.82 ppm (C2) and 191.44 ppm (C5).

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 thia-
diazole was done by using different concentrations
(100, 80, 50, 30) mg/ml from 4-[5-amino 1,3,4-thia-
diazole-2-yl] phenol (Thi-Hoa et al., 2000) The anti-
microbial 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 suscep-
tibility 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 de-
termined using the good diffusion method. The pro-
duced compounds were dissolved in various con-
centrations 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 sus-
pended in 20 milliliters of normal saline to make a

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<th>Kinds of bacteria</th>
<th>Inhibition zone (mm)</th>
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<td>100 mg/mL</td>
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<td><em>Bacillus cereus</em></td>
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<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>12</td>
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<tr>
<td><em>Escherichia coli</em></td>
<td>15</td>
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<tr>
<td><em>Pseudomonas</em></td>
<td>0</td>
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**FIGURE 2. Antibacterial activity against tested bacteria**

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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 tap 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, respec-

FIGURE 3. Minimum inhibitory concentration of the chemical compound
tively. 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 thidiazole compound

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

MIC of the prepared thidiazole compound

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

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 0.8 mg/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 (Shirinzadeh 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).

| TABLE 2. Minimal inhibitory concentration of the prepared thidiazole toward bacteria |
|---|---|---|---|---|
| Conc. mg/mL | B. cereus | Staphylococcus epidermidis | E. coli | Pseudomonas sp. |
| 20 | 13 | 10 | 9 | 0 |
| 10 | 13 | 10 | 9 | 0 |
| 5 | 12 | 10 | 8 | 0 |
| 4 | 12 | 9 | 8 | 0 |
| 3 | 11 | 9 | 8 | 0 |
| 2 | 9 | 8 | 7 | 0 |
| 1 | 7 | 7 | 5 | 0 |
| 0.8 | 5 | 5 | 5 | 0 |
| 0.7 | 0 | 0 | 0 | 0 |

| TABLE 3. Effect of the chemical compound on blood RBC. |
|---|---|---|---|---|---|---|---|
| 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 |
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.

Conflict of interest: none declared
Financial support: none declared

REFERENCES