Pharmacological and Biological Evaluation of 5,5'[(1,4-Phenelene) bis (1,3,4-thiadiazol-2-amine)]

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Pharmacological and Biological Evaluation of 5,5'[(1,4-Phenelene) bis (1,3,4-thiadiazol-2-amine)]

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Abstract: The thiadiazole 2-amines compound have been synthesis in this study. The biological activity of the compound was determined at 100, 150, 200 and 250 mg/ml against four types of bacteria i.e. *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Slmonella* sp. The results of MIC were 2,5,4,5 mg/ml for *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Slmonella* spp, respectively. Median lethal dose (LD_{50}) and cytotoxic activity were also investigated. The results showed that this compound did not affect the red blood until the concentration reach 30mg/ml or above. The LD_{50} for this compound is 0.01994 gm/kg bw.

1 Introduction

Due to the diversity of synthetic procedures, physiological and industrial significance, heterocyclic chemistry has been and continues to be one of the most active areas of organic chemistry. Heterocyclic compounds such as thiazoles, thiadiazoles were used successfully as antibacterial, anti-cancer, antipyretic, schistosomicidal, hypoglycemic, anti-hypertensive, anti-inflammatory and anti-HIV agents [1].

Compounds ' biological activity depends on their molecular structure. Due to the presence of N= C-S in the ring, thiadiazole exhibits a wide range of activity. Because of their broad types of biological activity, they have become an important class of heterocycles of great interest to research [2].

Resistance to available drugs is fast becoming a major problem worldwide. The main research areas today are the need to design new compounds to address this resistance. (Neelottama Kushwaha et. al., 2014). The purpose of this study is to prepare thiadaizol compound with determined MIC and study its biological activity on various types of bacteria. Besides determining the cytotoxic and the LD_{50} activity.

2 Materials and Methods

2.1 Synthesis of 5,5'[(1,4-Phenelene) bis(1,3,4-thiadiazol-2-amine)]

This compound was previously prepared according to [3] method; (1.661 gm, 0.01 mol.) of terphthalic acid and (3.646 gm, 0.02 mol.) of thiosemicarbazide were mixed in three neck round bottom flask fitted with condenser then 15ml of concentrated sulphuric acid was added slowly in cold condition (ice bath). The mixture was refluxed for 3hrs, poured into crushed ice, Ammonia solution was added cautiously for neutralization (PH<7). A yellow precipitate was formed which filtered off, washed with saturated sodium bicarbonate solution, then washed with water, dried, recrystallization from ethanol gave yellow precipitate with m.p. (342-344°C litraturer343-344°C), wt. 1gm Yield (36%), with R_f=0.85 using (3:7 Ethanol/Ethyl acetate).

2.2. Physical measurements

2.2.1 Melting point: The product compound's melting point was expressed in degree (0 ^{o}C). It was measured in the Department of Chemistry, University of Basrah College of Science. Use of digital SMP31 melting point device and was uncorrected.

2.2.2 FT-IR Spectra: FT-IR spectrum was recorded, using Shimadzu FT-IR-8400 affinity spectra photo meter made in Japan, in Department of Chemistry, College of Education for pure Science, University of Basrah using KBr disc, and expressed in cm^{-1}.

2.2.3 Thin Layer Chromatography: Thin layer chromatography of the starting materials and products was carried out using Eastman chromatography sheet (GERMANY) with the appropriate eluent ratio (methanol: ethyl acetate) (3:7); the spot was visualized by exposing the dry plate in UV light.

2.2.4 Elemental Analysis: Elemental micro analysis of Carbon, Hydrogen and Nitrogen were carried out in Al al-Bayt University, Al-Mafraq, Jordan using a Euro vector EA 3000A Elemental analysis (Italy).

2.3 Biological activity of chemical compounds

2.3.1 Bacterial isolates: In this test, four bacterial isolates have been used. These are *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* Sp. *Bacillus cereus* was isolated during this study. The other bacterial strains were collected from the Central Lab. of College of Veterinary Medicine University of Basrah. Bacteria were cultivated on MYP agar, manitol salt agar, EMB agar and XLD agar. Growth was transferred to a sterile tube with a normal saline of 5ml. The turbidity of the actively growing broth culture was adjusted with 0.5 McFarland standard [5] .

2.3.2 Antimicrobial activity test of the prepared compound: Different concentrations i.e. (100, 150, 200, 250) mg/ml from the synthesis compound were prepared and were used in this study [5]. The antimicrobial susceptibility was tested by agar well diffusion method according to [6].

2.3.3 Minimum inhibitory concentration (MIC) for the compound: Using concentrations (1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 40, 50) mg / ml, the four bacterial strains were subjected to the susceptibility tests to prepared compound. Well diffusion method was used to determine the MIC for prepared compounds. The prepared compounds were dissolved in DMSO into different concentrations [7]. Bacterial suspensions were adjusted to MacFarland standard 0.5($1.5*10^{8}$) CFU.

2.3.4 Blood cell cytotoxicity assay: The method of studying the cell toxicity of the prepared compounds [9]. A physiological saline (1.0 ml blood suspension in 20 ml saline) has been prepared. Different levels of prepared compounds have been used in DMSO. Two ml of erythrocyte suspension prepared in the first step were added to the sterile tubes, adding 0.1 ml of each concentration. Two ml of tab water with 0.1 ml of erythrocyte were used as positive control, and two of normal saline with 0.1 ml of erythrocyte were used as negative control. The turbidity was read at 10, 30 and 60 min. at 37 ° C. The concentrations that provided a clear solution due to RBC lysing are an indication of the degree of toxicity to the erythrocytes of the test compounds [9].

2.3.5 Median Lethal Dose (LD_{50}) assay: To determine the LD_{50} for each of the three compounds, a total number of seven male and female rats (Rattus norvegicus) were used. In the animal house of the College of Veterinary Medicine / University of Basrah, the animals aged 8-10 weeks and their body weight between 190-200 gm, each animal was isolated in one cage in a good air condition room. The animals fed on dried bread, pellet and given R.O. water. The LD_{50} steps used in this experiment was "up and down" method described by [11] as below;
The code that found was (XOXO) for the compound, and the LD_{50} was determined according to the formula described by [11].

$$\mathbf{LD_{50} = X_f + Kd}$$

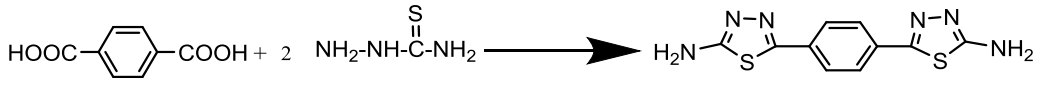
LD_{50} = Median lethal dose. X_f = Last dose used in the experiment.
K = Factor of change from the table. , D = Distance between doses.
O= Symbol of survival animal after 24 of hrs. of dosing., X= Symbol of dead animal within 24 hrs. of dosing.

3 Results

3.1 Synthesis of Thiadiazole Derivative

In this study, Thiadiazole compound was synthesized. The reaction scheme for the synthesis of this compound is shown in Figure 1. Elemental analysis; Found (calculated)= **C**: 43.35(43.46), **H**: 2.88(2.92), **N**: 30.69(30.41), **S**: 23.44(23.21). The FT.IR spectrum for compound using KBrdisk:υ $NH_{assym.}$3282cm^{-1}, υNH_{sym}3097cm^{-1}, υ$CH_{arom.}$3000cm^{-1}, υC=N1620cm^{-1}, υC=$C_{assym.}$1541cm^{-1}, υC=$C_{sym.}$1410cm^{-1}, υN-N1041cm^{-1}, υC-$N_{arom.}$1332cm^{-1}, υC-$S_{Het\cdot Cyclic}$688cm^{-1}.

[1], υC-$S_{Het\cdot Cyclic}$688cm^{-1}.



terephthalic acid hydrazinecarbothioamide 5,5'-(1,4-phenylene)bis(1,3,4-thiadiazol-2-amine)

Figure 1: Pathway for Chemical synthesis of the compound

3.2 Biological activity of chemical compounds

The antimicrobial activity was determined against four types of bacteria in four concs. 100, 150, 200 and 250 mg/ml Table (1), Figure (2).

Table 1: Biological activity of compound A

Chemical compounds	Types of bacteria	Inhibition zone (mm)			
		100 mg/ml	150 mg/ml	200 mg/ml	250 mg/ml
Compound A	*Bacillus cereus*	14	15	16	17
	Staphylococcus aureus	16	18	20	21
	Escherichia coli	16	17	18	19
	Salmonella spp.	12	13	15	17

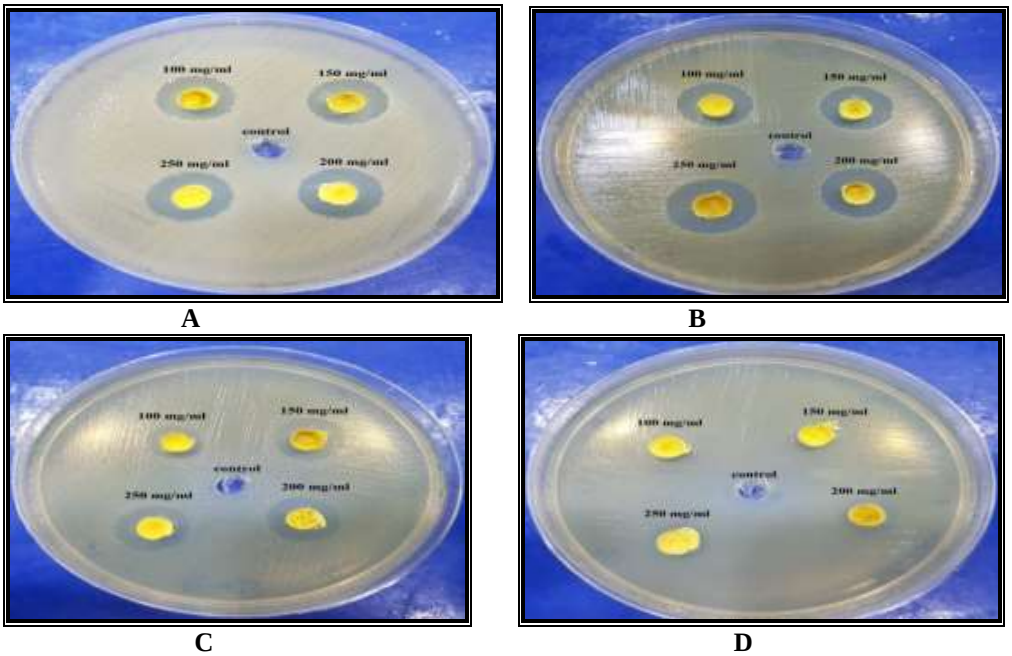


Figure 2: The biological activity of prepared compound against different types of bacteria, A= B. cereus, B= S. aureus, C= E. coli, D= Salmonella sp.

3.3 Minimum inhibitory concentration (MIC) of prepared compounds:
The minimum inhibitory concentration for this compound against four types of bacteria was listed below Table (2), Figure (3).

Table 2: The MIC of prepared compound against four types of bacteria

Conc. mg/ml	Inhibition zone (mm)			
	B. cereus	*S. aureus*	*E. coli*	*Salmonella* spp.
50	12	15	13	10
40	12	14	12	9
30	11	14	12	8
25	10	14	12	7
20	9	14	11	7
15	8	14	11	6
10	7	13	10	5
5	6	9	9	5
4	5	7	9	0
3	4	0	7	0
2	3	0	0	0
1	0	0	0	0

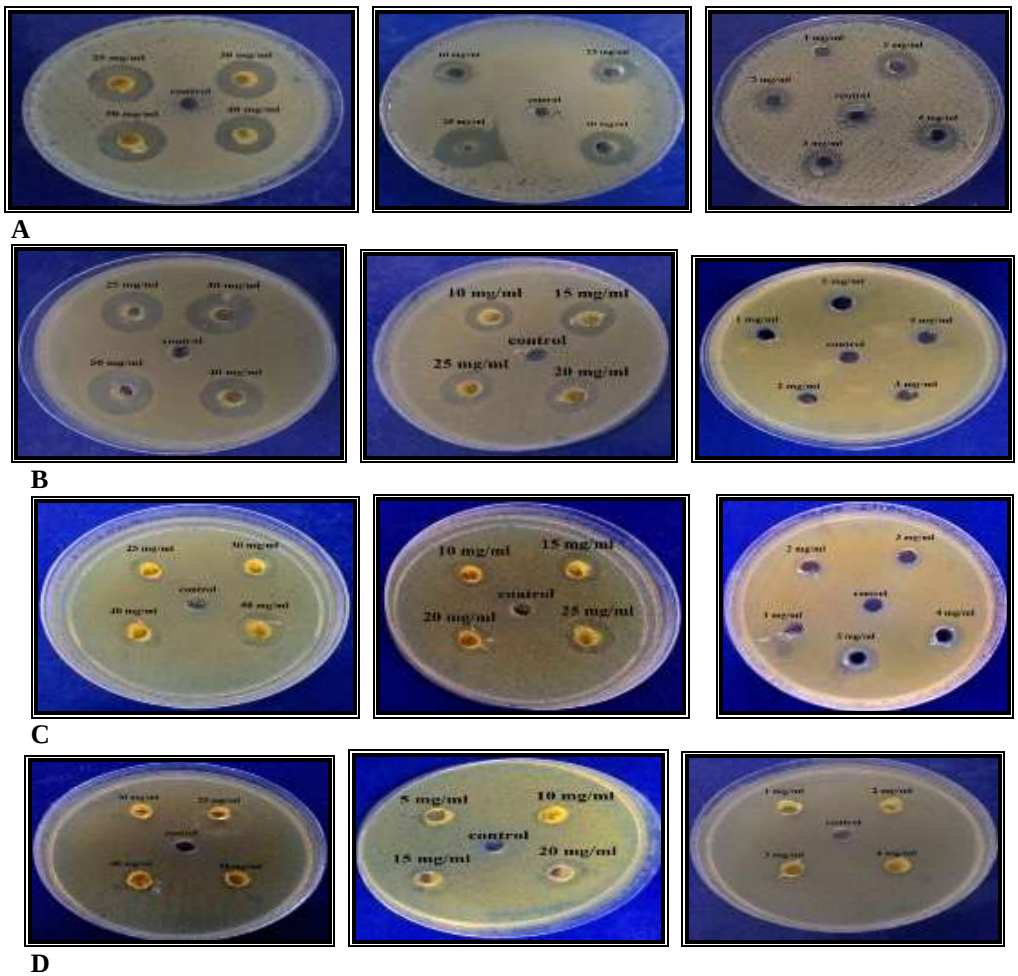


Figure 3: The MIC of prepared compound against A= *B. cereus* , B= *S. aureus* ,C= *E. coli* , D= *Salmonella* sp.

3.4 Determination of blood cytotoxicity of chemical compounds
The prepared Compound did not affect the red blood cells at the concentration 1,2,3,4. The concentrations 5, 10, 20 had few hemolysis; the concentrations 30, 40, 50, 60, 70, 80, 90, 100, 150, 200 and 250 mg/ml cause high hemolysis in red blood cells. Table (3), Figure (4)

Table (3) Toxicity of compound A on red blood cells

Conc. mg/ml	Toxicity
3 ; 4	-
5; 10; 20	+
30; 40; 50; 60; 70; 80; 90; 100; 150; 200; 250	+++

- = no hemolysis, += few hemolysis, ++ = Moderate hemolysis, +++ High hemolysis

Figure 4: Toxicity of the prepared compound

3.5 Lethal Dose (LD_{50}) assay:
The LD_{50} for the prepared compound is 0.01994 gm/kg bw.

4 Discussion
Previously, the investigated compound was prepared from the reaction of one mole terephthalic acid and tow moles of thiosemecarbazide in the presence of concentrated sulphuric acid as a dehydration agent in the thiosemecarbazide cyclization reaction to give 5,5'-[1,4-Phynlenebis(1,3,4-thiadiazol-2-amine)]. This method was generally used in preparation of 2-amino-5-substituted-1,3,4-thiadiazole which include the nucleophilic attack by the amine of thiosemecarbazide on electrophilic carbon atoms of aromatic acids. i.e. the reaction involves acylation of thiasemecarbazide then dehydration cyclization by using sulphuric acid. This compound has been characterized by melting point, FT-IR, and elemental analysis that confirms compound A's proper structure. The absence of any 3500-3560 cm^{-1} absorption bands for O-H str. and 1700-1725 cm^{-1} for C=O str. gave good evidence that both carboxylic groups in the terephthalic acid were converted to thiadiazole rings. The compound produced has biological activity against four bacterial strains, two of which are gram-positive bacteria, i.e. *Bacillus cereus, Staphylococcus aureus* and other bacteria are gram negative, i.e. *Escherichia coli* and the *spp* of *Salmonella.* Use four 100 mg / ml, 150 mg / ml, 200 mg / ml and 250 mg / ml concentrations. Results have been found leading to increased concentration in the compounds ' inhibition zone. The compound ' highest concentration had the highest biological activity. This results due to chemical structure of the compounds. The effect of compound at the lowest conc. 100 mg/ml had high activity against *E. coli* and *Staph aureus* but, at high conc. 250 mg/ml against *Staph. aureus*, and its MIC was (2, 5, 4, 5) mg/ml on *B. cereus*, *Staph aureus*, *E. coli* and *Salmonella* spp., respectively. The bacteria's cell wall is very important because many antibiotics have a concentrated cell wall effect. The cell wall differs between gram-positive and gram-negative bacteria in the chemical composition. Furthermore, number of wall layers, wall thickness and wall content of polyunsaturated fatty acids and multiple proteins. Any drug that has a toxic effect on cells; commonly used in chemotherapy to inhibit the proliferation of cancerous cells [9]. Any drug that affects cells toxically; commonly used in chemotherapy to inhibit cancer cell proliferation [9]. Product cytotoxicity was used as a control sample

at different concentrations of (5-250) mg / ml. Tab water, DMSO, normal salin. Blood hemolysis at concentration of 5-250 mg / ml was caused by the compound. The cells may undergo a loss of membrane integrity and die rapidly as a result of cell lysis [10].
Because the compound was not water soluble, it was soluble in DMSO and used intraperitoneal injection (IP) as a suspension of the rats ' abdominal cavity to determine the LD_{50}. Mortality recorded when rats are exposed to 0.01994 gm / kg body weight was shown by intraperitoneal injection of compound. We found increased mortality when compound doses were increased.

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