Egypt.J.Chem. **Vol. 63**, No.2. pp. 669-681 (2020)

Egyptian Journal of Chemistry
http://ejchem.journals.ekb.eg/

Synthesis, Characterization, Antimicrobial Activity And Acute Toxicity Test of Anovel 4,4- (4, 5, 6, 7-Tetrahydro- [1, 2, 3 -] Selenad Iazolo [4, 5 e] Pyridine-4, 6-Diyl) BIS(Benzene-1, 3-Diol)

Nadheerah F. Neamah *[1], Abdul-Razzak .Naaeem Khudair [2], Shaker.A.N.Al-Jadaan[3]

1-Pharmacology &Toxicology Department, College of Pharmacy, University of Basrah, Iraq.
2-Physiological department, College of Veterinary, University of Basrah, Iraq.
3-Pharmaceutical Chemistry Department, College of Pharmacy, University of Basrah, Iraq.

Novel selena-diazole compound (T) i.e. 4, 4 - (4 ,5 ,6 ,7- Tetrahydro- [1,2,3-] selenadiazolo [4, 5e] pyridine - 4, 6 - diyl) bis (benzene-1,3-diol) was prepared by reacting 2,4-hydroxy benzaldehyde with acetone in the presence of ammonium acetate through condensation reaction to form 2,6-bis(2,4-dihydroxyphenyl)piperidin-4-one, which on reaction with hydrazinecarboxamide in absolute ethanol in acidic medium gave 2-(2,6-bis(2,4-dihydroxyphenyl)piperidin-4-ylidene) hydrazine-1-carboxamide which converted to T by reaction with selenium dioxide in excellent yield. T was characterized by elemental analysis and spectroscopic data which confirmed the proposed structure.
The median lethal dose (LD50) of T compound was assayed to determine the median toxic dose also the lowest toxic dose. LD50 was found equal to 863.28 mg/kg which indicate that T considered slightly toxic based on Hodge and Sterner scale.
Antimicrobial activity of T at deferent concentrations showed some promising antibacterial activity against Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli, using filter paper disk method. The best minimum inhibitory concentration (MIC) was against Pseudomonas aeruginosa. Also, it has a potent antifungal effect against Candida albicans, Candida krusi, and Candida paras. It can be conclude that T more safe and has a good antibacterial and antifungal activity.

Keywords: Selena-diazole, LD50, Antibacterial, Antifungal, MIC

Introduction

Selenium(Se) can found in organic form in which directly attached to carbon such as selenomethionine ($C_5H_{11}NO_2Se$), and selenocysteine($C_3H_7NO_2Se$) or inorganic form like selenite and elemental Selenium. Environmental selenium species are mainly inorganic and in living organisms selenium commonly organic forms[1]. It is common that many of heterocyclic compounds containing nitrogen and selenium obtain different pharmacological properties. Heterocyclic ring systems having a piperidin-4-one nucleus, and their derivative have provoked great interest in the past and recent years due to their wide variety of biological properties such as antiviral, antitumor, local anesthetic, anticancer and antimicrobial activity [٢]. The biological character of selenium as a constituent of the enzyme glutathione peroxidase was first underlying in 1973. Subsequently, additional about 30 selenium-containing proteins, mainly

*Corresponding author e-mail: fanma_64@hotmail.com; (Scopus Affiliation ID: 60014618).
Received 15/4/2019; Accepted 15/6/2019
DOI: 10.21608/ejchem.2019.11702.1753

enzymes, have been recognized[3]. Organic selenium forms have larger bioavailability, with lower toxicity in comparison with inorganic compound. Firstly, unstable compounds were synthesized like selenols. Various studies concentrated on development of more stable and easily purified organic-selenium compounds[4]. In the human and animal body, Se deficiency is the main cause for dangerous chronic diseases such as necrosis of the liver [5]

Multi agent's resistance microbes are international spread in unusual manner, thus novel procedures, or therapeutics to eradicate micro-organisms are frequently needed[6]. Many methodologies and therapeutics are developed, such as Heterocyclic compounds comprising Selenium, and they have many biological and synthetic applications. Several 1,2,3-selena-diazoles derivatives have been prepared and practically some of them have a high antimicrobial action[7], potent antioxidant, Anti-proliferative action against several of human cancer cell lines[8]. The probability of "1, 2, 3-selena-diazole" has been extended in pharmaceutical chemistry. 1,2,3-selena-diazoles, furthermore to their high-tech requests, also have been broadly studied for their pharmacological applications e.g. biological activities and cytotoxicity[9]. The major objectives of this study were synthetization, characterization novel selena-diazole and to determine the median lethal dose of newly synthesized compound. Furthermore, to evaluation the antimicrobial activity of T compound.

Materials and Methods

Synthesis of Selena-diazole

Physical measurements

FT-IR spectra were recorded, using Shimadzu FT-IR affinity spectrophotometer made in Japan, using KBr disc, and expressed in cm^{-1}. The melting point of the product compound was expressed in degree (^0C) using melting point digital apparatus SMP31 and is uncorrected. Both measured in the department of Pharmaceutical Chemistry, College of Pharmacy, University of Basrah, Iraq. ^1H-NMR and ^{13}C-NMR spectra of (T) compound was recorded using Bruker Ultra shield spectrophotometer (300 MHz), University of Al-al-Bayt, Jordan. The Chemical shift was expressed as ppm. Tetramethylsilane (TMS) used as internal standard and DMSO-d_6 as a solvent. 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).

Synthesis of 2,6-bis(2,4-dihydroxyphenyl) piperidin-4-one:

In 500ml round bottom flask (20gm , 0.1449mol.) of 2,4-dihydroxy benzaldehyde was dissolved in 200ml absolute ethanol (4.20 gm, 0.0724mol.) of dry acetone was added and stirring with heating until all components were completely dissolved, to this solution (2.678gm, 0.0724mol.) of ammonium acetate was added, the reaction mixture were heating under reflux for 3hrs, the reaction mixture were monitoring under TLC, filtered hot and stand until cooling to room temperature orange-red viscous oily compound were collected by rotary evaporation of all solvents, wt.=20.12gm, 88% yield.

Synthesis of 2-(2,6-bis(2,4-dihydroxyphenyl) piperidin-4-ylidene) hydrazine-1-carboxamide:

Equimolar amounts of piperidin-4-one (20.12gm, 0.0638mol.) and hydrazine carboxamide (4.79gm, 0.0638mol.) was dissolved in absolute ethanol (30 ml) with 0.1gm of p-toluene sulphonic acid were refluxed for 3hrs, the content was cooled to room temperature, the precipitate was filtered off washed with water, dried and recrystallized from absolute ethanol gave orange crystals (14.9gm) yield 62.7%, with m.p. (69.7^oC).

Synthesis of 4', 4" -(4, 5, 6,7-Tetrahydro-[1, 2, 3-] selenadiazolo [4, 5e] pyridine-4, 6-diyl) bis (benzene-1, 3-diol):

A mixture of semicarbazone (24.31gm, 0.0653mol.) was dissolved in dioxane 25

ml was added to an aqueous solution of Selenium dioxide 10ml (7.25gm, 0.0653 mol.) with stirring at room temperature for 3hrs.The product obtained was filtered, dried and recrystallized from absolute ethanol to give bloody red compound (15.7gm), yield = 60%, with m.p. (178.7-180.6 ^{0}C) and have R_f value=0.89 (1:1) (Chloroform\DMSO). Elemental analysis; found (calculated)= C:50.38(50.51), H: 3.63 (3.73), N: 10.51 (10.39). The FT-IR spectrum of selena-diazole compound show characteristic bands in certain positions which indicates the proper functional groups for the prepared compound. 3433.29cm-1(ʊOH), 3309.85cm^{-1}(ʊN-H assym.), 3263.56cm^{-1}(ʊN-H sym.), 3128.54cm^{-1}(ʊ =C-Se-N), 3062.96cm^{-1}(ʊ C-H arom.), 2881.95cm^{-1}(ʊ C-Haliph.), 2657.91cm^{-1}(ʊ N=N cyclic), 1685.79cm^{-1}(N-H bending), 1585.49cm^{-1}(ʊ N=N assym.), 1531.48cm^{-1}(ʊ N=N sym.), 1485cm^{-1} (ʊ C=C arom.) and 1388.75cm^{-1}(ʊ C-Naliph.).

The ^{1}H-NMR spectrum for new (T) compound was recorded using DMSO-d_6 as a solvent(300Mz). In general, ^{1}H-NMR spectrum shows signal at1.13ppm(t) attributed to 2H(C7), signal at 2.30(s) may assigned to H(C4), and broad signal at 3.47(s, b) may assigned to NH proton, the signal at 3.93(d) may attributed to H(C6), 6.65ppm(s) may assigned to 2H (C2, C2), the signal at 7.13ppm(d) due to 2H (C5, C5), the signal at 7.48ppm(d) due to 2H (C6, C6), the signal at 8.86ppm(s) due to 2OH (C1, C1) while that at 9.85ppm(s) due to 2OH (C3, C3).

The ^{13}C-NMR spectrum for new (T) compound was recorded using DMSO –d_6 32.033(C7), 53.960(C6), 56.960(C4), 103.609(C2, C2), 108.658(C6, C6), 118.480(C4, C4), 127.717(C5, C5), 136.333(C9), 157.726(C8), 176(C1, C1, C3, C3).

Median Lethal dose (LD_{50})

Healthy female rats procured from Veterinary Medicine College/University of Basrah. The female rats kept in polypropylene cages lined with sawdust. Rats were provided usual rat pellet diet and tap water. At the beginning the rats were adapted to laboratory circumstance, natural day and light (12 hours days and 12 hours nights). Room temperature 21±4°C[10]

Acute oral toxicity test was done like OECD-423 guidelines (Organization for Economic Co-operation and Development). LD_{50} can be primarily measured as a pilot study by a Staircase method or up and down by using small number of animals [11]. Female rats were randomly dispersed into eight investigational groups; each group comprising ten rats weighted 120-150 g. The first group received only 2 mL of DW via oral gavage, other seven groups, received (T) compound at one of the doses: 64, 128, 256, 500, 1000, 1500, and 2000 mg/Kg body weight, each dose liquefied in 2ml of distilled water at 37 ℃, the responses are recorded after 48 hours by percentage of death in each group[12]. Percentage of mortality calculated by

% Mortality = (number of dead animals/Total number of animals) X 100.

A diagram was drawn to illustrate the relationship between the dose that given to the rats and the mortality rate. Then through the equation calculated on the basis of deviation in the results line by the Excel system, the dose at x-axis that gives any mortality rate can be calculated. Thus is calculated LD_{50} than estimate by the equation based on the relationship between the x-axis and the y axis[13]

Antimicrobial activity

Preparation of a stock solution of T compound

Five milligram of T dissolved in 100 mL of solution composed of 90 mL Distilled water with 10 mL DMSO. Through serial dilution of stock solution Several T concentrations were prepared like 50, 40, 30, 20, 10, 1, 0.5, and 0.25µg/ml. The lowest concentrations with no visible growth, is the minimum inhibitory concentration (MIC) for the test organisms were used to compare the antimicrobial activity of T on the microorganism.

Antibacterial effects

The synthetic(T) was assessed for antibacterial effect against Gram positive *S.aureus*, and Gram negative *E. coli*, and *P. aeruginosa* organisms at the concentrations of 0.25, 0.5,

1,10, 20, 30, 40and 50µg/ml(T) compound dissolved in DMSO1 mL: Distilled water 9mL. All bacteria were revealed resistant to the most antibacterial drugs, isolated from clinical labs of Al Basrah general hospital. And then identification of bacteria and all antimicrobial assays in microbiological lab in pharmacy college. Filter paper disks were used, inhibitory effects was indicated by the inhibition zone diameter[14]02115.
Nanomedicine Science and Technology Center, Northeastern University, Boston, Massachusetts, 02115.
Universitat Rovira I Virgili, Tarragona, Spain.</auth-address><titles><title>Synthesis and characterization of biogenic selenium nanoparticles with antimicrobial properties made by Staphylococcus aureus, methicillin-resistant Staphylococcus aureus (MRSA. A control disks (Negative controls)were similarly performed with DMSO at the same dilutions(DMSO1 mL: Distilled water 9mL)[15]. The method of filter paper disk was achieved as: filter paper Whatman III puncture disks with diameter of 5 mm were flooded with (T) compound, and then dried at 60ºC. The disks were sited on cultured Muller-Hinton agar[16]. The clear zone diameter (mm) was measured after incubation at 37°C for 24 hr.

Antifungal activity

The antifungal action of (T) compound was verified against the clinical strains: *C albicans*, *C krusi*, and *C paras*. These strains were obtained from Al Basrah general hospital. Disks were flooded with 0.25, 0.5, 1, 10, 20, 30, 40 and 50µg/ml concentrations of (T) compound, and then dried at 60ºC. A control disks was similar to that for antibacterial activity[15]. The disks placed on Sabouraud dextrose agar (SDA) plates were cultured with Candida. The plates incubated at 37°C for 24 hr. The results expressed as diameter of inhibition zone in mm.

Results and discussion:

3.1 Synthesis of Selena-diazole

In this work, T compound was synthesized by condensation of one mole of acetone with two moles of 2,4-dihydroxybenzaldehyde and one mole of ammonium acetate using Mannich reaction[1] which gave orange-red viscous compound with 88% yield, all attempt to crystalize this compound was failed. Semicarbazone derivative of 2,6-diaryl-4-piperidone was prepared by the reaction of equimolar amounts of piperidone with hydrazinecarboxamide[2,3] which gave orange crystal with 62.7% yield. Then the product was converted to selena-diazole by addition of selenium dioxide in dioxane as a solvent with 60% yield. Selenazdiazole compound prepared in this method was recrystallized twice by absolute ethanol to give pure bloody red crystals with one spot in TLC in different solvents. The product was solid, stable in air, unaffected by moisture and dissolved in common organic solvents. The reaction scheme for the synthesis of these compounds is shown in scheme (1).

The elemental analysis for CHN is within ±0.2% of the theoretical values.

The selena-diazole prepared in this study was characterized by FT-IR which shows the characteristic bands at 3433,28 cm^{-1} (υOH). Two bands were appeared at 3309.85cm^{-1} (υN-H assym.) and 3263.56cm^{-1} (υN-H sym.)due to asymmetrical and symmetrical stretching of N-H bond respectively [4,5]. The weak band was appeared at 3128.54cm^{-1} may attributed to (υ =C-Se-N) [6]. The band at3062.96cm^{-1}(υ C-H arom.), Two weak and medium bands appeared at 2981.95cm^{-1} and 2881.95cm^{-1} (υ C-H aliph.). Strong band appeared at 2657.91cm^{-1} may be attributed to cyclic N=N stretching [1,7]. The strong band at1685.79 cm^{-1}; due to N-H bending. Two strong bands appeared at 1585.49cm^{-1} and 1531.48cm^{-1} attributed to asymmetrical (υ N=N assym.) and symmetrical (υ N=N sym.) stretching of N=N bond respectively. Also, two strong bands at 1485.19cm^{-1} (υ C=C arom.) and 1388.75cm^{-1}(υ C-Naliph.). Strong band appeared at 1219.01cm^{-1} due to stretching of C-N bond [4,5]. As in figure 1.

The ^{1}H-NMR spectrum for new selena-diazole was recorded using DMSO-d_6 as a solvent (300Mz) at ambient temperature. Generally, a signal at 1.13ppm (m) may attribute to methylene protons of 2H(C7),

signal at 2.30ppm (S) attributed to 1H(C4) while signal at 3.92ppm(d) may due to 1H(C6) [8] 6.56ppm(s) allocated to 1H(N-H) the signal at 7.13ppm(d) credited to 2H (C6', C6"), (J= 7.90) while the signal at 7.13ppm(d) as a result of 4H (C6¯, C2¯, and C6=, C2=) the signal at 7.47ppm(d) attributable to 2H (C5¯, C5=), the signal at 8.86ppm(s) due to 2OH (C1¯, C1=) while that at 9.98ppm(s) due to 2OH (C3¯, C3=) which appear as singlet signal due to strong interhydrogen bond with other molecules [9,10].The results were illustrated in figures (2,3)

The ^{13}C-NMR spectrum (DMSO-d_6) gave a further support for the structure formation of prepared selena-diazole. In general, ^{13}C-NMR spectrum of selena-diazole compound shows the signals of cyclic methylene carbon atom at 32.033ppm (C7) 53.96ppm (C6) and 56.96ppm (C4). These values are in agreed well with previous literatures [4, 8, 11, 12], while signals at 157.726ppm and 136.333ppm attributed to (C8) and (C9) respectively. Comparatively, the low chemical shift of selenated carbon atom may be due to the polarity of C-Se bond [8, 13]. The spectrum shows signal at 176 ppm due to aromatic carbon atoms attached to hydroxyl groups, i.e., (C1¯, C1¯, C3¯and C3=), while other aromatic carbon atoms appeared at 103.609ppm (C2¯, C2=), 108.658ppm (C6¯, C6=), 118.480ppm (C4¯, C4=) and 127.717ppm (C5¯, C5=). The new selena-diazole prepared in this study which characterized by elemental analysis, IR, 1H-NMR and ^{13}C-NMR spectroscopy which confirmed the proposed structure. The results summarized in Table (1)

Median Lethal doses (LD_{50})

The female rats groups treated with single oral doses of (T) compound at concentration of 0, 64, 128, 256, 500, 1000, 1500, and 2000 mg/kg BW, via gavage administration. The mortality percentage up to 48 hours demonstrates that LD_{50} of (T) doses in female rats is 863.28 mg/kg, the dose calculated from slope equation of dose –response curve, the results documented in table: 2; no mortality was recorded at control group (0 dose), and 64 mg/kg B.W., however when doses increased response was observed from 10% at 128mg/Kg, to 100% at 2000 mg/Kg.

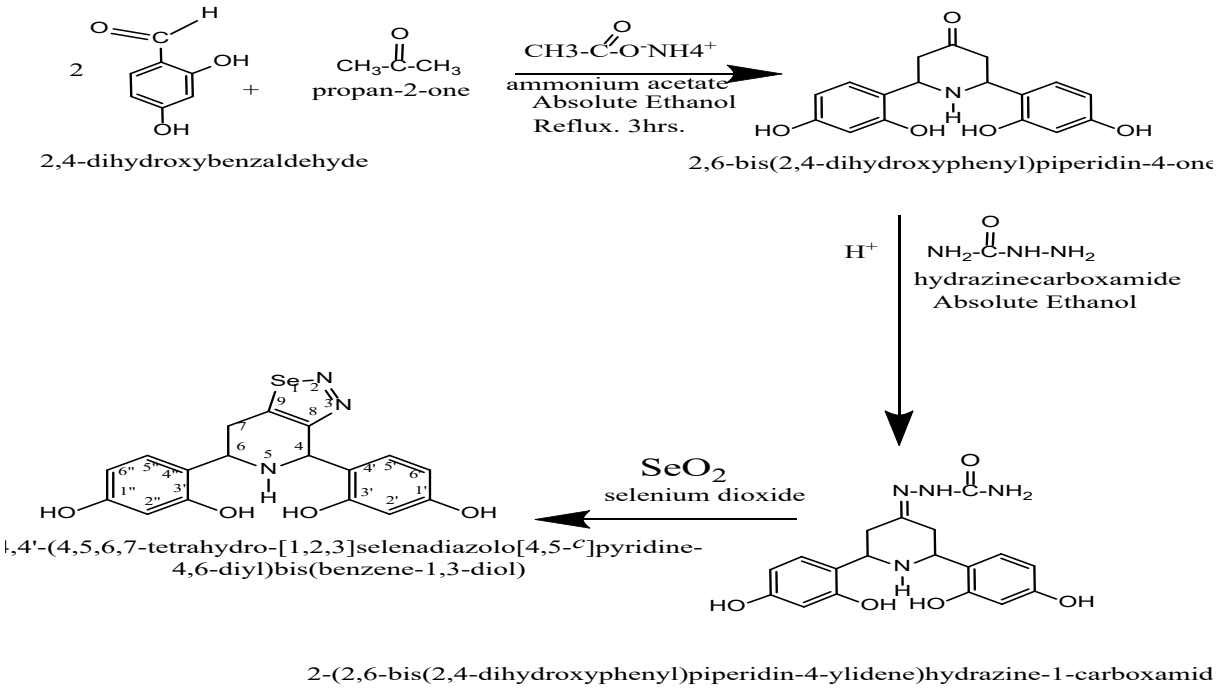
The results indicated that 50% of mortality between the concentration of 500-1000 mg/Kg, based on Hodge and Sterner scale that used for the evaluation of toxicity, (T) compound considered slightly toxic[13]. From the graphical design of percentage of mortality; vs concentration, LD_{50} was measured from equation of data straight line. A substance with a lower LD_{50} is more toxic than one which has a higher LD_{50}. Acute toxicity test provides evidences on the range of doses that could be toxic to the animal; it could also be used to estimate the therapeutic index (LD_{50}/ED_{50}) of drugs and xenobiotics[17].

In this study the LD_{50} of (T) compound was 896.60 mg/kg, which indicate that (T) is a slightly toxic substance, based on Hodge and Sterner scale that used for the evaluation of toxicity[13]. In contrast with many naturally organic and inorganic resorbed Selenium compounds have LD_{50} in the range of 2–5 mg/kg. In comparison with rat's dietary requirement for selenium, which about 0.20 mg/kg and the threshold for its toxicity from selenite, which about 0.50 mg/kg, simply it's about 2.5 times than the dietary requirement. Selenomethionine (LD_{50} 4.3 mg/kg) is less poisonous than selenite[18]. However, it was later shown by Yamashita that, selenoneine found naturally in some animals tissues was a non-toxic organic selenium , in comparison with the high toxicity of selenocysteine, selenomethionine and selenite [19]. Studies have similar results of the current study; found that some of the synthetic selenadiazol derivatives are less toxic than naturally selenium containing compounds. According to Arsenyan et al., 4-Methyl-1, 2, 3-selena-diazole-5-carboxylic acid amides were organized by suitable synthetic means. The association between compounds LD_{50} between 1113-1145mg/kg[20].

1.2 Antimicrobial activity

1.2.1 Antibacterial action

Evaluation of antimicrobial effect of T compound against *S aureus*, *P aeruginosa*, and *E coli* was studied and from table (3)



Scheme1: Synthesis path way for preparation of Novel Selena-diazole

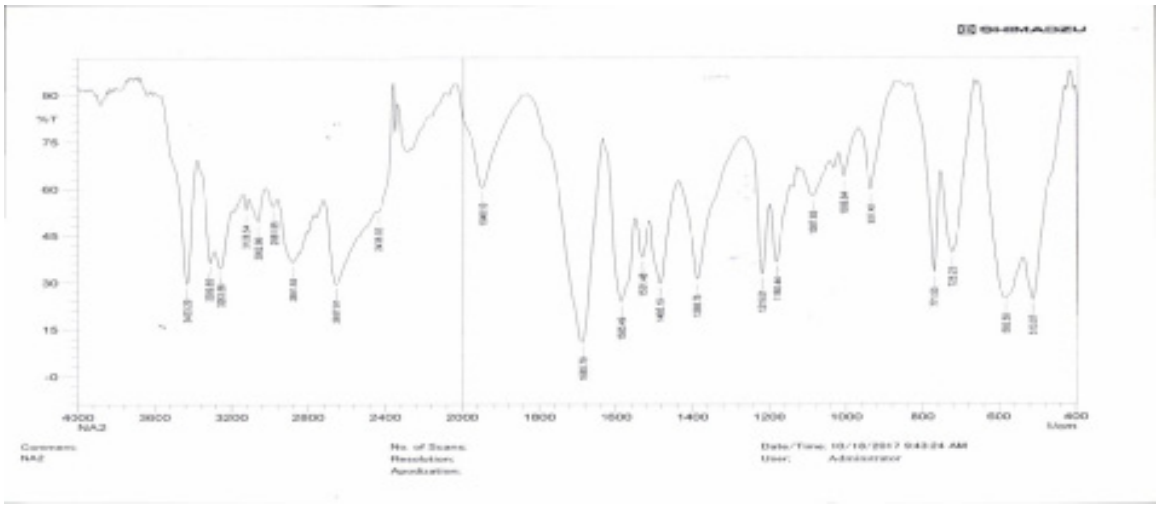


Fig.1: The IR spectrum for (T) Selena-diazole compound shows it's the functional groups.

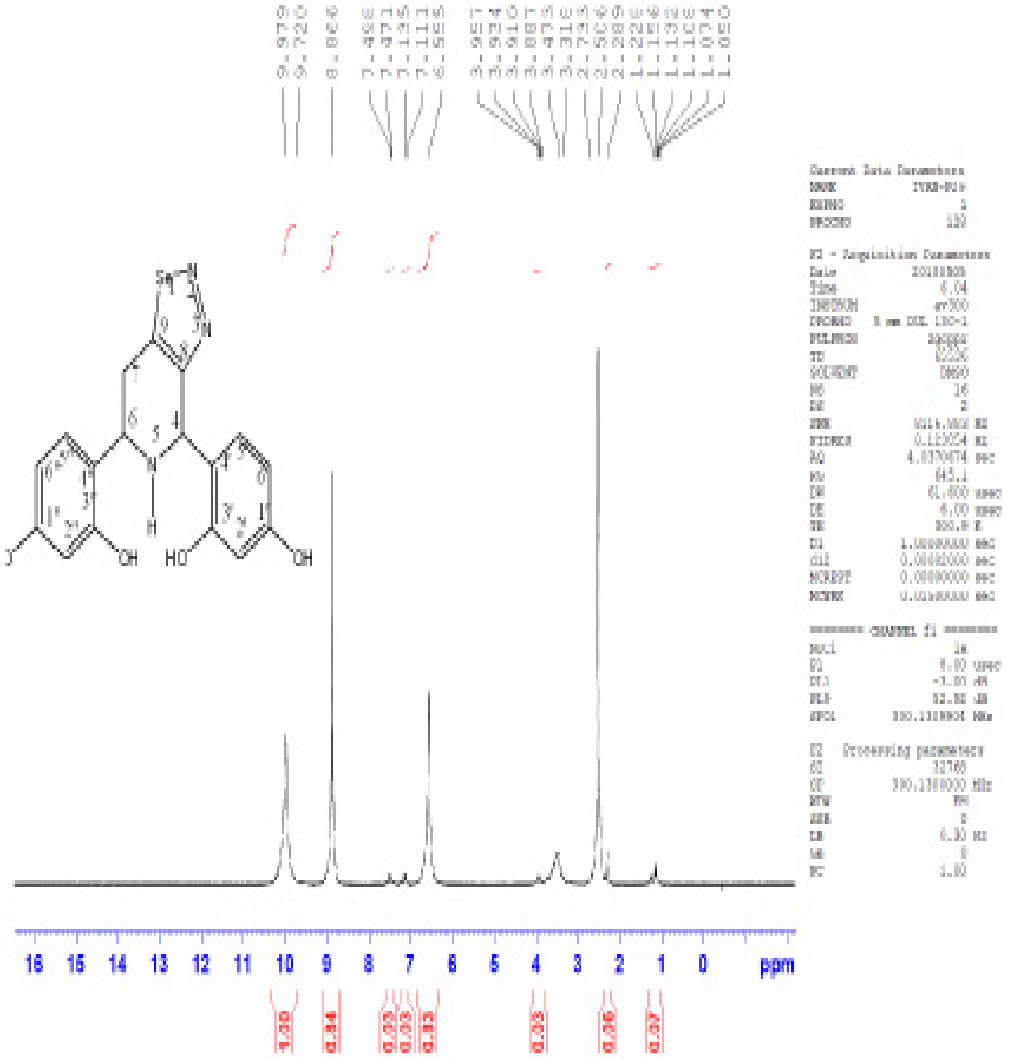


Fig.2 : 1H-NMR for Selena-diazole compound in DMSO-d_6

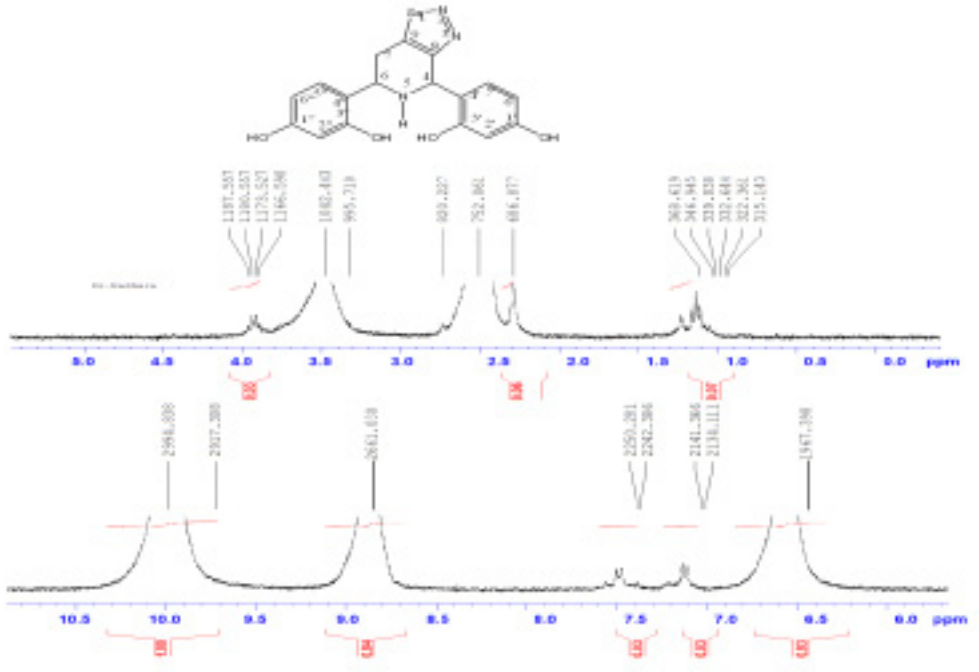
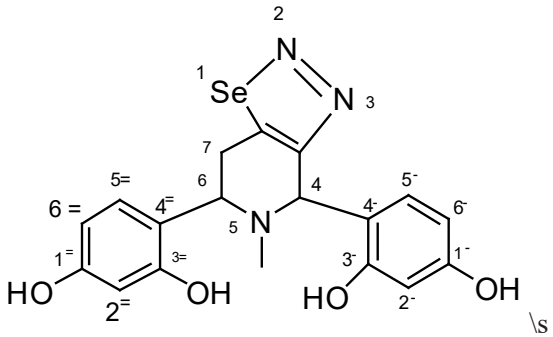


Fig.3: The expansion 1H-NMR spectrum for (T) selena-diazole compound Recorded using Bruker Ultra shield spectrophotometer (300 MHz), University of Al-al-Bayt, Jordan.

TABLE 1 :- properteis of T compound

Description	Properties of crystals		
Molecular Formula:- C17H15N3O4Se			
MW	404.2832		
Color	Bloody red crystals		
m.p.	178.7-180.6ºC		
Solubility	Insoluble in: Water	Partial soluble in: Ethanol, methanol	Soluble in Chloroform,DMSO at<30ºC

FT-IR	3433.29cm-1(υOH), 3309.85cm-1(υN-H assym.), 3263.56cm-1(υN-H sym.), 3128.54cm-1(υ =C-Se-N), 3062.96cm-1(υ C-H arom.), 2881.95cm-1(υ C-Haliph.), 2657.91cm-1(υ N=N cyclic), 1685.79cm-1(N-H bending), 1585.49cm-1(υ N=N assym.), 1531.48cm-1(υ N=N sym.), 1485cm-1 (υ C=C arom.). 1388.75cm-1(υ C-Naliph.).
1H-NMR	1.13ppm(m) attributed to 2H(C7), signal at 2.30(S) may assigned to H(C4), the signal at 3.92ppm (d)due to H(C6) proton, 6.65ppm(s) may assigned to H (N-H) the signal at 7.13ppm(d) due to 4H (C6¯, C2¯, C6= , C2=),the signal at 7.47ppm(d) due to 2H (C5¯, C5¯), signal at 8.86ppm(s) due to 2OH (C1¯, C1=) while that at 9.98 ppm(s) due to 2OH (C3¯, C3=).
13C-NMR spectrum	32.033(C7), 53.960(C6), 56.960(C4), 103.609(C2¯, C2¯), 108.658(C6¯, C6¯), 118.480 (C4¯, C4¯),127.717(C5¯,C5¯), 136.333(C9), 157.726(C8), 176(C1¯, C1¯, C3¯, C3¯).

TABLE2: shows the dose and related response (number of dead rats and mortality%)

NO.group	NO. Rats	Dose (T) mg/kg	No.Dead rats	Mortality%
1	10	64	0	0
2	10	128	1	10
3	10	256	2	20
4	10	500	3	30
5	10	1000	6	60
6	10	1500	8	80
7	10	2000	10	100

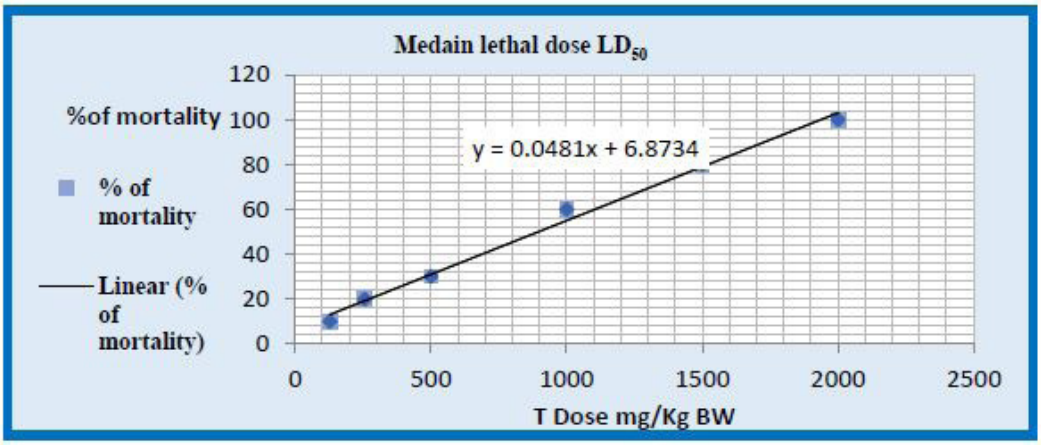


Fig.4: showed the relation between doses of (T) compound and percentage of mortality

The LD_{50} estimation from the equation as illustrated in figure 4:

$Y = 0.0481X + 6.8734$ LD_{50}=896.6mg/kg bw

it can be determined that (T) compound has an inhibitory effect against all three test bacteria, but the results are deferring according to deferent concentrations. In general the activity increased with increased T level. The best results are obtained at 50µg/mL concentration. (T) has high inhibitory effects against *P. aeruginosa* and *S. aureus* at 50µg/mL; the results were 35 and 36 mm respectively, but it has moderate effect against *E. coli* even at the higher concentration, the result, only 18 mm. At the lower concentration 0.25µg/mL only *P. aeruginosa* is affected by the test substance, then at 0.5µg/mL *S. aureus* exert some sensitivity to (T) substance, while *E coli* not affected at level 20µg/mL. Initial inhibition appeared when the concentration of the material reached to 30µg/mL.

***refers to MIC**

Resistance to actually available medicines is gradually reducing their value in treatment of bacterial infections, therefore to solve that problem novel therapeutics be discovered, that control novel target through identification of many genes which encoding for unique protein, such proteins can be a novel targets for a novel drug[21]. It was found that the (T) selena-diazole derivative most effective against Gram negative *P. aeruginosa* and Gram positive, *S. aureus*, even in a very low concentration 0.5µg/mL. But it had a moderate effect against E.coli. Similarly [22] It was found that the (T) selenadiazole derivative most effective against Gram negative *P. aeruginosa* and Gram positive, *S. aureus*, even in a very low concentration (0.25, and 0.5µg/mL). But it had a moderate effect against E.coli. Similarly [22]found that two of "2,6 disubstituted piperidine 4 one derivatives" demonstrated highly antibacterial action at 200µg/mL against *S aureus*, and *B subtillis*. The third studied compound is highly effective at 100µg/mL against *E coli*.

There are some studies that suggest that some selena-diazole derivatives have anti-bacterial effect. Research finding by [7] points that the selena-diazole derivative strictly effective against Gram positive bacteria (Sarcina sp.) and (Staphylococcus sp.) In contrast to the results in the present study [23] reported that antibacterial activity of Thiadiazole [1,2,3] selena-diazole and Thiadiazoline derivative compounds against *P aeruginosa, S aureus, E coli* and Enterococcus faecium, the preliminary screening results indicated that the total activity summaries were moderate or poor. Findings of the current study contradict the study by [15], in which the author examined antibacterial data revealed that the 1,2,3-selena-diazoles derivatives activity against Gram-positive bacteria was in contrast to the good antibacterial activity of norfloxacin against both Gram-positive and Gram negative bacteria. This difference may be due to the position and nature of the substituent stimuluses the extent of antibacterial action.

Similar results obtained by Narajji, Karvekar et al agreed with our results, the found that selenoxanthenes and selenopyrylium salts have antibacterial activity have potent action with respect to *S. aureus*, rather than *E. coli* [4]. However, interestingly, this is contrary to a study conducted by[24], antimicrobial action of " pyrazolo[3,4-b] pyridines and their spiro-heterocyclic derivatives" was tested against Gram positive strains as *S aureus, B cereus*, and *E coli , P aeruginosa*, as Gram -negative bacteria, the method used was "the ager well-diffusion", results documented that selena-diazole derivative had no antibacterial activity.

Recently many metallic nanoparticles used for their antimicrobial effect, a study by [25] *E coli, S. aureus*, Methicillinresistance *S aureus*, and *P aeruginosa* used for synthesis of selenium nanoparticles (90–150 nm/ diameter), with a good antibacterial action against both Gram negative and Gram positive bacteria including *S aureus,* Methicillinresistance *S aureus*, and *P aeruginosa* . Synthesize selenium nanoparticles mainly by microorganism enzymes such as selenite reductases from natural and synthesized Se containing compounds. Thus, biogenic Se nanoparticles represent a viable method to inhibit bacteria evolution overcoming the problems of synthetic methods that utilize toxic chemicals, therefore they are low toxic for animals and human besides resistance may not be developed as for antibiotics. Such results may explain the potent antibacterial even with low concentrations.

TABLE3:- Sensitivity of pathogenic microbes to the new synthetic (T) compounds using the filter paper disk method

T conc. µg/mL	Inhibition zone of T compound(Mean mm ±SD) dissolved in 5mL(DMSO): 95mL(DW) against bacterial and fungal pathogens							
Pathogens	**0.25**	**0.5**	**1**	**10**	**20**	**30**	**40**	**50**
E. coli	**0**	**0**	**0**	**0**	**0**	**9±2.12***	**12± 2.1**	**18±2.11**
P.aeruginosa	**10±2.11***	**15±2.12**	**18±2.12**	**20±2.1**	**22±2**	**12±2.12**	**32±2.01**	**35±2. 2**
S. aureus	**0**	**10±2.11***	**16±2.1**	**17±2.1**	**21± 2.11**	**18±2.11**	**27±2.1**	**36±2.11**
C. paras	**8±3.46***	**12±6.0**	**15±7.37**	**20±9.52**	**23±11**	**27±9.12**	**31±8.89**	**34±9.14**
C. albicans	**6±3.4***	**12±6.0**	**12±7.4**	**16±9.5**	**21±11**	**25±9.12**	**30±14.9**	**35±9.14**
C. krusi	**6± 3.5***	**12±6.0**	**16± 7.37**	**20±9.5**	**22± 11**	**25±9.12**	**28±8.14**	**31±9.14**

Antifungal activity

Antifungal effect of (T) compound in dimethyl sulfoxide (DMSO) is active against all the tested candida, the inhibition zone diameter increased directly with T concentration in comparison with control group (DW: DMSO, 95:5) which has no antifungal action. The existing data in table (3) indicated that synthetic (T) compound shows potential as novel antimicrobial agents. It was clear that even at very low concentration 0.25 µg/mL T has antifungal activity. Several selena-diazoles, and derivatives have been studied for their antimicrobial activity, many of them demonstrate potent antifungal activity. The Introduction of a 1,2,3- selena-diazole ring to particles of well-known bioactive compounds alters their actions and may leads to an enhance their biological effects[21].

Accordingly, the results of the present study illustrate good antifungal activity against *Candida, this results agreed with* Parveen, Mehdi who s fount that [2, 3 d] friedelin-3-selena-diazole The compound 3 showed high activity against *S.albus* (14) and *C. albicans* (12 mm) but moderately [26] active against fungus *A. niger* (10 mm). In contrast to the results in the present study Abdelmohsen reported that the one of synthetic selena-diazole showed only an excellent antifungal activity (›100% growth inhibition against *C. albicans and A. flavus*)[24].

It can be conclude that T less toxic than natural organic selenium compounds and has a good antibacterial and antifungal activity.

References

1. Thennarasu, S. and T.P. Perumal, *An Efficient Preparation of 1,2-Diamino-1-phenylheptane.* Molecules, 2002. **7**(6). doi: 10.3390/70600487

2. Gopalakrishnan, M., et al., *Design, synthesis, characterization, antibacterial and antifungal activities of a novel class of 5,7-diaryl-4,4-dimethyl-4,5,6,7-tetrahydropyridino[3,4-d]-1,2,3-selenadiazoles.* J Enzyme Inhib Med Chem, 2008. **23**(3): p. 347-51. https://doi.org/10.1080/14756360701611498 3. Zarczyñska, K., et al., *Effects of selenium on animal health.* J.Elem.s.,2012(2/2013):p.329–340. DOI: 10.5601/jelem.2013.18.2.12dr wet.

3. Narajji, C., M.D. Karvekar, and A.K. Das, *Biological importance of organoselenium compounds.* Indian J of pharmaceutical sciences, 2007. **69**(3): p. 344-351. DOI: 10.4103/0250-474X.34541

4. Shaban, E., et al., *Synthesis and Antibacterial Activity of Some New Azo Disperse Dyes Containing Selenium and their Application in Polyester Printing.* Egyptian Journal of Chemistry, 2017. **60**(Conference Issue (The 8th International Conference of The Textile Research Division (ICTRD 2017), National Research Centre, Cairo 12622, Egypt.)): p. 73-78. DOI: 10.21608/ejchem.2017.1561.1121

5. Naicker, L., et al., *Antimicrobial and antioxidant activities of piperidine derivatives.* African Journal of Pharmacy and Pharmacology, 2015. **9**(31): p. 783-792. DOI: 10.5897/AJPP2015. 4335

6. Sadek, M.M.E., et al., *1,3,4-oxadiazole and selenadiazole derivatives as new C-Glycosyl analogs with MAO-B, antibacterial and antifungal activities.* International Research Journal of Microbiology, 2013. **4**(9): p. 204-219. http:/dx.doi.org/10.14303/irjm.2013.038

7. Ruberte, A.C., et al., *Novel selenadiazole derivatives as selective antitumor and radical scavenging agents.* Eur J Med Chem, 2018. **157**: p. 14-27. https://doi.org/10.1016/j.ejmech.2018.07.063

8. Jadhav, A.A., et al., *Solventless synthesis of new 4,5-disubstituted 1,2,3-selenadiazole derivatives and their antimicrobial studies.* Cogent Chemistry 2016. **2**(1144670). http://dx.doi.org/10.1080/23312009.2016.1144670

9. Kumar, T.S., et al., *Toxicity evaluation of ammonium sulfate to albino rat.* Asian Journal of Pharmaceutical and Clinical Research, 2017. **10**(1).

10. Raj, J., et al., *Determination of median lethal dose of combination of endosulfan and cypermethrin in wistar rat.* Toxicol Int, 2013. **20**(1): p. 1-5. http://www.ncbi.nlm.nih.gov/pubmed/23833430

11. Chandra, M., et al., *Determination of median lethal dose of Triazophos with DMSO in wistar rats.* Asian J Pharm Clin Res, 2014. **7**(4). https://doi.org/**10.22159/ajpcr.2017.v10i1.15355**

12. Ahmed, M., *Acute Toxicity (Lethal Dose $_{50}$ Calculation) of Herbal Drug Somina in Rats and Mice.* Pharmacology & Pharmacy, 2015. **06**(03): p. 185-189. DOI: 10.4236/pp.2015.63019

13. Medina Cruz, D., G. Mi, and T.J. Webster, *Synthesis and characterization of biogenic selenium nanoparticles with antimicrobial properties made by Staphylococcus aureus, methicillin-resistant Staphylococcus aureus (MRSA), Escherichia coli, and Pseudomonas aeruginosa.* J Biomed Mater Res A, 2018. DOI: 10.1002/jbm.a.36347

14. Balasankar, T., M. Gopalakrishnan, and S. Nagarajan, *Synthesis and antibacterial activity of some 5-(4-biphenylyl)-7-aryl [3,4-d]-1,2,3-benzoselenadiazoles.* J Enzyme Inhib Med Chem, 2007. **22**(2): p. 171-5. http://dx.doi.org/10.1080/14756360601051365

15. Mhaidat, N.M., et al., *Synthesis, antimicrobial and in vitro antitumor activities of a series of 1,2,3-thiadiazole and 1,2,3-selenadiazole derivatives.* Drug Design, Development and Therapy, 2015. **9**: p. 3645-3652. https://doi.org/10.2147/DDDT.S86054

16. Sharma, V., R.C. Agrawal, and V.K. Shrivastava2, *Assessment of median lethal dose and anti-mutagenic effects of Glycyrrhiza glabra root extract against chemically induced micronucleus formation in Swiss albino mice.* International Journal of Basic & Clinical Pharmacology, 2014. **3**(2): p. 292. https://doi.org /10.2147/DDDT.S86054

17. Garousi, F., *The toxicity of different*

selenium forms and compounds – Review. AGRáRTUDOMányI KöZLEMényEK, 2015. **64**: p. 33-38. https://www.researchgate.net/publication/313869821

18. Yamashita, Y. and M. Yamashita, *Identification of a novel selenium-containing compound, selenoneine, as the predominant chemical form of organic selenium in the blood of bluefin tuna.* J Biol Chem, 2010. **285**(24): p. 18134-8.DOI:10.1074/jbc.C110.106377

19. Arsenyan, P., et al., *4-Methyl-1,2,3-selenadiazole-5-carboxylic acid amides: antitumor action and cytotoxic effect correlation.* Eur J Med Chem, 2007. **42**(5): p. 635-40. https://doi.org/10.1016/j.ejmech.2006.12.004

20. Al-Smadi, M. and F. Al-Momani, *Synthesis, Characterization and Antimicrobial Activity of New 1,2,3-Selenadiazoles.* Molecules, 2008. **13**(11): p. 2740-9. https://doi.org/10.3390/molecules13112740

21. Perumal, P., V.P. Pandey, and Y.L. Sarayu, *Antimicrobial activity of 2, 6 di-substituted piperidine 4-one derivatives.* International Journal of Pharmacy and Pharmaceutical Sciences, 2014. **6**(1). https://innovareacademics.in/journal/ijpps/Vol6Issue1/8435.pdf

22. Pawar, M.J., A.B. Burungaleb, and B.K. Karale, *Synthesis and antimicrobial activity of spiro[chromeno[4,3-d][1,2,3]thiadiazole-4,1'-cyclohexane, spiro[chromeno[4,3-d][1,2,3]selenadiazole-4,1'-cyclohexane and spiro[chroman-2,1'-cyclohexan]-4-one-5-spiro-4-acetyl-2-(acetylamino)-Δ2-1,3,4-thiadiazoline compounds.* ARKIVOC, 2009. **xiii**: p. 97-107. http://dx.doi.org/10.3998/ark.5550190.0010.d08

23. Emary, T.I.E. and S.A. Abdelmohsen, *Synthesis, characterization and antimicrobial activity of novel pyrazolo[3,4-b]pyridines and their spiro-heterocyclic derivatives.* Journal of Advances in Chemistry, 2014. **10**(7): p. 2901-2915. https://www.iasj.net/iasj?func=article&aId=98792

24. Cruz, D.M., G. Mi, and T.J. Webster, *Synthesis and characterization of biogenic selenium nanoparticles with antimicrobial properties made by Staphylococcus aureus, methicillin-resistant Staphylococcus aureus (MRSA), Escherichia coli, and Pseudomonas aeruginosa.* J Biomed Mater Res Part A, 2018. **00A**(00). DOI: 10.1002/jbm.a.36347

25. Parveen, M., et al., *Synthesis, Characterization And Antimicrobial Activity Of Friedelin [2, 3-D] Selenadiazole.* Indo. J. Chem., 2009. **9**(2): p. 285-288. *https://jurnal.ugm.ac.id/ijc/article/view/21554*