P- ISSN 1991-8941 E-ISSN 2706-6703
2024,(18), (01):165 – 179

Journal of University of Anbar for Pure Science (JUAPS)

Open Access

Preparation, and Molecular Docking Study of Some New Tris-Hetero Cyclic Compounds(Pyridyl, 1, 3, 4-Oxadiazole, and 1,3-Oxazepine) as Possible Antimicrobial Agent

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ARTICLE INFO

Received: 08 / 10 /2023
Accepted: 04 / 11 / 2023
Available online: 19 / 06 /2024

DOI: 10.37652/juaps.2023.143811.1146

Keywords:
Immune system, Probiotics, Interleukin, Optical density, Wistar Albino rats.

ABSTRACT

The study describes synthesis of compounds with a tris heterocyclic rings of (Pyridyl, 1, 3, 4-Oxadiazole, and 1,3-Oxazepine) derived from isoniazid (INH) (V2-V23). The structures of synthesized compounds were characterized using several spectroscopic approaches, including IR, 1H-NMR, and 13C-NMR, and the results are consistent with the assigned structures. Compounds (V1-V23) were examined for antibacterial activity in vitro, and the findings showed that some compounds are more active than conventional medicines. Molecular docking was used to investigate the inhibitory impact of synthesized compounds (V18-V23) (structure - reactivity relationship) on the activity of the *E-Coli* biotin carboxylase (PDB: ID:3jzf) and some compounds (V1-V17) on the activity of *S-aureus* biotin protein ligase (PDB: ID:4dq2). The tested and molecular docking results indicated that the introducing 1,3,4-oxadiazol, hydrazine group, and 1,3- Oxazepine in the structure of parent bioactive drug compound (Isoniazid) enhancing the antimicrobial activity.

Finally, in vitro, antioxidant of the synthesized compounds (V1, V5, V7, V9, V13, V14, and V16-V23) were evaluated, and molecular docking of compounds (V18-V23) with human heme oxygenase-1 (PDB code: 3CZY) were studied and compared with standard natural antioxidant [ascorbic acid and 1(adamantan1yl)2(1Himidazol1yl)ethanone (Std)] respectively and the results indicated that these compounds exhibit excellent to moderate activity.

Introduction

The most extensively studied anti-tubercular drug now available is isonicotinic acid hydrazide[1]. Isoniazid has a number of less well-known properties in addition to its ability to fight tuberculosis[2], including those that are antimycobacterial[3], antibacterial[4], anti-virus[5], antimicrobial[6], antimalarial[7], antifungal[8], anticancer[9], anti-analgesic[10], anti-convulsant[11], anticorrosive, and anti-inflammatory activities[12]. The oxyadiazole nucleus can be found in a variety of drug architectures, Numerous biological properties, including those that are anti-cancer, anti-microbial, anti-tuberculosis, anti-inflammatory, anti-oxidant, anti-convulsant, and anti-HIV, have been linked to the oxadiazole nucleus[13].

Hydrazones have been the subject of extensive research by medicinal chemists all over the world, culminating in the development of drugs with higher activity and decreased toxicity profiles[14]. Emerging bacterial resistance is a widespread issue in the treatment of many illnesses. As a result, the quest for antimicrobials is a neverending endeavor[15]. A variety of hydrazone derivatives have recently been produced and tested for antibacterial activity[16,17].

Otherwise, oxazepine 1,3-Dione is a seven-member unsaturated derivative having carbonyl groups in positions 3 and 7. Oxazepines are a well-known class in medicinal chemistry in which the seven heteroatoms are cyclesized, forming a seven heterocyclic ring and the presence of a group –N – C (=O)-like protein-amide bond with inherent physiological activities[18]. This is a topic of interest by researchers broadly because of its role in biological activities including anti-

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inflammatory[19], antifungal [20], antithrombotic[21], anti-epileptic[22], anti-convulsant[23], progesterone agonist[18], antagonist and analgesic[24].

Antioxidants are chemicals that protect your body from free radical damage. Compounds known as free radicals are substances that might harm your body if their quantities get too high. They are connected to a number of diseases, such as diabetes, heart disease, and cancer. Our body contains built-in antioxidant defenses that control free radicals[25].

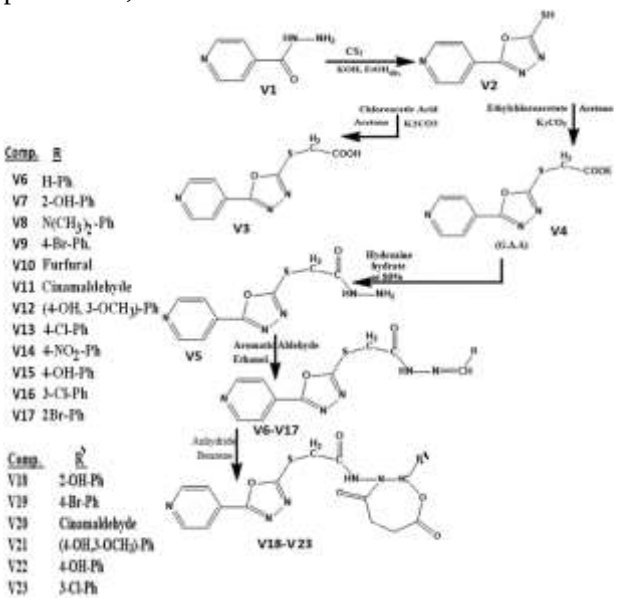
Finally, Molecular docking is a structure-based drug design approach that predicts the binding mechanism and affinity between receptors and ligands by simulating molecular interactions. Using the compounds database to screen possible pharmacophores not only makes it easier for researchers to acquire, synthesize, and conduct follow-up pharmacological testing, but it also increases efficiency and lowers research costs. Furthermore, the introduction of reverse molecular docking technology has the potential to considerably increase drug target prediction ability and understanding of the relevant molecular process for drug design[26].

According to our literature review, the synthesis of tris heterocyclic compounds (Pyridyl, 1, 3, 4-Oxadiazole, and 1,3- Oxazepine) are rare. As a result, our project aimed to prepare a series of substituted 1,3-oxazepine using isoniazid as the backbone for synthesized molecules and test their antibacterial activity against *(S. aureus* and *E-Coli*), and antioxidant activity.

Materials and Methods (for Heading, use Times New Roman 11 bold)

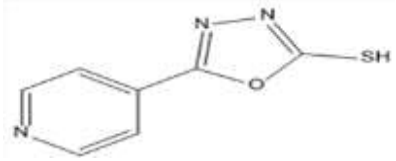
Isoniazid (isonicotinoyl hydrazide) was obtained from MEDIVER LTD, Kemp House, 160 City Roud London EC1V 2NX, United Kingdom. There have been no further improvements to the compounds; they were all acquired and obtained from Sigma-Aldrich, Merck, Aladdin, and Alfa aesar. Thin layer chromatography (TLC) was used to monitor the course of reactions on silica gel pre-coated metal sheets. The melting points were calculated using an uncorrected Stuart SMP10. IR spectra (KBr disc) were measured at the University of Sallahadin Erbil/Iraq- College of Science utilizing a SHIMADZU, Co., Germany spectrometer (4000-600 cm^{-1}). Spectra of ^{1}HNMR and ^{13}CNMR for compounds generated in the labs of Sanati Sharif University/Tehran/Iran were recorded in DMSO-d6 using a Bruker Ultershield 500MHz NMR spectrometer, Co., Germany.

Synthetic procedures: To synthesize intermediates and end products, the following procedures were performed, as illustrated in Scheme 1.



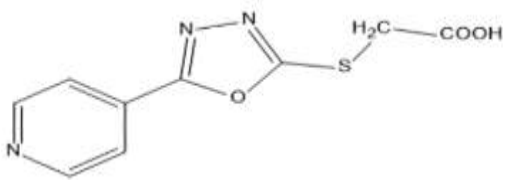
Scheme 1: Synthesis of the target compounds (V2-V23)

Synthesis of 5-(pyridine-4-yl)-1,3,4-oxadiazole-2-thiol(V2): Isoniazid (0.01mol, 1.371gm) was dissolved in 100ml ethanol then potassium hydroxide and Carbon Disulfide were added in equimolar amounts to this solution. After that, the mixture was refluxed at 78°C for 24 hr. Distilled water was then added, followed by neutralization with dilute HCl(1N). A solid mass appeared and was filtered and recrystallized from methanol to give bright yellow crystals. The yellow crystal, yield= 95%, M.P.= 273-275°C[27].



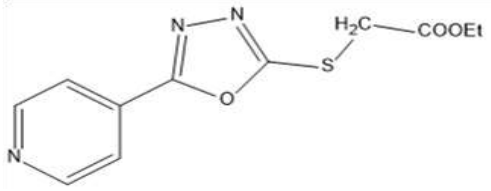
V2

Synthesis of 2-((5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl)thio)acetic acid(V3): The 5-pyridin-4-yl-1,3,4-oxadiazole-2-thiol (0.01 mole, 1.79 gm), (0.01 mole, 0.40 gm) NaOH in ethanol (40 mL) was refluxed for 1 h. Ethyl-chloroacetate (0.01 mol, 0.947 gm) was added to this solution, and the mixture refluxed for 4 hour. After a slow cooling process, the solution was poured into ice and recrystallization was performed with a mixture of ethanol and water(4:1). Yield: % 62, m.p: >375 ^{0} C (25).



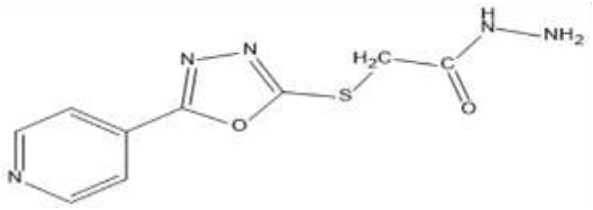
V3

Synthesis of ethyl 2-((5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl)thio)acetate(V4): Refluxing (V2)(0.0335mol, 6gm) with ethyl chloroacetate (0.0335mol, 4.104gm) in the presence of K_2CO_3(0.0335mol, 4.625gm) in acetone generated (V4), the precipitate filtered and washed with distilled water, drying and taking melting point 90-91^{0}c with 90% yield[28].



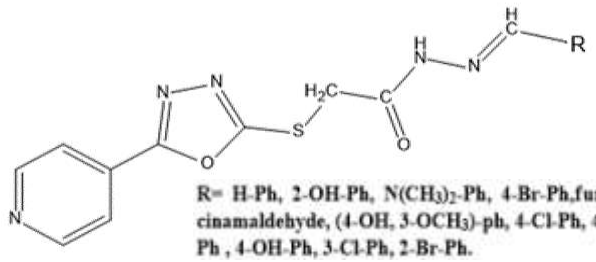
V4

Synthesis of 2- ((5-(pyridine-4-yl)- 1,3,4-oxadiazol - 2-yl) thio) acetohydrazide(V5): compound (V4) (0.04mol, 10.6gm) and hydrazine hydrate at 80%(20ml) was added to ethanol ,stirred for 2hrs at room temperature, and stirred under ice bath for 12hrs. the formed solid product was filtered, dried then crystallized from ethanol to give (V5) and taking m.p. 176–178°C with 70% yield[28].



V5

General Procedure for the synthesis of (Z/E) N′-Arylidene-2- [5-(pyridine-4-yl)-1,3,4- oxadiazol-2-ylsulphanyl]acetohydrazides (V6–V17) : A solution of equimolar amounts of V5 (0.001 mol, 0.25g) and the appropriate aromatic aldehyde (0.001 mol) in absolute ethanol (15 mL) and 2-3 drops of glacial acetic acid was heated under reflux for 3 h. After cooling, the formed solid product was filtered, dried then crystallized from ethanol to give(V6–V17) [29].As shown in table(1).



(V6-V17)

Table (1): Physical properties of acyl hydrazone derivatives (V6–V17)

Comp.	Molecular formula	Molecular weight	Description	M.p. (^{o}C)
V6	$C_{16}H_{12}O_2N_5S$	340	White powder	201–202
V7	$C_{16}H_{13}O_3N_5S$	356	White powder	191–192
V8	$C_{18}H_{18}O_2N_6S$	383	Yellow powder	270-272
V9	$C_{16}H_{12}O_2N_5SBr$	417	White powder	196–198
V10	$C_{14}H_{11}O_3N_5S$	330	White powder	195-196
V11	$C_{18}H_{15}O_2N_5S$	366	Yellow powder	240-242
V12	$C_{17}H_{15}O_4N_5S$	386	Orange powder	195–196
V13	$C_{16}H_{12}O_2N_5SCl$	375.5	White powder	203–205
V14	$C_{16}H_{12}O_4N_6S$	386	White powder	216–217
V15	$C_{16}H_{13}O_3N_5S$	357	White powder	221–222
V16	$C_{16}H_{12}O_2N_5SCl$	375.5	White powder	190-191
V17	$C_{16}H_{12}O_2N_5SBr$	417	White powder	186-188

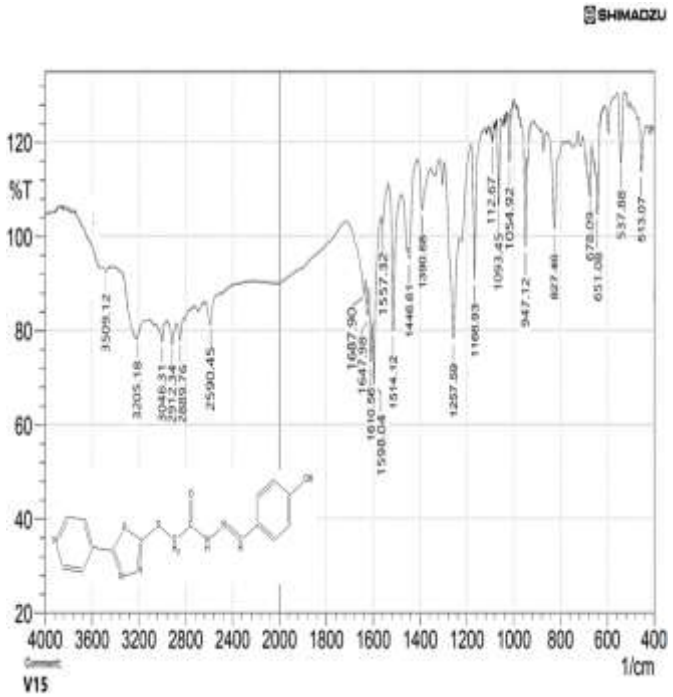


Figure 1: FT-IR spectrum of (V15) compound

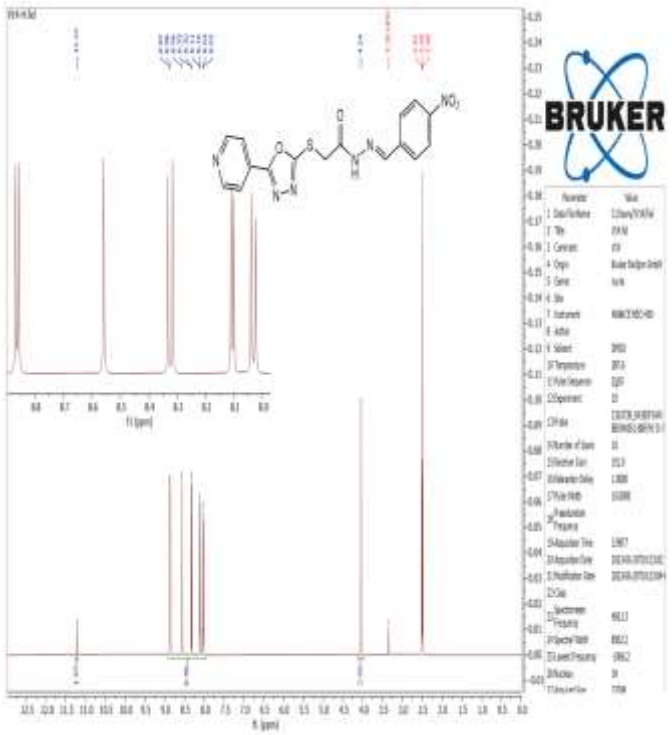


Figure 2: 1H-NMR spectrum of (V14) compound

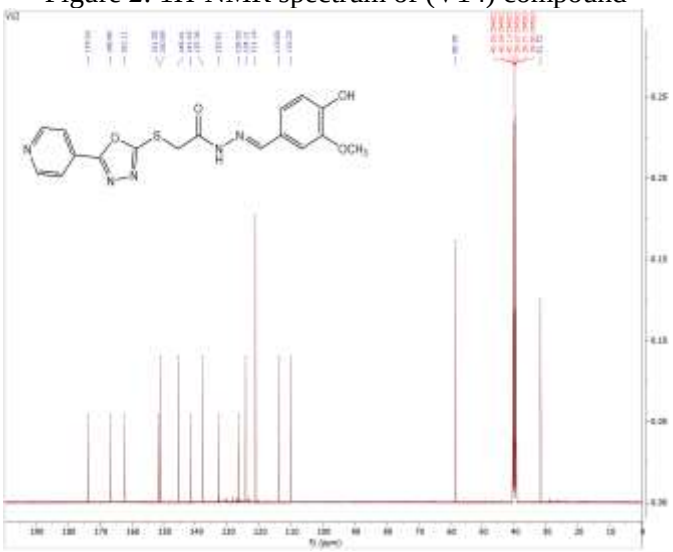


Figure 3: 13C-NMR spectrum of (V12) compound

(E)-N'-benzylidene-2-((5-(pyridine-4-yl)-1,3,4-oxadiazol-2-yl) thio) acetohydrazide(V6) ^{1}H-NMR (500 MHz, [D6] DMSO): δ = 4.05 (s, 2 H, -S-C$\underline{H}_2$-), 7.43-8.73 (m, 9 H, Aro. Protons), 8.24 (s, 1 H, -N=C$\underline{H}$-Ar), 11.15 (s, 1 H, -N$\underline{H}$-C=O) ppm. ^{13}C NMR (125 MHz) δ = ppm. 30.53(-S-$\underline{C}H_2$-), 121.14-165.65 (Aro. Carbons), 146.40 (-N=$\underline{C}$H-Ar), 173.97 (-$\underline{C}$=O). IR (KBr), v_{max}/cm^{-1} = 3212.90 (νN–H); 1687.45(νC = O); 1599.59 (νC = N); 1573.91, 1492.90, (νC = C); 1211.30 (νC-O); 956.69 (νN-N).

(E)-N'-(2-hydroxybenzylidene)-2-((5-(pyridine-4-yl)-1,3,4-oxadiazol-2-yl)thio)acetohydr- azide (V7) ^{1}H NMR (500 MHz, [D6] DMSO): δ = 4.25 (s, 2 H, -S-C$\underline{H}_2$-), 6.92-8.86 (d,d, 8 H, Aro. Protons), 8.77 (s, 1 H, -N=C$\underline{H}$-Ar), 11.22 (s, 1 H, -O-H), 11.27 (s, 1 H, -NH-C=O) ppm. ^{13}C NMR (125 MHz) δ = 29.75(-S-$\underline{C}H_2$-), 115.74-167.86 (Aro. Carbons), 147.95 (-N=$\underline{C}$H-Ar), 174.01 (-$\underline{C}$=O) ppm. IR (KBr), v_{max}/cm^{-1} = 3279.13(νN–H); 1671.09 (νC = O); 1597.19 (νC = N); 1572.95, 1487.12(νC = C);1271.09 (νC-O); 970.19(N-N).

(E)-N'-(4-(dimethylamino)benzylidene)-2-((5-(pyridine-4-yl)-1,3,4-oxadiazol-2-yl)thio) -acetohydra- zide (V8) ^{1}H NMR (500 MHz, [D6] DMSO): δ = 3.13 (s, 6H, -N(C$\underline{H}$3)), 4.16 (s, 2 H, -S-C$\underline{H}_2$-), 6.89-8.83 (d,d, 8 H, Aro. Protons), 8.54 (s, 1 H, -N=C$\underline{H}$-Ar), 11.13 (s, 1 H, -NH-C=O) ppm. ^{13}C NMR (125 MHz) δ =36.54 (-N($\underline{C}H_3$)$_2$), 33.45(-S-$\underline{C}H_2$-), 113.65-164.39 (Aro. Carbons), 145.33 (-N=$\underline{C}$H-Ar), 172.35(-$\underline{C}$=O) ppm. IR (KBr), v_{max}/cm^{-1} = 3211.02 (νN–H); 1672.88 (νC = O); 1561.23 (νC = N); 1521.84, 1458.09(νC = C); 1226.73 (νC-O); 945.78 (νN-N).

(E)-N'-(4-bromobenzylidene)-2-((5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl)thio)acetohydraz- ide (V9) ^{1}H NMR (500 MHz, [D6] DMSO): δ = 4.25 (s, 2 H, -S-C$\underline{H}_2$-), 7.55-8.86 (d,d, 8 H, Aro. Protons), 8.58 (s, 1 H, -N=C$\underline{H}$-Ar), 11.16 (s, 1 H, -NH-C=O) ppm. ^{13}C NMR (125 MHz) δ = 31.25(-S-$\underline{C}H_2$-), 121.14-164.95 (Aro. Carbons), 148.16 (-N=$\underline{C}$H-Ar), 171.80 (-$\underline{C}$=O) ppm. IR (KBr), v_{max}/cm^{-1} = 3197.98 (νN–H); 1698.90 (νC = O); 1591.27 (ν C = N); 1552.70, 1487.12, (νC = C); 1294.24 (ν C-O); 960.55 (ν N-N).

(E)-N'-(furan-2-ylmethylene)-2-((5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl)thio)acetohydraz- ide(V10) ^{1}H NMR (500 MHz, [D6] DMSO): δ = 4.26 (s, 2 H, -S-C$\underline{H}_2$-), 6.69-8.84 (m, 8 H, Aro. Protons), 8.55 (s, 1 H, -N=C$\underline{H}$-Ar), 11.25 (s, 1 H, -NH-C=O) ppm. ^{13}C NMR (125 MHz) δ = 30.30 (-S-$\underline{C}H_2$-), 116.88-165.89 (Aro. Carbons), 150.32 (-N=$\underline{C}$H-Ar), 171.11 (-$\underline{C}$=O) ppm. IR (KBr), v_{max}/cm^{-1} = 3210.04 (νN–H); 1694.03 (νC = O); 1554.08 (νC = N); 1522.01, 1503.09, (νC = C); 1226.73 (νC-O); 947.05 (νN-N).

(E)-N'-((E)-3-phenylallylidene)-2-((5-(pyridine-4-yl)-1,3,4-oxadiazol-2-yl)thio)acetohyd- razide (V11)^{1}H NMR (500 MHz, [D6] DMSO): δ = 3.34 (s, 2 H, -S-C$\underline{H}_2$-), 6.95-6.98(t, 1H, -C$\underline{H}$=CH-),7.30-7.33(d, 1H, -CH=C$\underline{H}$-) 7.40-8.82 (m, 9H, Aro. Protons), 7.99-8.00 (d, 2H, -N=C$\underline{H}$-Ar), 10.67 (s, 1 H, -NH-C=O) ppm. ^{13}C

NMR (125 MHz) δ = 34.24(-S-CH$_2$-), 121.14-163.74 (Aro. Carbons), 136.23 (-CH=CH-Ar), 128.93 (-CH=CH-Ar), 150.89 (-N=CH-Ar), 171.56 (-C=O) ppm. IR (KBr), v_{max}/cm^{-1} = 3201.09 (νN–H); 1692.78 (νC = O); 1597.23 (νC = N); 1576.44, 1484.32, (νC = C); 1301.80 (νC-O); 975.98 (νN-N).

(E)-N'-(4-hydroxy-3-methoxybenzylidene)-2-((5-(pyridine-4-yl)-1,3,4-oxadiazol-2-yl)thi- o)acetohyd-razide (V12):^{1}H NMR (500 MHz, [D6] DMSO): δ = 4.17 (s, 3H, -O-CH$_2$-), 4.36 (s, 2H, -S-CH$_2$-), 6.95-8.86 (d,d, 7H, Aro. Protons), 8.53 (s, 1 H, -N=CH-Ar), 9.85 (s, 1H, -O-H), 11.19 (s, 1H, -NH-C=O) ppm. ^{13}C NMR (125 MHz) δ = 33.45 (-S-CH$_2$-), 58.35 (-O-CH$_3$), 110.23-166.68 (Aro. Carbons), 145.41 (-N=CH-Ar), 173.53 (-C=O) ppm. IR (KBr), v_{max}/cm^{-1} = 3219.67 (νN–H); 1689.24 (νC = O); 1564.87 (νC = N); 1534.01, 1488.34, (νC = C); 1278.34 (νC-O); 971.34 (νN-N).

(E)-N'-(4-chlorobenzylidene)-2-((5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl)thio)acetohydraz- ide(V13): ^{1}H NMR (500 MHz, [D6] DMSO): δ = 4.04 (s, 2 H, -S-CH$_2$-), 7.59-8.86 (d,d, 8 H, Aro. Protons), 8.57 (s, 1 H, -N=CH-Ar), 11.19 (s, 1 H, -NH-C=O) ppm. ^{13}C NMR (125 MHz) δ = 29.51 (-S-CH$_2$-), 121.14-165.89 (Aro. Carbons), 145.25 (-N=CH-Ar), 171.09 (-C=O) ppm. IR (KBr), v_{max}/cm^{-1} = 3199.98 (νN–H); 1689.45 (νC = O); 1597.05(νC = N); 1575.84, 1489.05(νC = C); 1294.24 (νC-O); 1012.63 (νN-N).

(E)-N'-(4-nitrobenzylidene)-2-((5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl)thio)acetohydrazid- e (V14):^{1}H NMR (500 MHz, [D6] DMSO): δ = 4.04 (s, 2 H, -S-CH$_2$-), 8.02-8.87(d,d, 8 H, Aro. Protons), 8.56 (s, 1 H, -N=CH-Ar), 11.22 (s, 1 H, -NH-C=O) ppm. ^{13}C NMR (125 MHz) δ = 32.51 (-S-CH$_2$-), 121.14-163.74(Aro. Carbons), 146.90 (-N=CH-Ar), 173.53 (-C=O) ppm. IR (KBr), v_{max}/cm^{-1} = 3174.10 (νN–H); 1662.84 (νC = O); 1596.06 (νC = N); 1521.84, 1498.34 (νC = C); 1256.93 (νC-O); 920.05 (νN-N).

(E)-N'-(4-hydroxybenzylidene)-2-((5-(pyridine-4-yl)-1,3,4-oxadiazol-2-yl)thio)acetohyd- razide (V15):^{1}H NMR (500 MHz, [D6] DMSO): δ = 4.10 (s, 2 H, -S-CH$_2$-), 6.97-8.78 (d,d, 8 H, Aro. Protons), 8.53(s, 1 H, -N=CH-Ar), 9.86 (s, 1 H, -O-H), 11.14 (s, 1 H, -NH-C=O) ppm. ^{13}C NMR (125 MHz) δ = 33.22 (-S-CH$_2$-), 115.01-166.13 (Aro. Carbons), 145.29 (-N=CH-Ar), 170.85 (-C=O) ppm. IR (KBr), v_{max}/cm^{-1} = 3205.18 (νN–H); 1687.90 (νC = O); 1598.04 (νC = N); 1557.32, 1446.61 (νC = C); 1257.59 (νC-O); 947.12 (νN-N).

(E)-N'-(3-chlorobenzylidene)-2-((5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl)thio)acetohydraz -ide (V16):^{1}H NMR (500 MHz, [D6] DMSO): δ = 4.45 (s, 2 H, -S-CH$_2$-), 7.52-8.73 (d,d, 8 H, Aro. Protons), 8.40 (s, 1 H, -N=CH-Ar), 11.19 (s, 1 H, -NH-C=O) ppm. ^{13}C NMR (125 MHz) δ = 33.69 (-S-CH$_2$-), 119.33-165.42 (Aro. Carbons), 143.99 (-N=CH-Ar), 171.80 (-C=O) ppm. IR (KBr), v_{max}/cm^{-1} = 3256.98 (νN–H); 1697.36 (νC = O); 1599.34 (νC = N); 1575.84, 1489. 05, (νC = C); 1263.37 (νC-O); 969.58 (νN-N).

(E)-N'-(2-bromobenzylidene)-2-((5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl)thio)acetohydraz- ide(V17): ^{1}H-NMR (500 MHz, [D6] DMSO): δ = 4.10 (s, 2 H, -S-CH$_2$-), 7.44-8.73 (d,d, 8 H, Aro. Protons), 8.35 (s, 1 H, -N=CH-Ar), 11.15 (s, 1 H, -NH-C=O) ppm. ^{13}C NMR (125 MHz) δ = 33.22 (-S-CH$_2$-), 121.14-159.75 (Aro. Carbons), 148.64 (-N=CH-Ar), 171.80 (-C=O) ppm. IR (KBr), v_{max}/cm^{-1} = 3248.90 (νN–H); 1668.43 (νC = O); 1589.34(νC = N); 1558.48, 1463.97, (νC = C); 1211.30 (νC-O); 1024.20 (νN-N).

General procedure for the synthesis of (Z/E) N′-(4,7-dioxo-2- Arylidene-1,3-oxazepin-3(2H,4H,7H)-yl)-2-((5-(pyridin-4-yl) -1,3,4- oxadiazol-2-yl) thio) acetamide (V18-V23): appropriate hydrazone compounds (0.0016 mol) dissolved in 15 ml of dried benzene. Was mixed with (0.0016 mole, o.153 gm) of appropriate anhydride (succinic anhydride) and charged into the round bottom flask. Then, stirred until homogeneous. The mixture was refluxed for 5 h. afterwards; the mixture was allowed to cool at room temperature. The crystals formed were filtered, washed it with distilled water. The resulting solid was recrystallized with ethanol[30], as shown in table (2).

Table (2): Physical properties of 1,3- Oxazepine (V18–V23)

Comp.	Molecular formula	Molecular wt.	Description	M.P.(°C)
V18	$C_{20}H_{16}O_6N_5S$	455	Light yellow	142-144
V19	$C_{20}H_{16}O_5N_5SBr$	516	White	161-162
V20	$C_{22}H_{19}O_5N_5S$	465	White	157-159
V21	$C_{21}H_{18}O_7N_5S$	485	Fire orange	151-153
V22	$C_{20}H_{16}O_6N_5S$	485	Yellowish orange	162-164
V23	$C_{20}H_{16}O_5N_5SCl$	485	White	155-157

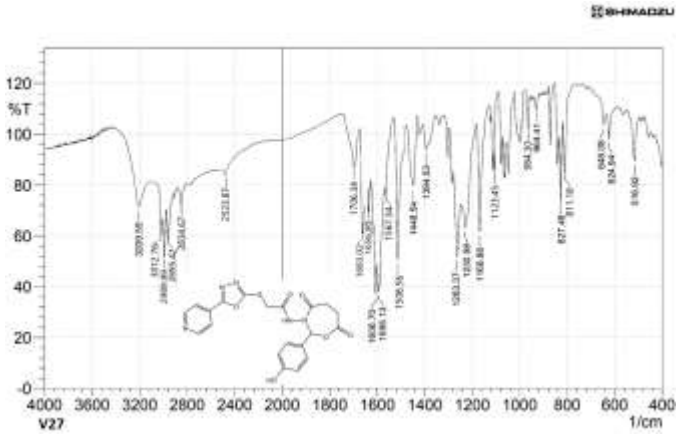


Figure 4: FT-IR spectrum of (V22) compound

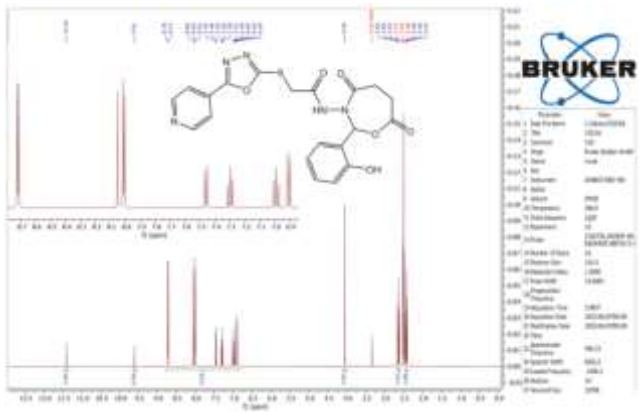


Figure 5: 1H-NMR spectrum of (V18) compound

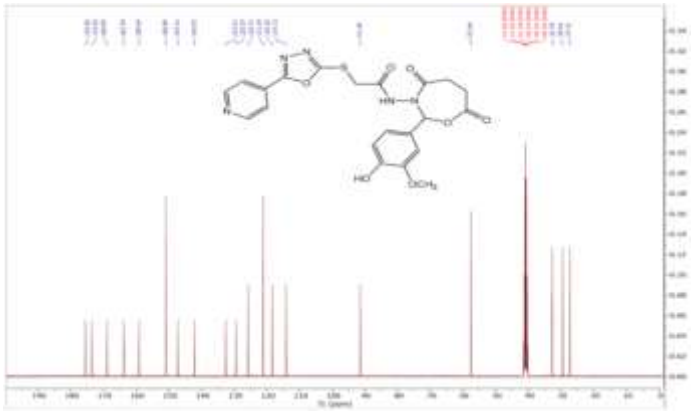


Figure 6: 13C-NMR spectrum of (V21) compound

N-(2-(2-hydroxyphenyl)-4,7-dioxo-1,3-oxazepan-3-yl)-2-((5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl)thio)aceta mide (V18): ^{1}H NMR (500 MHz, [D6] DMSO): δ =2.41-2.45(t, 2 H, -N(CO)-C<u>H</u>$_2$-), 2.62-2.66(t, 2 H, -O(CO)-C<u>H</u>$_2$) 4.05 (s, 2 H, -S-C<u>H</u>$_2$-), 6.90-8.73 (m, 8 H, Aro. Protons), 8.05 (s, 1 H, -N-C<u>H</u>-O $_{Oxazpin}$), 9.61 (s, 1 H, -O-<u>H</u>), 11.41 (s, 1 H, -N<u>H</u>-C=O) ppm. ^{13}C NMR (125 MHz) δ = 25.57 (-N(C=O) –<u>C</u>H$_2$-), 27.07 (-O(C=O) -<u>C</u>H$_2$-) , 31.72 (-S-<u>C</u>H$_2$-), 91.20 (N-<u>C</u>H-O $_{Oxazpin}$), 118.07-163.74 (Carbon. Protons), 168.10 -N(<u>C</u>=O), 172.59-O(<u>C</u>=O), 176.29 (-NH-<u>C</u>=O) ppm. IR (KBr), v_{max}/cm^{-1} = 3205 (νN–H); 1612.49 (νC = O); 1589.34(νC = N); 1558.48, 1463.97, (νC = C); 1211.30 (νC-O); 1024.20 (νN-N).

N-(2-(4-bromophenyl)-4,7-dioxo-1,3-oxazepan-3-yl)-2-((5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl)thio)aceta mide (V19):^{1}H NMR (500 MHz, [D6] DMSO): δ =2.38-2.42(t, 2 H, -N(CO)-C<u>H</u>$_2$-), 2.70-2.73(t, 2 H, -O(CO)-C<u>H</u>$_2$) 4.10 (s, 2 H, -S-C<u>H</u>$_2$-), 7.33-8.84 (d,d , 8 H, Aro. Protons), 8.11 (s, 1 H, -N-C<u>H</u>-O $_{Oxazpin}$), , 11.37 (s, 1 H, -N<u>H</u>-C=O) ppm. ^{13}C NMR (125 MHz) δ = 25.33 (-N(C=O) –<u>C</u>H$_2$-), 27.78 (-O(C=O) -<u>C</u>H$_2$-) , 31.01 (-S-<u>C</u>H$_2$-), 87.26 (N-<u>C</u>H-O $_{Oxazpin}$), 121.14-163.74 (Carbon. Protons), 167.86 -N(<u>C</u>=O), 174.01-O(<u>C</u>=O), 177.26 (-NH-<u>C</u>=O) ppm. IR (KBr), v_{max}/cm^{-1} = 3205 (νN–H); 1612.49 (νC = O); 1589.34(νC = N); 1558.48, 1463.97, (νC = C); 1211.30 (νC-O); 1024.20 (νN-N).

(E)-N-(4,7-dioxo-2-styryl-1,3-oxazepan-3-yl)-2-((5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl)thio)acetamide (V20): ^{1}H NMR (500 MHz, [D6] DMSO): δ =2.39-2.43 (t, 2 H, -NH-C=O), 2.64-2.68(t, 2 H, -O(CO)-C<u>H</u>$_2$) 4.03(s, 2 H, -S-C<u>H</u>$_2$-), 6.35-8.81 (m , 11 H, Aro. Protons&-CH=C<u>H</u>-$_{Ethlene\&\ Oxazpin}$), 7.47-7.48 (d, 1 H, -N-C<u>H</u>-O $_{Oxazpin}$), , 11.30 (s, 1 H, -N<u>H</u>-C=O) ppm. ^{13}C NMR (125 MHz) δ = 25.27 (-N(C=O) –<u>C</u>H$_2$-), 27.54 (-O(C=O) -<u>C</u>H$_2$-) , 32.27 (-S-<u>C</u>H$_2$-), 88.68 (N-<u>C</u>H-O $_{Oxazpin}$), 121.14-163.74 (Carbon. Protons), 166.13 -N(<u>C</u>=O), 172.82-O(<u>C</u>=O), 176.55 (-NH-<u>C</u>=O) ppm. IR (KBr), v_{max}/cm^{-1} = 3205 (νN–H); 1612.49 (νC = O); 1589.34(νC = N); 1558.48, 1463.97, (νC = C); 1211.30 (νC-O); 1024.20 (νN-N).

N-(2-(4-hydroxy-3-methoxyphenyl)-4,7-dioxo-1,3-oxazepan-3-yl)-2-((5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl)thio)acetamide (V21):^{1}H NMR (500 MHz, [D6] DMSO): δ =2.32-2.36(t, 2 H, -N(CO)-C<u>H</u>$_2$-), 2.70-2.73(t, 2 H, -O(CO)-C<u>H</u>$_2$), 3.98 (s, 3 H, -OC<u>H</u>$_3$-) 4.05 (s, 2 H, -S-C<u>H</u>$_2$-), 6.84-8.70 (d,d , 7 H, Aro. Protons), 8.08 (s, 1 H, -N-C<u>H</u>-O $_{Oxazpin}$), 10.07 (s, 1 H, -O-<u>H</u>), 11.34 (s, 1 H, -N<u>H</u>-C=O) ppm. ^{13}C NMR (125 MHz) δ = 27.31 (-N(C=O) –<u>C</u>H$_2$-), 29.51 (-O(C=O) -<u>C</u>H$_2$-) , 32.74 (-S-<u>C</u>H$_2$-), 57.64 (-O<u>C</u>H$_3$), 91.44 (N-<u>C</u>H-O $_{Oxazpin}$), 114.13-163.74 (Carbon. Protons), 168.89 (-N(<u>C</u>=O)), 173.53 (-O(<u>C</u>=O)), 175.50 (-NH-<u>C</u>=O) ppm.IR (KBr), v_{max}/cm^{-1} = 3205 (νN–H); 1612.49 (νC = O); 1589.34(νC = N); 1558.48, 1463.97, (νC = C); 1211.30 (νC-O); 1024.20 (νN-N).

N-(2-(4-hydroxyphenyl)-4,7-dioxo-1,3-oxazepan-3-yl)-2-((5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl)thio)aceta mide (V22): ^{1}H NMR (500 MHz, [D6] DMSO): δ =2.38-2.42(t, 2 H, -N(CO)-C$\underline{H}_2$-), 2.63-2.67(t, 2 H, -O(CO)-C$\underline{H}_2$), 4.05 (s, 2 H, -S-C$\underline{H}_2$-), 6.83-8.69 (d,d , 8 H, Aro. Protons), 8.02 (s, 1 H, -N-C$\underline{H}$-O $_{Oxazpin}$), 9.13 (s, 1 H, -O-$\underline{H}$), , 11.31 (s, 1 H, -N$\underline{H}$-C=O) ppm. ^{13}C NMR (125 MHz) δ = 28.56 (-N(C=O) –$\underline{C}$H$_2$-), 29.75 (-O(C=O) -$\underline{C}$H$_2$-) , 32.74 (-S-$\underline{C}$H$_2$-), 89.00 (N-$\underline{C}$H-O $_{Oxazpin}$), 116.00-163.74 (Carbon. Protons), 167.47 (-N($\underline{C}$=O)), 171.09 (-O($\underline{C}$=O)), 173.08 (-NH-$\underline{C}$=O) ppm. IR (KBr), v_{max}/cm^{-1} = 3205 (νN–H); 1612.49 (νC = O); 1589.34(νC = N); 1558.48, 1463.97, (νC = C); 1211.30 (νC-O); 1024.20 (νN-N).

N-(2-(3-chlorophenyl)-4,7-dioxo-1,3-oxazepan-3-yl)-2-((5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl)thio)aceta mide (V23):^{1}H NMR (500 MHz, [D6] DMSO): δ =2.41-2.44(t, 2 H, -N(CO)-C$\underline{H}_2$-), 2.62-2.65(t, 2 H, -O(CO)-C$\underline{H}_2$), 4.14 (s, 2 H, -S-C$\underline{H}_2$-), 7.21-8.77 (m , 8 H, Aro. Protons), 8.07 (s, 1 H, -N-C$\underline{H}$-O $_{Oxazpin}$), , 11.34 (s, 1 H, -N$\underline{H}$-C=O) ppm. ^{13}C NMR (125 MHz) δ = 26.04 (-N(C=O) –$\underline{C}$H$_2$-), 29.04 (-O(C=O) -$\underline{C}$H$_2$-) , 34.47 (-S-$\underline{C}$H$_2$-), 90.73 (N-$\underline{C}$H-O $_{Oxazpin}$), 121.14-163.74 (Carbon. Protons), 170.38 (-N($\underline{C}$=O)), 173.06 (-O($\underline{C}$=O)), 174.34 (-NH-$\underline{C}$=O) ppm. IR (KBr), v_{max}/cm^{-1} = 3205 (νN–H); 1612.49 (νC = O); 1589.34(νC = N); 1558.48, 1463.97, (νC = C); 1211.30 (νC-O); 1024.20 (νN-N).

Antibacterial activity

The disk diffusion technique was used to test the antibacterial activity of some synthesized compounds (V1-V23) against Escherichia coli, Gram (-) ve and Staphylococcus aureus Gram (+) ve. After being submerged in DMSO, the disks were dried in an incubator before being employed in cultures of bacteria. As a pessimistic monitor, DMSO was utilized. At 37°C, the plates were incubated for two days. For of the maximal inhibition zone diameter (IZD) of the test microorganism form was measured and computed. Using three dosages, As monitoring samples, ampicillin were employed[31].

Anti-oxidant activity

The anti-oxidant activity of produced compounds (V18–V23) is assessed in the laboratory using the free radical scavenging properties of 1,1-diphenyl-2-picrylhydrazyl (DPPH). Gallic acid was produced in a variety of concentrations (25, 50, 100, 150, 200, and 400 g/mL) and used as standard solutions. DPPH solution preparation is another step. Additionally, test sample solutions (V18–V23) were prepared. After 30 minutes, the UV-visible spectrophotometer measures the absorbance at 515 nm with methanol serving as a blank[32]. The percentage of inhibition was determined according to the equation given below.

$$DPPH\ radical\ scavenging\ activity\ \%=[ODBlank-ODSampleODBlank]\times 100$$

Molecular Docking simulation:

The patterns of organic chemical binding to active sites in bacterial proteins are clearly shown by molecular docking. From the Protein Data Bank (RCSB), the crystal structures of the proteins from Escherichia coli (3jzf), S-aureus biotin protein ligase (PDB: ID:4dqz), and human heme oxygenase-1 (PDB code: 3CZY) were retrieved. All hetero molecules were deleted from the docking experiments and the crystal structure, along with any water molecules with a radius more than 5 A^{0} and unimportant molecules. Charges were also added the produced compounds (V1-V23) had their geometry adjusted, which resulted in a drop in energy and an increase in stability. Utilizing the Auto Dock v4.2 Program, the molecular docking was completed [33].

Results and Discussion (for Heading, use Times New Roman 11 bold)

Synthesis of acyl-hydrazone derivatives (V6-V17): The creation of acyl-hydrazone compounds was initially demonstrated by changes in physical qualities such as color, and melting point. The newly produced chemicals were not the same as the initial components. The structures of the synthesized compounds were validated using available spectral techniques, where FT-IR spectroscopy, revealed the lack NH_2) group's peak at (3215.44-3302.24) cm^{-1}. In addition to changing the sharp peak of carbonyl group from frequency 1666.55 cm^{-1} to various stretching vibration frequency of the range (1698-1600) cm^{-1} of (C=N). The appearance of novel stretching vibration frequency bands of the range (3270-3160) cm^{-1} indicates the (N-H) hydrazone.

The ^{1}H-NMR spectra of acyl-hydrazone compounds (V6-V17) indicated the existence of a singlet signal at a range (10.67-11.27) ppm indicating one proton of the (-NH-N=C-). the presence of singlet signal at a range of (8.00-8.77) ppm with an integral value equal to one proton representing the proton of the azomethine group

(-N=CH-), and the singlet signal at approximately δ= (3.13-4.45) ppm represents the protons of the (-S-C$\underline{H}_2$-).

The ^{13}C-NMR spectra of acyl-hydrazone derivatives (V6-V17) revealed the following spectrum information: the presence of a signal at δ = (170.85-174.01) ppm corresponding to a carbon of the (C=O) group, the presence of a signal at = (143.99-150.89) ppm corresponding to a carbon of the (C=N) group, the aromatic carbons were resonated at = (110.23-167.86) ppm.

synthesis of (Z/E) N′-(4,7-dioxo-2- Arylidene-1,3-oxazepin-3 (2H ,4H,7H)-yl)-2-((5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl) thio)acetamide(V18-V24): The prepared oxazepines derivatives were identified (V18-V24) through some physical properties such as (color and melting point) and by means of infrared spectra measurements (IR). Where it was observed that the spectrua of the absorption band appeared within the range (1784.15-1705.07)cm^{-1} due to the stretching of the (O-CO) lacton carbonyl group, in addition to the appearance of an absorption band at the range (1667.36-1697.36) cm^{-1} dating back to the stretching of the (N-CO) lactam carbonyl group, In addition to the absorption bands at the range (1492.90-1595.13) cm^{-1} related to the stretching of the aromatic (C=C), as it was observed that the absorption bands at the range (3005.10-3098.45) due to the stretching of the aromatic (C-H), In addition to the absorption bands at the range (3201.56-325) cm^{-1} related to the stretching bond of the (N-H), as well as the absorption bands at the range (1597.06-1657.03) cm^{-1} belonging to the stretching bond of the (C-N).

The proton magnetic resonance spectrum of compounds (V18-V24) showed a sharp single signal at the position δ (2.50) ppm belonging to the protons of the solvent (DMSO), and a single signal at the chemical shift δ (6.35) ppm due to the protons of the double bond (CH=CH) in the oxazepine heterocyclic ring, a single signal at the chemical shift at the range δ (7.47-8.15) ppm due to the protons of the (N-CH-O) in the oxazepine heterocyclic ring, a multipet signal at the chemical shift at the range δ (6.51-8.87) ppm due to the protons of the aromatic ring (Ar-CH). The ^{13}C-NMR spectra of 1, 3- Oxazepine derivatives (V18-V24) revealed the following spectrum information: the presence of a signal at δ (113.10-166.36) ppm corresponding to a carbon of the (Carbon. Protons & -CH=CH-$_{Oxazpin}$) group, the presence of a signal at = (83.80-91.67) ppm corresponding to a carbon of the (N-CH-O $_{Oxazpin}$) group, the presence of a signal at the range (25.27-28.56) ppm corresponding to a carbon of the (-N(C=O) -CH$_2$-) group, the presence of a signal at the range (27.07-29.75) ppm corresponding to a carbon of the (-O(C=O) -CH$_2$-) group.

3. Biological Part:

This part offers the following discussing of the results of the biological experiments:

Antibacterial activity:

As part of the current study, the well-diffusion [34] method was used to assess the in vitro antibacterial activity of most prepared compounds (V2-23) and ampicillin against two type's bacteria:

1. Antibacterial activity against E. coli: The derivatives (V17 and V20) were shown to have a good to moderate inhibitory action against E. coli when compared to Ampicillin, whereas other chosen compounds did not exhibit any inhibitory activity.
2. Antibacterial activity of S. aureus: Compared to Ampicillin and, the derivatives (V17) showed good to moderate inhibitory action against S. aureus, however the other chosen compounds don't exhibit any inhibitory activity. Examining the screening information in the subsequent table (3):

Table 3: Antibacterial activity of compounds (V17, V20 and ampicillin) a gains tested bacteria:

Comp.	Conc. (µg/ml)	Inhibition zone(mm)	
		Gram-positive	Gram-negative
		Staphylococcus aureus	E. coli
Ampicillin	10	23	27
V17	1024	28	31
	512	26	25
	256	26	26
	128	28	28
	64	26	24
V20	1024	18	35
	512	15	33
	256	17	33
	128	14	33
	64	15	33

Antioxidant activity by DPPH

The radical scavenging properties of most compounds from (V1-23) and ascorbic acid were studied using a DPPH assay in methanol; the DPPH is widely used to evaluate the effectiveness of antioxidants. In general, the DPPH has absorption at 517 nm. At which the absorption decreases in the presence of antioxidants, as a result the color changes from purple to yellow upon

the quenching of the radical in the presence of an antioxidant. The change in color is taken as an indication of the ability of hydrogen donates to tested compounds (V1-V23). The scavenging activity result of our synthesized compounds is tabulated in Table (4):

Table 4: Percentage of antioxidant activity of compounds (V1, V5, V7, V9, V13, V14, and V16-V23) and Ascorbic acid:

Concp. (µg/ml)	Percentage of antioxidant activity								
	V1	V5	V7	V9	V15	V16	V17	V18	V19
1024	95.0	87.7	92.3	83.2	66.6	80.1	96.5	76.6	78.7
512	93.0	87.8	92.7	86.4	67.1	71.7	95.0	78.2	72.7
256	92.8	86.3	92.0	31.7	68.2	76.4	93.4	77.4	73.9
128	95.5	95.1	92.3	30.2	93.9	65.1	93.5	82.2	82.1
64	95.4	83.2	91.0	28.8	85.4	64.8	89.4	73.4	71.1

Conc. (µg/ml)	Percentage of antioxidant activity				
	V20	V21	V22	V23	Ascorbic acid
1024	71.2	98.7	73.4	76.4	99.1
512	64.9	99.3	71.7	61.1	98.9
256	73.9	99.4	75.2	74.7	98.8
128	82.1	98.1	73.0	59.4	98.7
64	68.6	98.9	73.7	58.9	98.6

Table 5: Scavenging activity result of compounds (V1, V5, V7, V9, V13, V14, and V16-V23)

Comp.	IC_{50}	Comp.	IC_{50}	Comp.	IC_{50}
V1	1640.8	V15	6493.9	V21	1659.00
V5	2056.00	V16	6745.95	V22	790.900
V7	1622.7	V17	1639.4	V23	835.330
V9	2908.5	V18	1600.00	Vit. C	1658.00
V13	928.11	V19	1909.00		
V14	652.40	V20	1202.00		

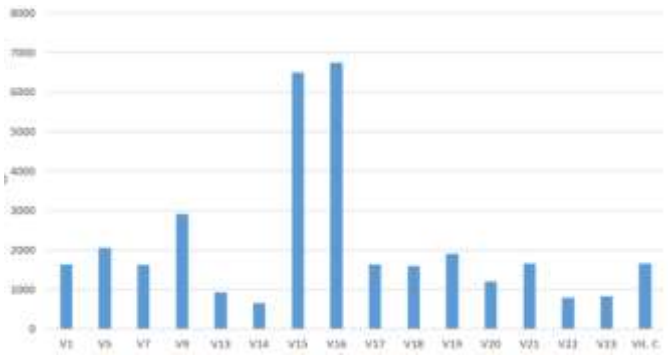


Figure**7**: Histogram representation of antioxidant activity with IC_{50} values of synthesized compounds (V1, V5, V7, V9, V13, V14, and V16-V23)

From the results in Table (5) and figure (1) the concentration (128µg/ml) is the most scavenging activity compared with other concentration.

The limiting capability of the examined synthesized compounds were determined by their reaction with stable free- standing 1, 1-diphenyl-2-picryl-hydrazine (DPPH) in five special concentration (1024, 512, 256, 128, and 64 µg/ml). From these results the following points are observed:

•The highest scavenges activity observed in compounds (V1, V7, V17, V18, and V21), this is probably due to the presence of the hydroxyl, nitro, and bromo group.

•The presence of electron withdrawing substituents deactivate aromatic ring and have no capability to bind the free radical.

•Among all synthesized compounds, compounds (V1, V7, V17, V18, and V21) showed the best antioxidant properties exhibiting an IC_{50} of approximately (1640.8, 1622.7, 1639.4, 1600.0, and 1659.0) respectively and being comparable to IC_{50} of Vitamin C (1659.20µg/ml).

•The order of the property depends on the radical stability formed in the derivatives.

•The lowest activity can be attributed to the influence of the electron withdrawing group while compounds (V1, V7, V17, V18, and V21) as a DPPH radical scavenges can be attributed to its strong hydrogen or electron donor ability.

Molecular docking study:

A. Molecular docking of compounds (V18-V23) with human heme oxygenase-1(3CZY)(as antioxidant):

1- The synthesized compounds (V18-V23) showed good docking scores ranging from (-8.81_-9.79) and were comparable with the binding score of the standard drug 1(adamantan1yl) 2 (1Himidazol1yl)ethanone (Std) (-7.35) Kcal/mole, and

the docking score of all compounds (V18-V23) was found to be the higher than the standard ligand.

2- Arg136, ASP140, PHE166, SER53, LEU54, LEU213, GLY139, VAL50, PHE214, SER142, HIS25, TYR134, THR135, PHE167, and LEU147 were lying near (Pyridyl, 1,3,4-Oxadiazole, and 1,3-Oxazepine) heterocyclic ring systems.

3- The H-bond is one of the most widely used parameters for the evaluation of the docking results, the number of H- bond interactions in the standard drug is (1) was compared with that of the synthesized compounds, (V18 and V19) showed (1) hydrogen bond like a standard ligand.

Finally, compound (V20) showed a higher five hydrogen bonds, the high number of the intermolecular hydrogen bonds N-H……X (X=O, N, S) which indicated these H- bonds play a key role in crystal packing.

4- The higher value of docking score for compounds (V18-V23) compared with the standard ligand (1(adamantan1yl)2(1Himidazol1yl)ethanone (Std)) due to the replacing (-NH=C-) moiety by oxazepine ring, which lead to enhancement the made type of interaction pyridyl, 1,3,4- oxadiazole, and oxazepine ring can interact with amino acids enzyme via its H-bond acceptor properties, it has been used instead of ester and amide functional group, leading to an increase of the biological activity. Moreover, 1, 3, 4-oxadiazole is a flat aromatic ring that gives an appropriate orientation.

5- The docking study showed good compatibility with in vitro study for compound (V21).as shown in table (6).

Table 6: Docking scores Kcal/mole and interactions of compounds (V20 and V**2**1) with human heme oxygenase-1 (PDB code: 3CZY):

Comp. No.	Docking Score (Kcal/ mol)	Interaction residues				
		Hydrogen Bonds				Other interactions
		Atom of comp.	Amino acid	No. of H. bond	Distance (A^0)	
1(adamantan1yl)2(1Himidazol1y)ethanone (Std)	-7.35	N- of oxadiazole ring	PHE79	1	3.03	Conventional Hydrogen Bond
		Oxadiazole ring	TYR137		4.99	Pi-Pi Stacked
V20	-9.79	N- of pyridine ring	LEU54	5	3.29	Conventional Hydrogen Bond
		N- of oxadiazole ring	ARG136		2.78	Conventional Hydrogen Bond
		(O-)ester in oxadiazole ring	SER142		3.37	Conventional Hydrogen Bond
		N- of pyridine ring	SER53		2.90	Carbon Hydrogen Bond
		Oxadiazole ring	ARG136		3.34	Pi-Cation;Pi-Donor Hydrogen Bond
		Oxadiazole ring	ASP140		4.78	Pi-Anion
V21	-9.25	O- of lactum in oxazepine ring	ARG136	5	2.73	Conventional Hydrogen Bond
		Between H- of lactum and N- of oxadiazole ring			2.10	Conventional Hydrogen Bond
		H- of hydroxyl	HIS25		2.02	Conventional Hydrogen Bond
		C- of methoxy group	GLU29		3.41	Carbon Hydrogen Bond
		N- of oxadiazole ring	GLY139		2.90	Carbon Hydrogen Bond
		Oxadiazole ring	HIS25		5.05	Pi-Pi T-shaped

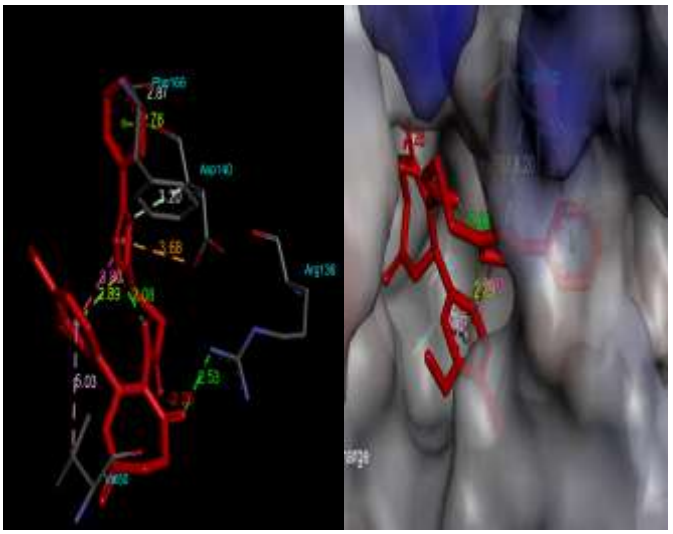


Figure **8**: 3D and 2D docking models structure of V21 into binding pocket of human heme oxygenase1 (PDB code:3CZY)

B. Molecular docking of compounds (V1-V17)and Ampicillin with S. aureus biotin protein ligase

1.The synthesized compounds (V1-V17) showed good docking scores ranging from (-5.25- -9.26) and were comparable with the binding score of the standard drug ampicillin (-4.16) Kcal/mole, the docking score of compounds (V9, V10, V11, V13, and V15) was found to be the higher than the other compounds, and compound (V9) was found to have the highest docking score - 9.26 Kcal/mole.

2.TRP127, GLY190, PHE191, LEU192, GLY208, ILE209, ARG120, PHE191, LEU192, SER93, GLN95

were lying near (Pyridyl and 1,3,4-Oxadiazole) heterocyclic ring systems.

3.The H-bond is one of the most widely used parameters for the evaluation of the docking results, the number of H- bond interactions in the standard drug (ampicillin) was compared with that of the synthesized compounds (V1, V2, V3, V5, V7 and V8) was found to be four, and compounds (V9, V11, V13, V15, V16) showed five hydrogen bonds and compounds (V10, V17) showed six hydrogen bonds.

4.The docking study showed good compatibility with in vitro study for compound (V17).as shown in table(7).

Table 7: Docking scores Kcal/mole and interactions of compounds (V9, V17 and Ampicillin) with biotin protein ligase (S-aureus) (PDB: ID: 4dqz)

Comp. No.	Docking Score (Kcal/ mol)	Interaction residues				
		Hydrogen Bonds				Other interactions
		Atom of comp.	Amino acid	No. of H. bond	Distance (A^0)	
Ampic-illin	-4.16	O–atom on azetidine ring	GLN116	4	3.04	Conventional Hydrogen Bond
		O-atom	GLN116		3.31	Conventional Hydrogen Bond
		H- of caroboxylic group	ARG120		2.98	Conventional Hydrogen Bond
		H- of amine group	GLY208		2.09	Conventional Hydrogen Bond
V9	-9.26	S-atom	THR94	5	2.69	Conventional Hydrogen Bond
		N- oxadiazole ring	GLN116		2.90	Conventional Hydrogen Bond
		N- oxadiazole ring	ARG120		3.12	Conventional Hydrogen Bond
		Br- atom	ARG122		3.53	Conventional Hydrogen Bond
		O- amidic group	GLY210		2.72	Conventional Hydrogen Bond
		N- amidic group	TRP127		4.64	Pi-Cation
V17	-7.42	N- oxadiazole ring	SER93	6	3.10	Conventional Hydrogen Bond
		O- of amidic group	GLN116		3.12	Conventional Hydrogen Bond
		N- oxadiazole ring	SER138		3.00	Conventional Hydrogen Bond
		Between N- atom of oxadiazole ring and H- atom of carbone schiff base	-		2.27	Conventional Hydrogen Bond
		O of amidic group	GLY208		3.60	Conventional Hydrogen Bond
		Br – substituted group	GLY208		3.42	Conventional Hydrogen Bond

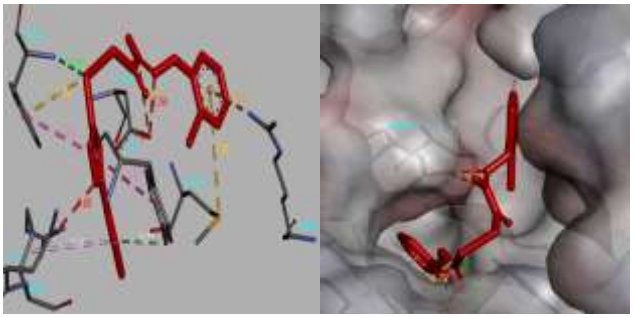


Figure **9**:3D and 2D docking models structure of (V17) into binding pocket of biotin carboxylase enzyme (E.Coli) (PDB:ID:3jzf)

C. Molecular docking of compounds (V18-V23) with E-Coli biotin carboxylase (PDB: ID: 3jzf):

1.The synthesized compounds (V18-V23) showed good docking scores ranging from (-7.99, -9.13) Kcal/mole and were comparable with the binding score of the standard drug (ampicillin) (-8.23) Kcal/mole, and the docking score of compounds (V19, V20, V21, and V22) was found to be higher than the standard drug (ampicillin), and compound (V19) was found to have the highest docking score –9.13 Kcal/mole.

2.LYS238, ASN290, ARG292, GLU87, ARG338, ASN340, SER383, ASN9, ARG10, ASP382, PHE84, GLN294, GLY83, TYR82, SER142, SER53, ARG136, ASP140. LEU54, LEU213, PHE214, PHE166, SER86, HIS209, GLU12, HIS370, MET384, and ARG10 were lying near (Pyridyl and 1,3,4 –Oxadiazole, and 1,3-oxazepine) heterocyclic ring systems.

3.The H-bond is one of the most widely used parameters for the evaluation of the docking results, the number of H- bond interactions in the Ampicillin drug is (7) was compared with that of the synthesized compound (V23) showed (10) hydrogen bonds, compound (V19) showed eleven hydrogen bonds, compounds (V20, and V21) showed four hydrogen bonds, compounds (V27) showed six hydrogen bonds.

Finally, compound (V19) showed a higher eleven hydrogen bonds, the high number of the intermolecular hydrogen bonds N-H……X (X=O, N, S) which

indicated these H- bonds play a key role in crystal packing.

4.Because of the presence of (pyridyl, 1,3,4-oxadiazole, 1,3-oxazepine) groups in compounds (V18-V23), the intermolecular interactions (H-bonds, π-π, π-cation, and salt bridge), added more stability and more bonding affinity, due to their constitution containing two nitrogen atoms and imine bond that is considered as electron donors causing increasing chelating ability of the ligands to sites of enzyme. as shown in Table 8.

Table 8: Docking scores (Kcal/mole) and interactions of compounds (V18,V23 and ampicillin) with protein E-Coli biotin carboxylase (PDB: ID:3jzf)

Comp. No.	Docking Score (Kcal/ mol)	Interaction residues				
		Hydrogen Bonds				Other interactions
		Atom of comp.	Amino acid	No. of H. bond	Distance (A^0)	
Ampic-illine	-8.23	O- atom on azetidine	ASN290	7	2.78	Conventional Hydrogen Bond
		O- atom of carbonyl group in carboxylic group	ARG292		2.92	Conventional Hydrogen Bond
		H- atom of hydroxyl group in carboxylic acid	GLU87		2.45	Conventional Hydrogen Bond
		H- atom of amine	GLU276		2.05	Conventional Hydrogen Bond
		Between O- atom on azetidine ring and H- atom of amine			2.12	Conventional Hydrogen Bond
		anotherH-atom of amine group	GLU276		2.25	Conventional Hydrogen Bond
		One H-atom of amine	GLU288		2.41	Conventional Hydrogen Bond
V19	-7.99	O- of ester group in oxazepine ring	ARG338	8	2.89	Conventional Hydrogen Bond
		O- of ester group in oxazepine ring	ASN340		2.71	Conventional Hydrogen Bond
		O- of amidic group in oxazepine ring	SER383		3.11	Conventional Hydrogen Bond
		O- of amidic group in oxazepine ring	SER383		2.88	Conventional Hydrogen Bond
		H- of hydroxyl group	ASP382		2.26	Conventional Hydrogen Bond
		O- of ester group in oxazepine ring	ARG338		2.82	Carbon Hydrogen Bond
		C- of pyridine ring	ASN9		3.16	Carbon Hydrogen Bond
		C- of pyridine ring	ARG10		3.54	Carbon Hydrogen Bond
		Benzene ring	ARG338		3.72	Pi-Cation

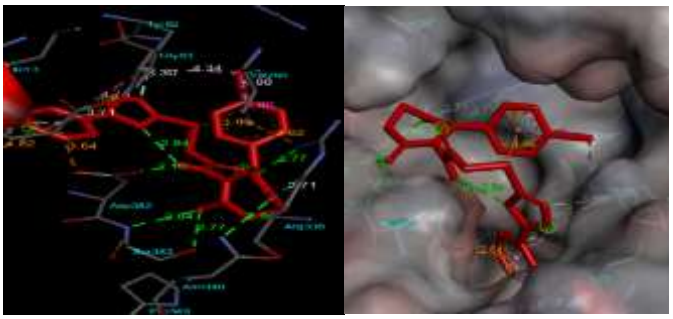


Figure **10**:3D and 2D docking models structure of V19 into binding pocket of biotin carboxylase enzyme (E.Coli) (PDB:ID:3jzf)

Conclusions

1. The methods used to obtain 1,3,4-oxadiazole and 1,3-oxazepine offer an easy experimental work up with satisfactory product yield.
2. Spectral data obtained from (FT-IR, ^{1}H-NMR, and ^{13}C-NMR) gives a good evidence for successful condensation reaction for formation compounds (V1-V23).
3. In comparison to ampicillin, the compounds (V17 and V20) shown high activity against E. coli, whereas the compounds (V17) demonstrated high to moderate activity against S. aureus.
4. The findings demonstrated the high antioxidant activity of the derivatives (V21) with an IC_{50} of approximately (1659.00 g/ml), which is comparable to the value of vitamin C (IC_{50} =1659.20). The hydroxyl group was thought to be responsible for this.
5. The docking score of the anti-oxidant active derivatives (V20 and V21) was found to be the higher than the standard ligand 1(adamantan1yl) 2 (1Himidazol1yl)ethanone (Std) and compound (V20 and V21) was found to have (5) hydrogen bonds.
6. The bioactive derivatives' docking simulation findings were reasonable, and there was agreement between predicted outcomes and actual outcomes.

7. Docking simulation provided accurate findings for the bioactive derivatives and demonstrated a correlation between predictions and actual outcomes.
8. The findings of the molecular docking analysis revealed that some biologically active substances had higher binding affinity energies than co-crystallized medications. Inferences and Suggestions.

Yielding compound with higher anti-microbial activity compared with parent isoniazid drug, these indicated the importance of development new pro-drug candidates from the parent drug as a safer template.

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P- ISSN 1991-8941 E-ISSN 2706-6703 *Journal of University of Anbar for Pure Science (JUAPS)* Open Access
2024,(18), (01):165 – 179

تحضير، ودراسة النمذجة الجزيئية لبعض المركبات ثلاثية الحلقة غير المتجانسة (بيرديل ، 4,3,1- اوكسادايازول و 3,1- اوكسازبين) كمضادات محتملة للميكروبات

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الخلاصة:

تصنف هذه الدراسة تحضير مركبات ثلاثية الحلقة غير المتجانسة (Pyridyl , 1,3- Oxazepine و1, 3, 4-Oxadiazole) والمشتقة من الايزونيزايد (INH) (V2-V23). تراكيب المركبات المحضرة تم تشخيصها باستخدام بعض التقنيات الطيفية والمتضمنة (IR, 1H-NMR, and 13C-NMR) وقد دلت النتائج على صحة التراكيب المسندة لهذه المركبات (V1-V23). تم تقييم الفعالية ضد البكتريا لهذه المركبات وقد دلت النتائج ان بعض هذه المركبات امتلكت فعالية اكبر من الدواء القياسي المستخدم . وتم استخدام تقنية النمذجة الجزيئية للتعرف على الخاصية التثبيطية لهذه المركبات((V18-V23)) على فعالية E-Coli biotin carboxylase (PDB:ID:3jzf) وبعض مركبات (V1-V17) على فعالية S-aureus biotin protein ligase (PDB: ID:4dq2) نتائج التقييم البايولوجي والنمذجة الجزيئية قد دلت على ان ادخال (1,3,4-oxadiazol, hydrazone group, and 1,3- Oxazepine) في تركيب المركب الدوائي (الايزونيزايد) قد عزز الفعالية ضد الميكربات .

واخيرا تم تقييم الفعالية ضد الاكسدة عمليا لبعض المركبات المحضرة (V1,V5,V7,V9,V13,V14, V16-V23) وكذلك دراسة النمذجة الجزيئية (V18-V23) مع human heme oxygenase-1(PDB:3CZY) ومقارنة القيم مع القيمة المرجعية للمركب الدوائي [1(adamantan1yl)2(1Himidazol1yl)ethanone (Std) وascorbic acid)] على التوالي النتائج المستحصلة دلت على امتلاك فعالية متوسطة الى عالية ضد الاكسدة