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Synthesis, Molecular Docking and Biological activity of Novel Compounds Containing three heterocyclic units derived from Isoniazid

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Abstract
The research describes the synthesis series of Hydrazone compounds containing 1,3,4- oxadiazole ring system derived from isoniazid (INH)) (V6-V17). The characterization of the structures of test compounds was elucidated by different spectroscopic methods IR, ^{1}H-NMR, and ^{13}C-NMR. The results obtained are in agreement with the assigned structures. Some of the synthesized compounds were tested in vitro for their antimicrobial activity *E-Coli* and *S-aureus*. The results indicated that compounds (V17) have activity against E-Coli. In contrast, the compounds (V14 and V17) showed good to moderate activity against *S. aureus*; these results are compared with standard Ampicillin drugs and indicated that the presence of 1,3,4-oxadiazole ring and hydrazone system enhance antibacterial activity. The inhibitory impact of the synthesized compounds (V1-V17) (structure – reactivity relationship) on the activity of the *E-Coli* biotin carboxylase (PDB:ID:3jzf) and *S-aureus* biotin protein ligase (PDB: ID:4dq2) were investigated using Molecular docking, the tested and molecular docking results indicated that the introducing 1,3,4-oxadiazol and hydrazone group in the structure of parent bioactive drug compound (Isoniazid) enhancing the antimicrobial 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, anticorrosive, and anti-inflammatory activities [11].

The oxyadiazole nucleus can be found in a variety of drug architectures, Numerous biological properties, including those that are anti-cancer, anti-microbial[12], anti-tuberculosis, anti-inflammatory, anti-oxidant, anti-convulsant [13], and anti-HIV, have been linked to the oxadiazole nucleus[14].

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 [15]. Emerging bacterial resistance is a widespread issue in the treatment of many illnesses. As a result, the quest for antimicrobials is a neverending endeavor. A variety of hydrazone derivatives have recently been produced and tested for antibacterial activity [16].

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 [17].

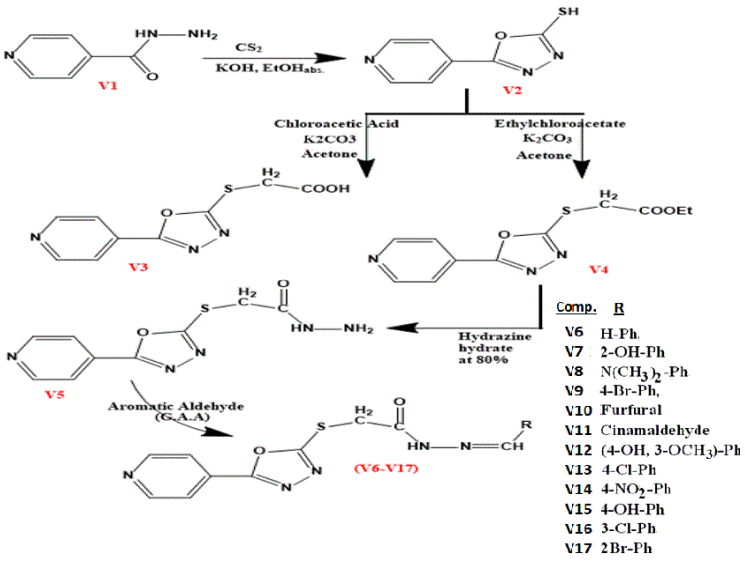
According to our literature review, the synthesis of tris heterocyclic compounds (Pyridyl, 1, 3, 4-Oxadiazole) is rare. As a result, our project aims to prepare a new series of hydrazones using isoniazid as the backbone for synthesized molecules and test their antibacterial activity against (***E-Coli***)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

Materials and methods

The drug isoniazid (isonicotinic hydrazide) was bought from MEDIVER LTD, Kemp House, 160 City Road London EC1V 2NX, United Kingdom. No chemicals have been further refined; they were all purchased and obtained from Sigma-Aldrich, Merck, Aladdin, and Alfa Aesar. Thin layer chromatography (TLC) was used to monitor the completion of reactions using silica gel pre-coated aluminum sheets. Melting points were measured using an uncorrected Stuart SMP10. IR spectra (KBr disc) were recorded with the use of a SHIMADZU, Co., Germany spectrometer (4000-600 cm^{-1}) at the University of Salahaddin Erbil/Iraq- College of Science. Spectra of ^{1}HNMR and ^{13}CNMR were recorded for the compounds prepared in the laboratories of Sanati Sharif University/Tehran/Iran using a device Bruker Ultershield 500MHz NMR spectrometer, Co., Germany in DMSO-d6.

1. Chemical synthesis:

The following processes, which are shown in Scheme 1, were used to create intermediates and final product



Scheme 1: Synthesis of the final compounds (V6-V17)

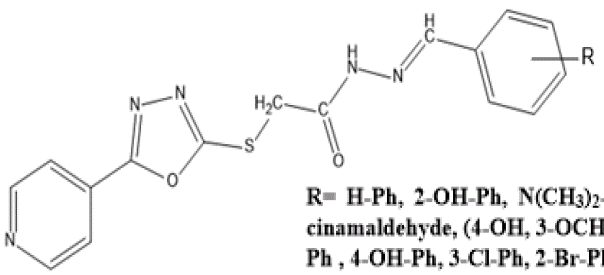
Synthesis of 5-(pyridine-4-yl)-1,3,4-oxadiazole-2-thiol (V2): Isoniazid (0.01mol, 1.371gm) was dissolved in 100ml ethanol, then potassium hydroxide and Carbon Disulfide were added in equimolar amounts to this solution. After that, the mixture was refluxed at 78°C for 24 hr. Distilled water was then added, 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. IR: KBr (cm^{-1}): 2368(S-H str.), 1594(C=N str. of 1,3,4-oxadiazole), 1007(C-O str. of oxadiazole nucleus)[18].

Synthesis of 2-((5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl)thio)acetic acid(V3) : prepared compound (V2)(0.0287mol, 4gm) dissolved in 30 ml of acetone by stirring for a 5 minute, then added chloroacetic acid(0.0287mol, 2.719gm), followed by adding (0.0287mol, 3.960gm)K_2CO_3, kept on the reflex for a night, followed by adding it to crushed ice water with stirring, causing a precipitate to form. This precipitate was then filtered and washed with distilled water, dried, and given a melting point 131-132^{0}c. IR : KBr (cm^{-1}): 3250-3000(O-H str.), 1590(C=N str. of 1,3,4-oxadiazole), 1689 (C=O str.) [19].

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% yeild. IR: KBr (cm^{-1}): 3250-3000(O-H str.), 1599 (C=N str.) of 1,3,4-oxadiazole, 1673 (C=O str.) The bands at 1587 attributed to (C=C str.)[19].

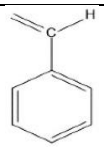
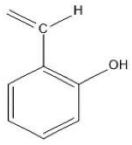
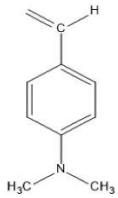
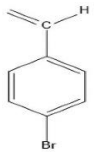
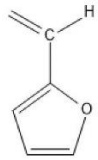
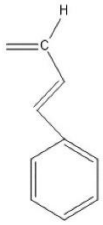
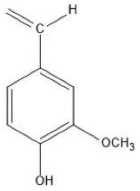
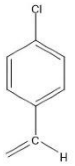
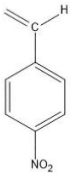
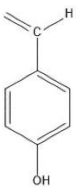
Synthesis for Preparation of 2-((5-(pyridine-4-yl)-1,3,4-oxadiazol-2-yl)thio)acetohydrazide (V5): compound (V4) (0.04mol, 10.6gm) was added to hydrazine hydrate at 80%(20ml), stirred for 2hrs at room temperature, and reflexed for 3hrs. the formed solid product was filtered, dried, and then crystallized from ethanol to give (V5) and taking m.p. 176–178°C with 70% yield. The FT-IR spectrum: the presence of a band at 1621cm^{-1} to the C=O bond of acid hydrazide. Also, the bands at 3260 cm^{-1} correspond to the (-NH-NH2) of the acid hydrazide. In addition, the bands at 3007, 2918, and 2854 cm-1 appeared due to stretching vibration of (C-H) aromatic and aliphatic, respectively. The bands at 1585 cm^{-1} to (C=C) [20].

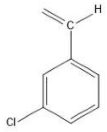
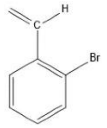
General Procedure for the synthesis of (Z/E) N′- Arylidene-2- [5- (pyridine-4-yl)-1, 3, 4-oxadiazol-2-ylsulphanyl] acetohydrazides (V6–V17) : A solution of equimolar amounts of V5 (0.001 mol, 0.25g) and the appropriate aromatic aldehyde (0.001 mol) in absolute ethanol (15 mL) and 2-3 drops of glacial 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)[21].As shown in table(1).



R= H-Ph, 2-OH-Ph, $N(CH_3)_2$-Ph, 4-Br-Ph,furfural, cinamaldehyde, (4-OH, 3-OCH_3)-ph, 4-Cl-Ph, 4-NO_2-Ph , 4-OH-Ph, 3-Cl-Ph, 2-Br-Ph.

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

Comp.	R	Molecular formula	Molecular weight	Description	M.p. (°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

(E)-N'-benzylidene-2-((5-(pyridine-4-yl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide(V6): 1H-NMR (500 MHz, [D6] DMSO): δ = 4.05 (s, 2 H, -S-C<u>H</u>$_2$-), 7.43-8.73 (m, 9 H, Aro. Protons), 8.24 (s, 1 H, -N=C<u>H</u>-Ar), 11.15 (s, 1 H, -N<u>H</u>-C=O) ppm. ^{13}C NMR (125 MHz) δ = ppm. 30.53(-S-<u>C</u>H$_2$-), 121.14-165.65 (Aro. Carbons), 146.40 (-N=<u>C</u>H-Ar), 173.97 (-<u>C</u>=O). IR (KBr), ν_{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)acetohydrazide (V7) 1H-NMR (500 MHz, [D6] DMSO): δ = 4.25 (s, 2 H, -S-C<u>H</u>$_2$-), 6.92-8.86 (d,d, 8 H, Aro. Protons), 8.77 (s, 1 H, -N=C<u>H</u>-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-<u>C</u>H$_2$-), 115.74-167.86 (Aro. Carbons), 147.95 (-N=<u>C</u>H-Ar), 174.01 (-<u>C</u>=O) ppm. IR (KBr), ν_{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 (νN-N).

(E)-N'-(4-(dimethylamino) benzylidene)-2-((5-(pyridine-4-yl)-1,3,4-oxadiazol-2-yl)thio) acetohydrazide (V8): 1H-NMR (500 MHz, [D6] DMSO): δ = 3.13 (s, 6H, -N(C<u>H</u>3)), 4.16 (s, 2 H, -S-C<u>H</u>$_2$-), 6.89-8.83 (d,d, 8 H, Aro. Protons), 8.54 (s, 1 H, -N=C<u>H</u>-Ar), 11.13 (s, 1 H, -NH-C=O) ppm. ^{13}C NMR (125 MHz) δ =36.54 (-N(<u>C</u>H$_3$)$_2$), 33.45(-S-<u>C</u>H$_2$-), 113.65-164.39 (Aro. Carbons), 145.33 (-N=<u>C</u>H-Ar), 172.35(-<u>C</u>=O) ppm. IR (KBr), ν_{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): 1H-NMR (500 MHz, [D6] DMSO): δ = 4.25 (s, 2 H, -S-C<u>H</u>$_2$-), 7.55-8.86 (d,d, 8 H, Aro. Protons), 8.58 (s, 1 H, -N=C<u>H</u>-Ar), 11.16 (s, 1 H, -NH-C=O) ppm. ^{13}C NMR (125 MHz) δ = 31.25(-S-<u>C</u>H$_2$-), 121.14-164.95 (Aro. Carbons), 148.16 (-N=<u>C</u>H-Ar), 171.80 (-<u>C</u>=O) ppm. IR (KBr), ν_{max}/cm^{-1} = 3197.98 (νN–H); 1698.90 (νC = O); 1591.27 (νC = N); 1552.70, 1487.12, (νC = C); 1294.24 (νC-O); 960.55 (νN-N).

(E)-N'-(furan-2-ylmethylene)-2-((5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl) thio)acetohydrazide (V10): ^{1}H NMR (500 MHz, [D6] DMSO): δ = 4.26 (s, 2 H, -S-C<u>H</u>$_2$-), 6.69-8.84 (m, 8 H, Aro. Protons), 8.55 (s, 1 H, -N=C<u>H</u>-Ar), 11.25 (s, 1 H, -NH-C=O) ppm. ^{13}C NMR (125 MHz) δ = 30.30 (-S-<u>C</u>H$_2$-), 116.88-165.89 (Aro. Carbons), 150.32 (-N=<u>C</u>H-Ar), 171.11 (-<u>C</u>=O) ppm. IR (KBr), ν_{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): 1H-NMR (500 MHz, [D6] DMSO): δ = 3.34 (s, 2 H, -S-C<u>H</u>$_2$-), 6.95-6.98(t, 1H, -C<u>H</u>=CH-),7.30-7.33(d, 1H, -CH=C<u>H</u>-) 7.40-8.82 (m, 9H, Aro. Protons), 7.99-8.00 (d, 2H, -N=C<u>H</u>-Ar), 10.67 (s, 1 H, -NH-C=O) ppm. ^{13}C NMR (125 MHz) δ = 34.24(-S-<u>C</u>H$_2$-), 121.14-163.74 (Aro. Carbons), 136.23 (-CH=<u>C</u>H-Ar), 128.93 (-<u>C</u>H=CH-Ar), 150.89 (-N=<u>C</u>H-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)thio)acetohydrazide (V12 : 1H-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) acetohydrazide(V14): 1H-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): 1H-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): 1H-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).

2. Antibacterial activity:

Sallahadin University, College of Education, Iraq, Department of Biology. 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. The maximal inhibition zone diameter (IZD) of the test, microorganism form was measured and computed. Using three dosages, ampicillin was employed as monitoring samples [22].

3. 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 of more than 5 A^0 and unimportant molecules. Charges were also added, and the produced compounds (V1-V17) 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[23].

Results and Discussion

1. 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. As shown in figure 1:

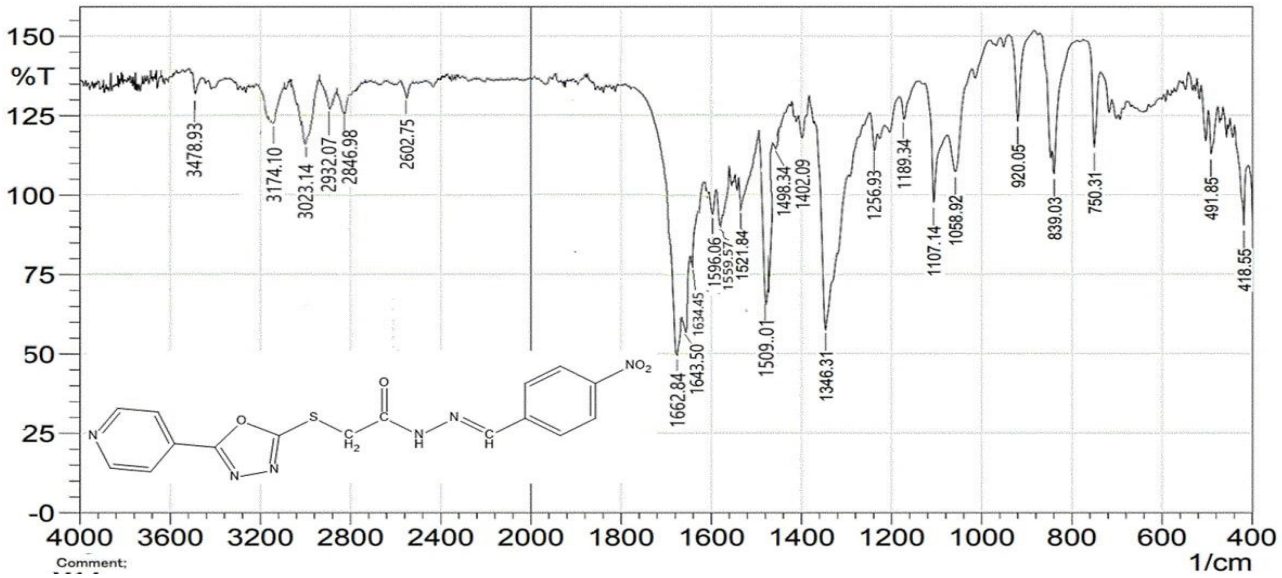


Fig. 1: FT-IR spectrum of compound (V14)

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 a 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$\underline{H_2}$-). As shown in Fig. 2:

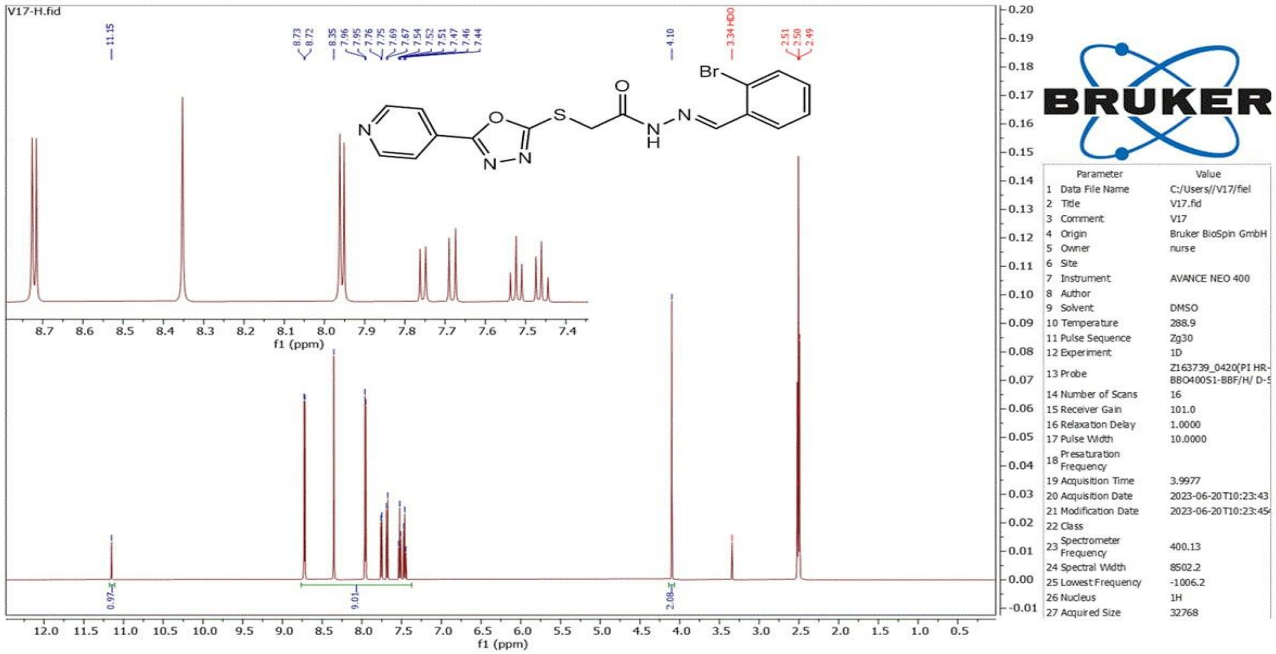


Fig. 2: ^{1}H-NMR spectrum of compound (V17)

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. As shown in figure 3.

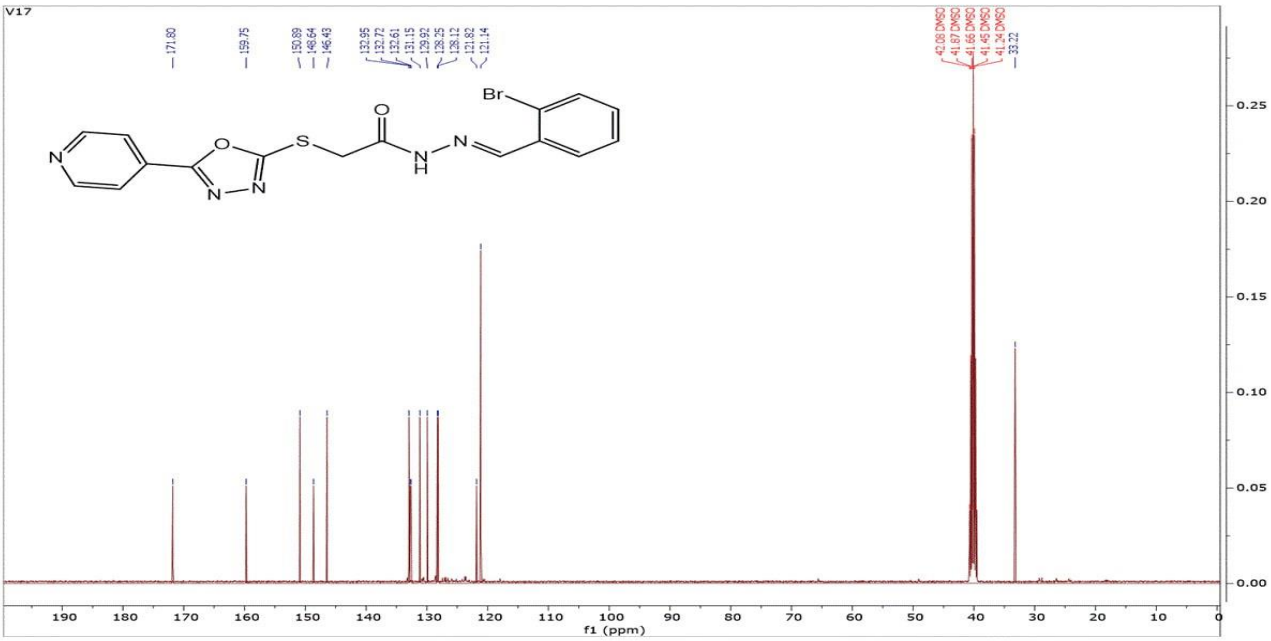


Fig. 3: ^{13}C-NMR spectrum of compound (V17)

2. 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 [24] method was used to assess the in vitro antibacterial activity of the derivatives (V1, V5, V7, V9, V13, V14, V16, and V17) and ampicillin against two type's bacteria:

1. Antibacterial activity against ***E-Coli***: The derivatives **(V17)** 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, the derivatives (V14 and 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 (2):

Table 2: Antibacterial activity of compounds (V14 and V17) and ampicillin against tested bacteria:

Compound	**Conc. (µg/ml)**	**Inhibition zone(mm)**	
		Gram-positive *S-aureus*	**Gram-negative (*E-Coli*)**
Ampicillin	10	23	27
V14	1024	26	13
	512	18	12
	256	20	11
	128	20	12
	64	20	11
V17	1024	28	31
	512	26	25
	256	26	26
	128	28	28
	64	26	24

3. Results of molecular docking studies:

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

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.

TRP127, GLY190, PHE191, LEU192, GLY208, ILE209, ARG120, PHE191, LEU192, SER93, GLN95 were lying near (Pyridyl and 1,3,4-Oxadiazole) heterocyclic ring systems.

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.

The docking study showed good compatibility with the in vitro study for compound (V17), as shown in Table (3).

Table 3: Docking scores Kcal/mole and interactions of compounds (V9, V17 and Ampicillin) with biotin protein ligase (***S-aureus***) (PDB: ID: 4dq2)

Comp. No.	**Docking Score (Kcal/ mol)**	**Interaction residues**				
		Hydrogen Bonds				**Other interactions**
		Atom of comp.	**Amino acid**	**No. of H. bond**	**Distance (A^0)**	
Ampicillin	-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
		oxadiazole ring	SER93		3.61	Pi-Donor Hydrogen Bond
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
		S – atom	TRP136		4.84	Pi-Sulfur

Fig.4 (***S-aureus*** Biotin Protein Ligase with Ampicillin, PDB:4dq2): Ampicillin uses its carboxyl and amine groups to create four typical hydrogen bonds with residues like GLN116, ARG120, and GLY208. These interactions, which are bolstered by polar attractions because of the hydrophilic environment, strengthen the binding inside the active site of the enzyme.

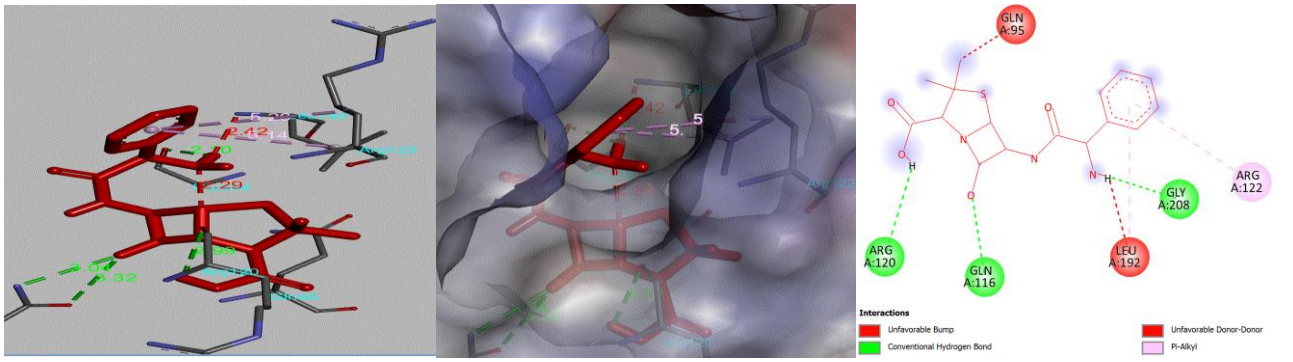


Fig. 4: 3D and 2D docking models structure of ampicillin into binding pocket of biotin carboxylase enzyme (***S-aureus***) (PDB:ID:4dq2)

S-aureus Biotin Protein Ligase (PDB: 4dq2) in Fig.5 (V9): Compound V9 demonstrates robust interactions with residues such as THR94, ARG120, and GLY210 via five hydrogen bonds. Additional Pi-cation and Pi-donor interactions from the oxadiazole and bromobenzyl groups provide persistent non-covalent attractions, improving its enzyme-binding affinity.

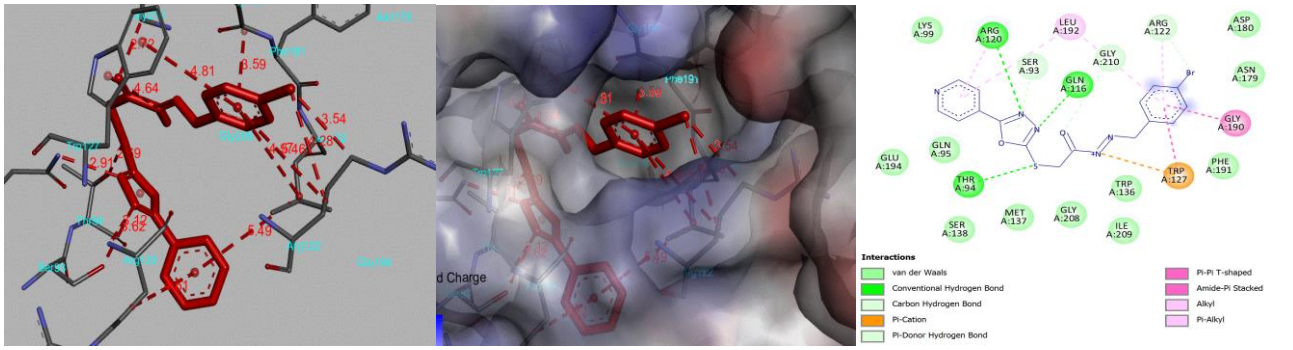


Fig. 5: 3D and 2D docking models structure of V9 into binding pocket of biotin protein ligase (PDB:ID:4dq2)

V17 with ***S-aureus*** Biotin Protein Ligase (PDB: 4dq2) in Figure 6: SER93, GLN116, and GLY208 are among the residues with which V17 makes six hydrogen bonds. The oxadiazole ring contributes to hydrogen bonding and Pi-stacking, showing strong electrostatic attraction, whereas the sulfur atom from the thioether group engages in Pi-sulfur interactions.

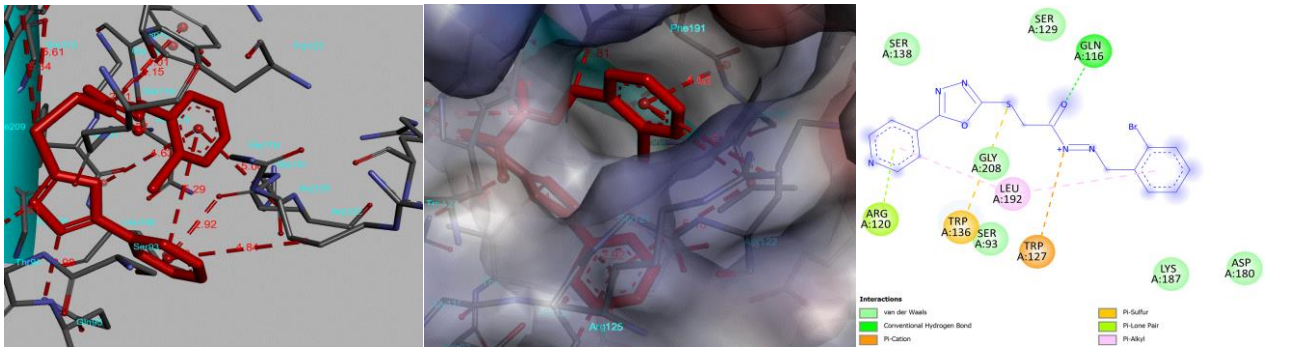


Fig. 6: 3D and 2D docking models structure of V17 into binding pocket of biotin protein ligase (PDB:ID:4dq2)

B. Molecular docking of compounds (V1-V17) and ampicillin with (*E-Coli*) biotin carboxylase: After the docking process using the activity of the (***E-Coli***) biotin carboxylase (PDB: ID: 3jzf) with the prepared compounds (V1-V17), the energy value was found between (-4.45_-8.75), when compared with the standard compound ampicillin, the docking score value of ampicillin was found (-8.23) Kcal/mol and the docking score of the compounds (V4, V6, V7, V9, V10, V11, V12, V13, V15, V16 and V17) was found to be less than that of ampicillin, specifically a higher diagnosis was compound (V14) docking score(-8.75) Kcal/mol.

MET384, ARG10, TYR82, ASP382, PHE84, LYS238, ILE13, GLU12, HIS370, ARG338, HIS209, GLU276, and GLY165 were found near heterocyclic ring systems (Pyridyl and 1, 3, 4-Oxadiazole).

The H-bond is one of the most widely used parameters for evaluating docking results; the number of H-bond interactions in the ampicillin was found to be seven when compared to that of the synthesized compounds (V3, V7, V9, V12, and V14), compound (V10) showed (8) eight hydrogen bonds, compound (V6) showed (6) six hydrogen bonds, compounds (V8, V11, and V13) showed (5) five hydrogen bonds, compounds (V1, V15) showed (4) four hydrogen bonds, compound (V4) showed (10) ten hydrogen bonds, compound (V5) showed (5) five hydrogen bonds, compound (V16) showed (3) three hydrogen bonds, compounds (V2nd V17) showed (2) two hydrogen bonds

Finally, compound (V10) showed a higher (8) eight 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 of the presence of hydrazones groups in compounds (V6-V17), the intermolecular interactions (H-bonds, π-π, π-cation, and Attractive Charge), 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 the chelating ability of the ligands to sites of the enzyme. As shown in table(4)

Table 4: Docking scores Kcal/mole and interactions of compounds (V10, V14 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)**	
Ampicillin	-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 group	GLU276		2.05	Conventional Hydrogen Bond
		Between O- atom on azetidine ring and H- atom of amine group			2.12	Conventional Hydrogen Bond
		anotherH- atom of amine group	GLU276		2.25	Conventional Hydrogen Bond
		One H- atom of amine group	GLU288		2.41	Conventional Hydrogen Bond
		With benzene ring and o- atom of hydroxyl group in carboxylic	LYS238		4.32	Pi-Cation

		acid				
V10	-7.23	H- of amidic group	ASP382	8	2.44	Salt Bridge;Attractive Charge
		N- of pyridine ring	GLU12		3.09	Conventional Hydrogen Bond
		O- of amidic group	ARG338		2.73	Conventional Hydrogen Bond
		O- of furan ring	ARG338		2.96	Conventional Hydrogen Bond
		Between H- of amidic group and O- of oxadiazole ring			2.11	Conventional Hydrogen Bond
		O- of furan ring	ARG338		3.00	Carbon Hydrogen Bond
		N- of pyridine ring	HIS370		3.25	Carbon Hydrogen Bond
		C- of furan ring	LYS238		3.27	Carbon Hydrogen Bond
		Oxadiazole ring	ASP382		3.19	Pi-Anion
		Furan ring	ASN340		3.48	Pi-Donor Hydrogen Bond
		Pyridine ring	MET384		5.65	Pi-Sulfur
V14	-8.75	N- of amidic group	ASP382	7	5.29	Attractive Charge
		O- of amidic group	PHE84		3.02	Conventional Hydrogen Bond
		O- of nitro group	ARG292		2.82	Conventional Hydrogen Bond
		Another O- of nitro group	GLN294		3.16	Conventional Hydrogen Bond
		O- of nitro group	VAL295		2.86	Conventional Hydrogen Bond
		O- of amidic group	GLY83		3.00	Carbon Hydrogen Bond
		C- of pyridine ring	MET384		3.67	Carbon Hydrogen Bond
		Pyridine ring	ARG10		4.77	Pi-Cation

In Fig.7 (***E.Coli*** Biotin Carboxylase with Ampicillin, PDB: 3jzf):Ampicillin uses its carboxyl, hydroxyl, and amine groups to create seven hydrogen bonds with residues such as ASN290, GLU276, and ARG292. Within the active site, these polar attractions produce a stable binding.

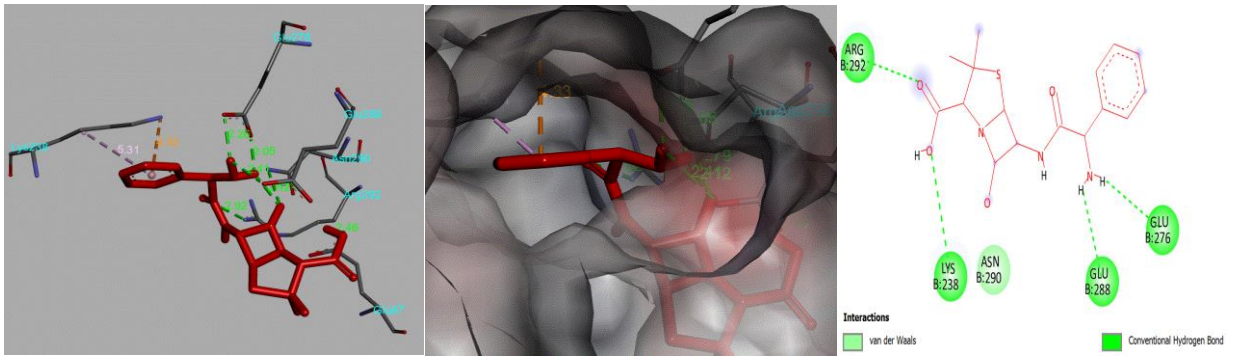


Fig. 7: 3D and 2D docking models structure of Ampicillin into binding pocket of biotin carboxylase enzyme (***E-Coli***) (PDB:ID:3jzf)

In Fig.8 (V10, PDB: 3jzf, ***E. Coli*** Biotin Carboxylase): V10 uses its amidic, pyridine, and oxadiazole groups to create eight hydrogen connections with important residues, including ASP382, ARG338, and GLU12. Strong polar and electrostatic attractions are reflected in the docking stability, which is strengthened by Pi-anion and Pi-sulfur interactions involving aromatic rings.

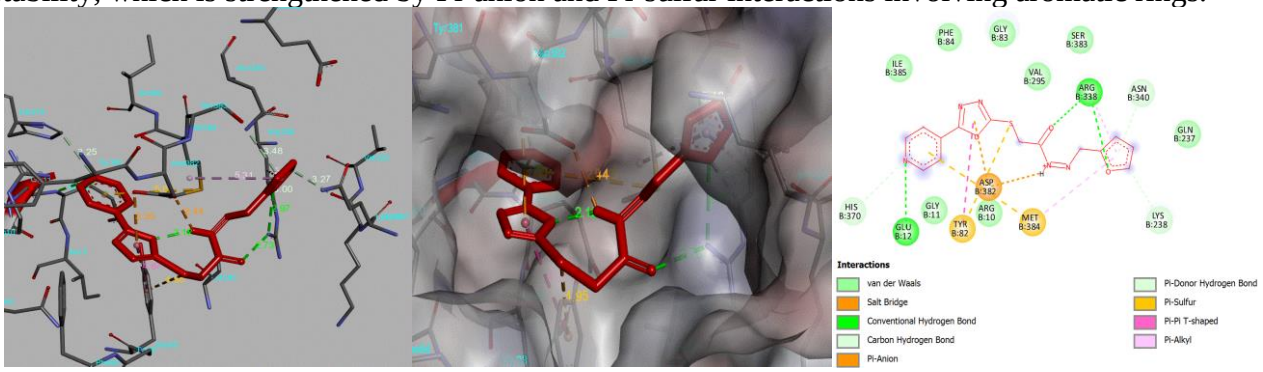


Fig. 8: 3D and 2D docking models structure of V10 into binding pocket of biotin carboxylase enzyme (***E-Coli***) (PDB:ID:3jzf)

In Fig.9 (V14 with ***E.Coli*** Biotin Carboxylase, PDB: 3jzf), V14 forms seven hydrogen bonds with residues like ASP382, ARG292, and GLN294 using its amidic and nitro groups to create strong binding. The binding affinity is increased by Pi-cation and Pi-anion interactions involving polar groups and aromatic rings, suggesting efficient molecule recognition and stability within the active site.

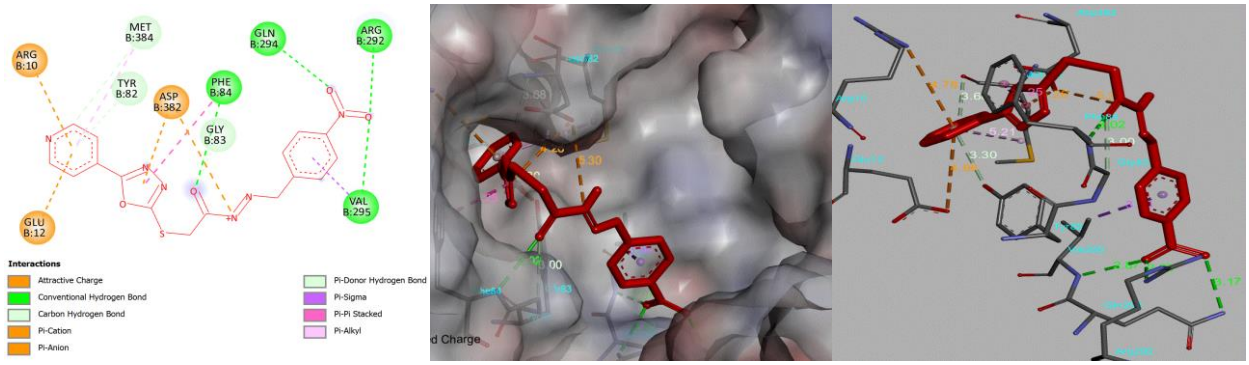


Fig. 9: 3D and 2D docking models structure of V14 into binding pocket of biotin carboxylase enzyme (***E-Coli***) (PDB:ID:3jzf)

Conclusions:

Isonicotinic acid hydrazide serves as a key nucleus for the synthesis of various heterocyclic rings. Through conventional esterification, 5-(pyridine-4-yl)-1,3,4-oxadiazole-2-thiol was successfully esterified. Hydrazine compounds were synthesized by reacting substituted benzaldehyde with the acid hydrazide compound (V5), yielding good results with simple experimental procedures. Spectral data from FT-IR, ^{1}H-NMR, and ^{13}C-NMR confirmed the successful formation of hydrazone compounds (V6-V17) through condensation reactions. Several hydrazone compounds were biologically screened for in vitro antibacterial activity, demonstrating effectiveness against both Gram-positive and Gram-negative bacteria. Molecular docking studies revealed that incorporating the 1,3,4-oxadiazole ring and hydrazone group into the isoniazid structure enhanced antibacterial activity. Compound V17 exhibited promising activity against ***E. coli***, while derivatives V14 and V17 showed good to moderate inhibitory effects against ***S. aureus*** compared to ampicillin. Docking simulation results aligned well with experimental findings, showing reasonable accuracy and correlation. Notably, some biologically active derivatives demonstrated higher binding affinities than co-crystallized drugs, emphasizing the potential of developing new pro-drug candidates from isoniazid as safer, more effective antimicrobial agents.

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تحضير، النمذجة الجزيئية الفعالية البايولوجية لبعض المركبات الجديدة الحاوية على ثلاث حلقات غير متجانسة والمشتقة من دواء أيزونزايد

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معلومات المؤلف
الايميل:
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الخلاصة:
يصف البحث تحضير سلسلة من مركبات هايدرازونات المحتوية على حلقة 4,3,1- اوكسادايازول والمشتقة من الايزونزايد (INH) (V6-V17) تشخيص المركبات المحضرة قد تم بواسطة تقنيات طيفية مختلفة (IR, 1H-NMR, 13C-NMR) والنتائج المستحصلة قد توافقت مع الصيغ التركيبية المسنده . تم تقييم الفعالية الحيوية للمركبات المحضرةضد (*E-Coli*) و (*S-aureus*) وقد بينت النتائج ان المركب (V17) قد اعطت فعالية جيدة ضد البكتريا (*E .coli*) بينما المركبات (V14و(V17 قد اعطت فعالية جيدة الى متوسطة ضد البكتريا (S.aureus). تم دراسة الفعالية التثبيطية للمركبات المحضرة (V1-V17) ضد فعالية E-Coli biotin carboxylase (PDB:ID:3jzf) و S-aureus biotin protein ligase (PDB:ID:4dqz) باستخدام تقنية النمذجة الجزيئية. نتائج التقيم البايلوجي والنمذجة الجزيئية قد بينت ان ادخال حلقة 4,3,1- اوكسادايازول ومجموعة الهايدرازون على الدواء الاصلي قد عززت الفعالية ضد الميكروبات.