Synthesis of Azo Derivatives Linked 1,2,3-Triazols Ring and Study of Their Biological Activity

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Received 3 May. 2025, Accepted 1 Jun. 2025, Published 30 June. 2025.

DOI: 10.52113/2/12.01.2025/104-116

Abstract: In this study, a series of azo derivatives incorporating a 1,2,3- triazole moiety were synthesized and evaluated for biological activity. The synthetic pathway began with N,N-diaminodiphenylmethane, which was diazotized using sodium nitrite ($NaNO_2$) and hydrochloric acid, by coupling with 1-methyl-1H-1,2,3-triazole-4-carbaldehyde in the presence of sodium hydroxide (NaOH) to yield the azo compound. This azo compound was then reacted with p-aminoacetophenone in ethanol, using NaOH as a catalyst, to form a chalcone derivative. Subsequent reactions were carried out under reflux conditions with urea (in basic medium), phenylhydrazine (using a little of glacial acetic acid as catalyst), and hydrazinium hydroxide to obtain Oxazine and pyrazole derivatives, respectively. Structural confirmation of the prepared compounds was thorough using FT-IR, ^{13}C-NMR, and ^{1}H-NMR spectroscopy. Biological screening revealed that all synthesized compounds exhibited promising antimicrobial activity**.**

Keywords :Azo derivative, triazol ring , N,N-diaminodiphenylmethan , chalcone, synthesis , biological activity.

1. Introduction

Azo dyes are important compounds due to the diversity chemical structures of chromophores, this important is result from its ease of preparation, great diversity in composition, Low cost. It can be used for different purposes natural colors, manufactured materials, cosmetics, ink, foodstuffs, leather, dyes. Azo compounds have a distinctive azo group in their stricture such as R1-N=N-R2, R1 and R2 can be either alkyl or aryl compounds [1].

The Azo derivatives can be mono-azo group, di-azo, or tri-azo. Azo derivatives are powerful therapeutic substances that can be used as anticancer, anti-diabetics, anti-inflammatory [2]. Because of their mechanism, azo derivatives can be either bacteriostatic or bactericidal and inhibit or kill the growth of bacterial [3]. Azo compounds are commonly prepared through the diazotization of primary aromatic amines, which are then coupled with nucleophilic agents—typically electron-rich groups like hydroxyl or amino groups. Several alternative methods also exist for synthesizing azo derivatives, including the reduction of nitroso compounds using lithium aluminium hydride ($LiAlH_4$), the reduction process of nitroaromatic compounds

by alkaline media, and the oxidation of primary amines using oxidizing agents such as potassium permanganate or lead tetraacetate. etc [4-5]. In this study, we synthesized an azo dye compound incorporating a triazole ring and characterized it using physical properties along with ^{1}H-NMR and ^{13}C-NMR spectroscopy. The synthesized compound was tested for its antimicrobial activity against two bacterial strains: s.*aureus* (a positive - Gram) and *E- coli* (a negative - Gram)[6].

2. Materials and methods

The chemicals and every solvent utilized were procured from reputable suppliers, including Aldrich, T.C.I., Merck, and B.D.H., and were purified according to established procedures documented in the literature. The melting points were measured using a Stuart apparatus at the Department of Chemistry, College of Education, University of Al-Qadisiyah. It was used thin-layer chromatography (TLC), and spot detection was achieved using iodine vapor and UV light as needed. FTIR spectra were recorded in the 400–4000 cm^{-1} region employing a Shimadzu spectrophotometer with KBr pellets at the Chemistry Department, College of Science, Al-Muthanna University. NMR spectra were acquired at the Central Laboratory of the University of Tehran using a Bruker Elemental INOVA spectrometer (^{1}H at 500 MHz and ^{13}C at 125.65 MHz), with TMS as an internal standard and DMSO as the solventChemical shifts (δ) are reported in ppm (parts per million), while coupling constants (J) are expressed in hertz (Hz).

2.1 Synthetic procedure

2.1.1 Preparation of compound (1)

5,5'-((1E,1'E)-(MPDMT -4-carbaldehyde) [7-8].

methylenebis(4,1-phenylene))bis(diazene-2,1-diyl))bis(1-methyl-1H-1,2,3-triazole (MPDMT) .

The first step is to prepare the diazonium salt by preparing a cold solution of sodium nitrite by dissolving (10 mmol- 0.68 g) of $NaNO_2$ in 20 ml of distilled water. Which was added in drops of the amine solution prepared by dissolving (5mmol-0.99g) of N,N-diaminodiphenylmethane in 40ml of distilled water and 20ml of concentrated HCl and cooled at (0 -5 ºC). After completing the addition of the sodium nitrite solution, stir the solution for half an hour at the same temperature. The second step is to add the prepared diazonium salt directly to a cooled solution of 1-methyl-1H-1,2,3-triazole-4-carbaldehyde (10 mmol-1.11 g) in 30 mL of absolute CH_3CH_2OH and 20 ml of 10% NaOH, and stir the reaction mixture for 2 hours at (0 ºC -5 ºC) and pH=6, to give compound (1) where the the reaction was validated using the TLC approach using (4: 1) (v: v) of mobile phase (hexane - ethyl acetate), then the precipitate compound was filtered, rinsed by distilled water, dried, after that recrystallized by CH_3OH to give a precipitate with crystals brown in color. M.W:446, Yield:87%, mp (220) ºC, R_f*:* 0.58.

2.1.2 Preparation of compound (2)

((2E,2'E)-3,3'-(((1E,1'E)-(MPDMT)-5,4-diyl))bis(1-(4-aminophenyl)prop-2-en-1-one) [9-10].

Compound (2) was prepared according to the general method for preparing chalcones by adding (1mmol - 0.44g) of Compound (1) and (2mmol - 0.27 g) of 4- aminoacetophenone and

completely dissolved in 25 mL of absolute CH_3CH_2OH with continuous stirring until completely dissolved. 14ml of 10% sodium hydroxide was added gradually and without heating. The reaction was reflux for 18 h to obtain compound (2). Where the completion of reaction was validated using TLC and (4: 1) (v: v) of mobile phase (hexane - ethyl acetate)], then the precipitate compound was filtered, rinsed by distilled water, dried, after that recrystallized by CH_3OH to give a precipitate with crystals red in color. M.W:680.78, Yield:70%, mp (310) ^oC, R_f: 0.52.

2.1.3 Preparation of compound (3)

4,4'-(((1E,1'E)- (MPDMT)-5,4-diyl))bis(6-(4-aminophenyl)-2H-1,3-oxazin-2-amine) [11-12].

Compound (3) was prepared by dissolving (0.5 mmol-0.33 g) of chalcone (2) in 25 ml of absolute CH_3CH_2OH with stirring until completely dissolved. Subsequently, urea (1 mmol, 0.06 g) was gradually add to the mixture under continuous stirring. The substrates were subjected to reflux to 12 h. After that the reflux, 25 mL of cold distilled water was introduced slowly with continued stirring for an additional hour, leading to the formation of the desired compound (3). Where the completion of the reaction was validated using the TLC by using mobile phase (4: 1) (v: v) (ethyl acetate-hexane) , then the precipitate compound was filtered, rinsed by distilled water, dried, after that recrystallized by CH_3OH to give a precipitate with crystals white in color. M.W:774.86, Yield:76%, mp (298) ^oC, R_f: 0.61.

2.1.4 Preparation of compound (4)

44,4'-((((1E,1'E)-((MPDMT)-5,4-diyl))bis(4,5-dihydro-1H-pyrazole-3,5-diyl))dianiline [13-14].

Compound (4) was prepared by dissolving (0.5 mmol-0.33 g) of chalcone (2) in 25 mL by absolute CH_3CH_2OH in a 100 mL by flask until completely dissolved, then (1 mmol-0.05 g) of Hydrazine hydrate was introduced to mixture, then refluxed to 15 h. The solution was filtered to obtain compound (4). where the completion of the reaction was validated using the TLC by using (4: 1) (v: v) (ethyl acetate-hexane), then the precipitate compound was filtered, rinsed by distilled water, dried, after that recrystallized by CH_3OH to give a precipitate with crystals yellow in color. M.W:708.84, Yield:74%, mp (259) ^oC, R_f: 0.63.

2.1.5 Preparation of compound (5)

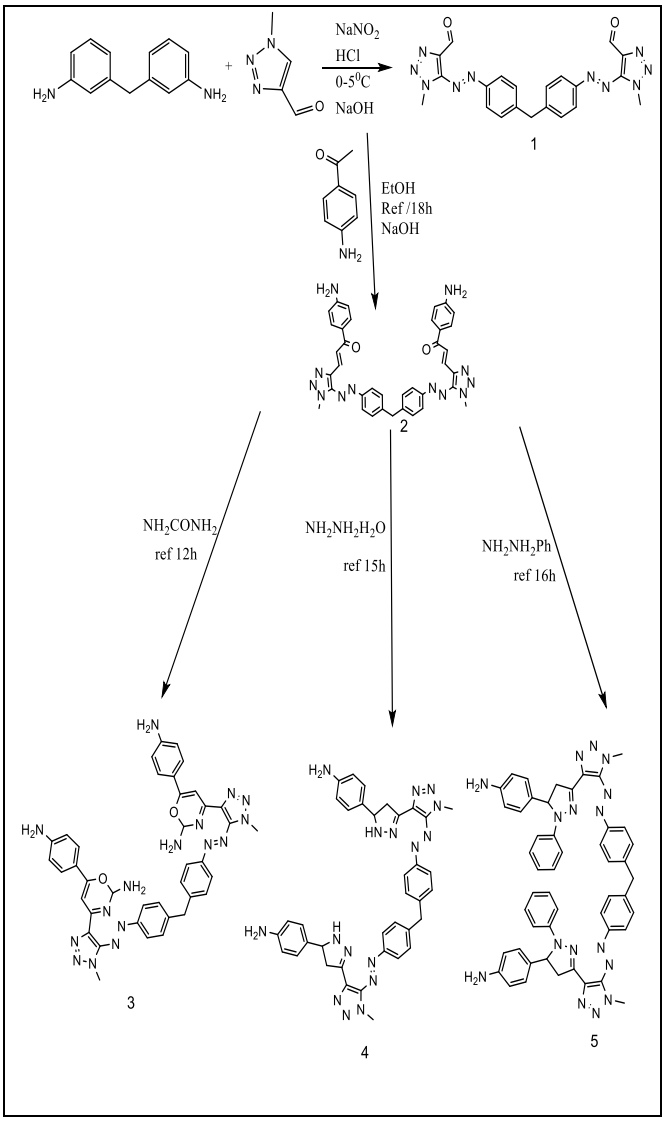
3,3'-((((1E,1'E)-(MPDMT)-5,4-diyl))bis(1-phenyl-4,5-dihydro-1H-pyrazole-3,5-diyl))dianiline

[15-17].

Compound (5) was prepared by dissolving (0.5 mmol-0.33 g) of chalcone (2) in 25 ml of absolute CH_3CH_2OH in a 100 ml by flask until completely dissolved, then (1 mmol-0.10 g) of phenylhydrazine was added, a some of glacial acid were add, the mixture was refluxed for 16 h as obtain compound (5). where the completion of the reaction was validated using the TLC by (4: 1) (v: v) of hexane - ethylacetate, then the precipitate compound was filtered, rinsed by distilled water, dried, after that recrystallized by CH_3OH to give a precipitate with crystals brown in color. M.W:861.04, Yield:73%, mp (315) ^oC, Rf: 0.61.

3. Antibacterial activity [18-21]

A study was carried out on the biological activities of several produced chemicals. The study included two types of pathogenic bacteria of Gram (-ve) bacteria *(Escherichia coli)* and (+ve) bacteria *(Staphylococcus aurous)* which was obtained from Research Unit at College of Science by Al-Qadisiyah University to determine the inhibitory effect. These compounds affected on bacterial growth and the diameters of the zones of inhibition were assessed after an incubation period of 24 hat a temperature of 37. Where All of the produced compounds' solutions were made by dissolving 0.02 grams of each compound in DMSO solvent (5 ml of volume) then the prepared bacteria were spread on the culture media in Petri dishes using (Loopful) and then Drilling was done in these dishes using a cork drill (borer-cork) sterilized with ethyl alcohol after that the prepared solutions was added to the holes using a micropipette while the prepared dishes were transferred to an incubator set at 37 °C and maintained under these conditions for a 24 h the amount Inhibition zone diameters for the prepared compounds was measured.



Scheme 1. Synthesis of (1,2,3,4,5) Azo derivatives.

4. Result and Discussion

5,5'-((1E,1'E)-((MPDMT)-4-carbaldehyde)(1):-

The spectrum FT-IR of derivative (1) displayed a distinct band at 1686 cm^{-1}, attributed to aldehydic C=O (carbonyl) group. The appearance of this new data band provides clear evidence for the successful formation of the derivative.,(3194 cm^{-1}) for (Ar-H) ,(2998cm^{-1}) for (C-H) aliphatic , (1391 cm^{-1}) for (N=N) Azo

, (1454cm^{-1}) for (C=C) aromatic and and (3437 cm^{-1}) for H-aldehyde Figure (1). (^{1}H-NMR) data of compound (a) show 2.50 DMSO-d6 , 3.56 ppm (s , 2H ,H-2) , 2.47 ppm (s ,3H ,CH_3) , 7.85-6.80 (Ar-H) , 10.43ppm for H-aldehyde Figure (2). ^{13}C-NMR spectrum data of compound (a) show: 39.57 DMSO-d6 ,195.37 ppm (C=O) aldehyde , 25.82 ppm (C^1,CH_3) , ,36.15 ppm (C^2) ,111.43 ppm(C^3) triazol , 112.46ppm (C^4) triazol , 162.41 ppm-112.83ppm for (C) aromatic Figure (3) .

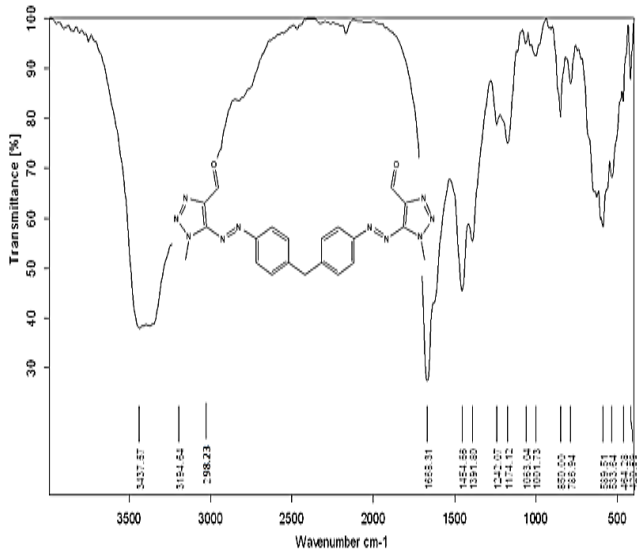


Fig. (1): FT-IR Measurement of (1) Compound.

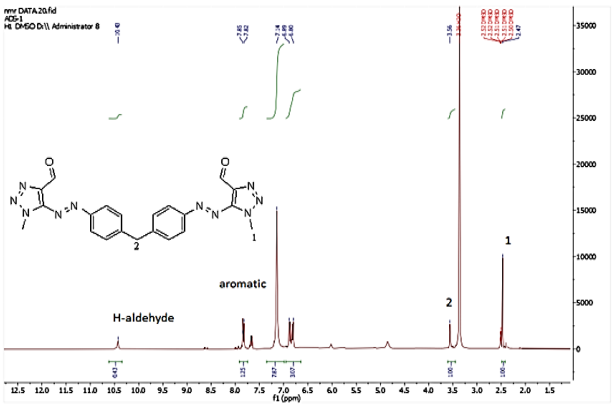


Fig. (2): ^{1}H-NMR Measurement of (1) Compound.

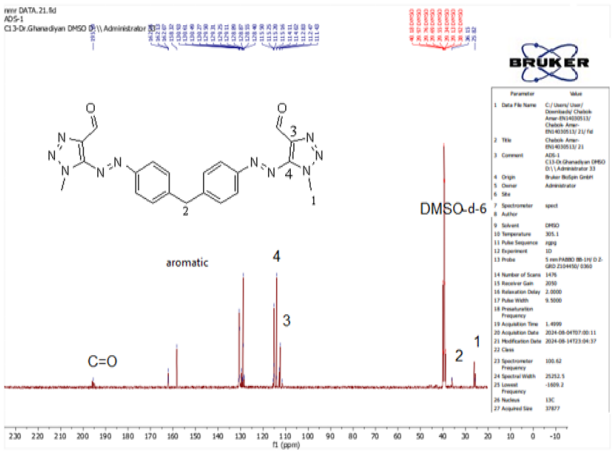


Fig. (3): ^{13}C--NMR Measurement of (1) Compound.

(2E,2'E)-3,3'-(((1E,1'E)- (MPDMT)-5,4-diyl))bis(1-(4-aminophenyl)prop-2-en-1-one)

The FT-IR spectrum of derivative (2) exhibited a characteristic absorption band at (1649 cm^{-1}) a noticeable decrease in the carbonyl stretching frequency was observed, which is consistent with the structure of the formed chalcone. This reduction can be attributed the resonance effect linked with chalcone moiety, (3099 cm^{-1}) for H-aromatic, (2995cm^{-1}) for H- aliphatic, (1280cm^{-1}) for (N=N) Azo, (1444cm^{-1}) for (C=C) chalcone and (3390 cm^{-1}) for (NH_2), the appearance of this new band provides clear evidence for the successful formation of the derivative Figure (4). (^{1}H-NMR) line data of compound (2) show 2.50 DMSO-d6 , 2.40 ppm (s,3H ,CH_3) , 3.57 (s ,2H ,CH_2) ,6.06 ppm (d,1H,H-3) chalcone,6.47 ppm (d,1H,H-4) ,7.73ppm-6.11 ppm for (Ar-H) , 8.81 ppm for NH_2 Figure (5). ^{13}C-NMR spectrum data of compound (2) show: 39.57 DMSO-d6, a decrease in the carbonyl carbon frequency was also observed, shifting from 195.37 ppm in the azo derivative to 184.47 ppm in the chalcone derivative. = This decrease is likely due to to resonance effect linked with the chalcone moiety which is consistent with the structure of the formed chalcone, 25.82 ppm (C-1,CH_3) ,

,36.50ppm (C-2, CH_2) ,36.63 ppm(C^3) triazol , 39.70 ppm (C^4) triazol ,145.92ppm (C^5=C) chlcone, 151.56ppm (C^5=C) chlcone,153.58ppm for C-NH_2, 151.31ppm -112.42 ppm for (C) aromatic Figure (6).

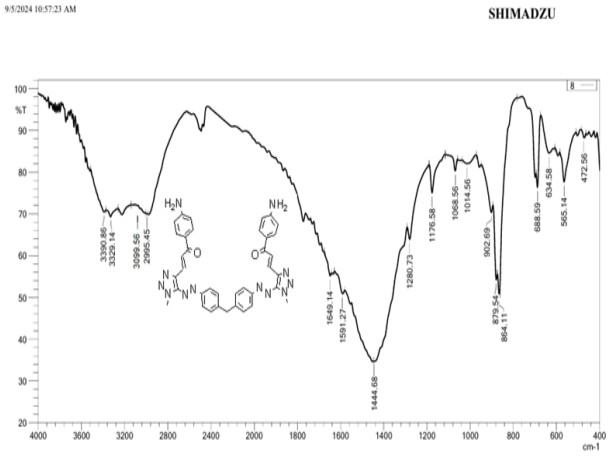


Fig. (4): FT-IR Measurement of (2) Compound.

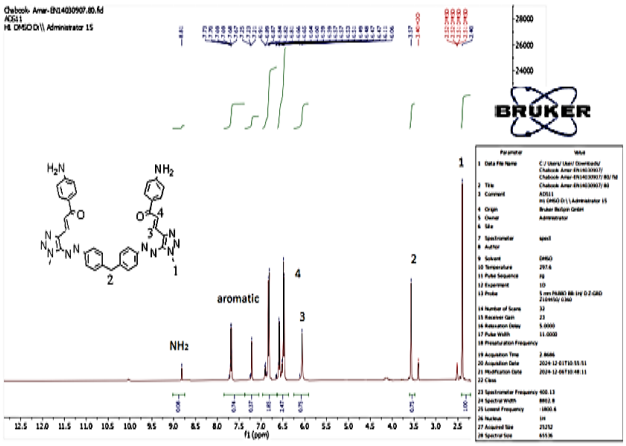


Fig. (5): ^{1}H-NMR Measurement of (2) Compound.

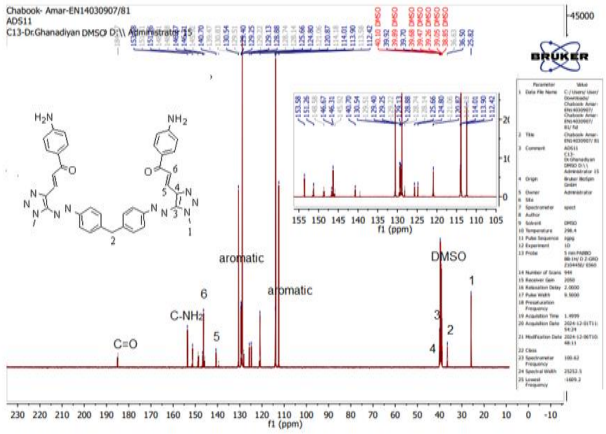


Fig. (6): ^{13}C-NMR Measurement of (2) Compound.

4,4'-(((1E,1'E)- (MPDMT)-5,4-diyl))bis(6-(4-aminophenyl)-2H-1,3-oxazin-2-amine).

The spectrum of FT-IR of derivative (3) exhibited a characteristic absorption band at(3390cm-1) for (NH_2), (3322) to (NH_2), (3130 cm^{-1}) to H-aromatic, (2995cm^{-1}) to H- aliphatic, (1591 cm^{-1}) to (C=N), (1444cm^{-1}) to (N=N), (1280cm^{-1}) for (C=C) aromatic, (1176) cm^{-1} to (C-O) in the newly synthesized derivative, the absence of the carbonyl group signal in the spectrum indicates the successful formation of the oxazine derivative Figure (7). ^{1}H-NMR spectra of (3) compound show 2.50 DMSO-d6, 3.76 ppm (s , H-1,3H ,) triazol, 3.98 ppm (s , H-2,2H ,), 4.12 (s ,2H ,H-3) oxazine , 6.06 ppm (s,1H,H-4) oxazine, 8.65 (s ,2H,NH2), 6.67 ppm (s,2H,NH_2) and 7.73 ppm-6.49 ppm for aromatic hydrogen Figure (8). ^{13}C-NMR spectrum line of compound (3) show: 39.94 ppm DMSO-d6, 25.82 ppm (C-1 ,CH_3) triazol, 169.57 ppm (C=N) oxazine,151.52-113.88 ppm for (C) aromatic, 40,04 ppm (C-4) triazol, 36.83 ppm (C-3) triazol, 36.80 ppm (C -2 ,CH2), 109.50 ppm (C-5) oxazine, 112.40ppm (C-6) oxazine also in the newly synthesized derivative, The disappearance of the C=O stretching signal

in the spectrum confirms the successful synthesis of the oxazine derivative, as shown in Figure (9).

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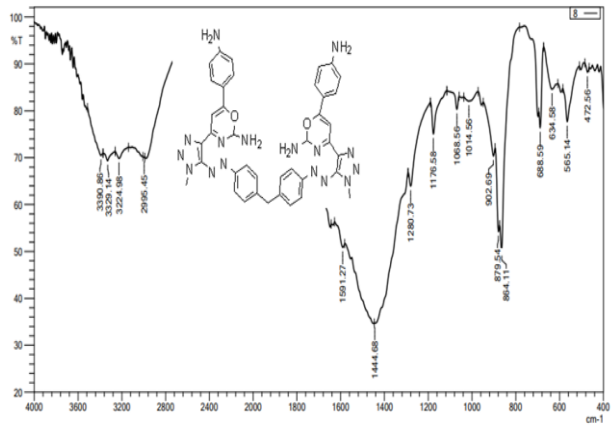

Fig. (7): FT-IR Measurement of (3) Compound.

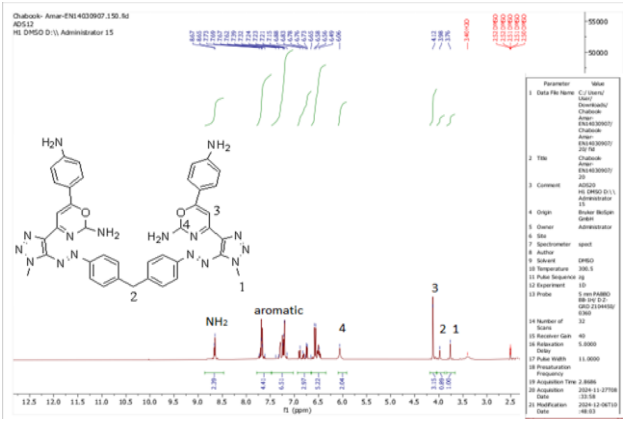

Fig. (8): ^{1}H-NMR Measurement of (3) Compound.

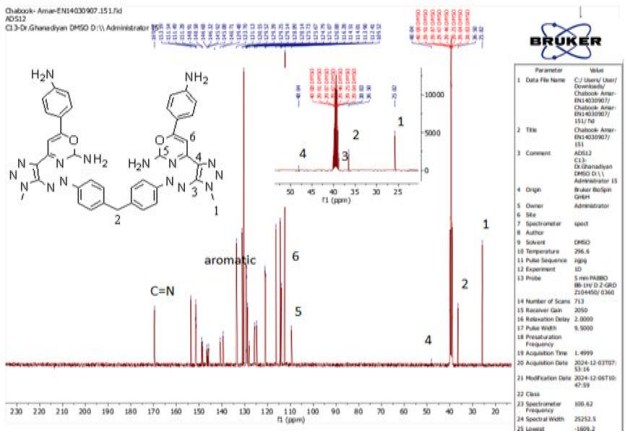

Fig. (9): ^{13}C-NMR Measurement of (3) Compound.

4,4'-((((1E,1'E)- (MPDMT)-5,4-diyl))bis(4,5-dihydro-1H-pyrazole-3,5-diyl))dianiline.

The spectrum FT-IR of derivative (4) exhibited a characteristic absorption band at(3612cm^{-1}) for (NH_2), 3423 cm^{-1}) for (NH), (3025cm^{-1}) for H-aromatic, (2937cm^{-1}) to H-aliphatic, (1612cm^{-1}) to (C=N), (1523cm^{-1}) to N=N, (1575) cm^{-1} to N=N azo in the newly synthesized derivative, the absence of the carbonyl group signal in the spectrum indicates the successful formation of the pyrazole derivative Figure (10). (^{1}H-NMR) data of (4) compound show 2.50 DMSO-d6, 3.31 ppm (s,3H ,H-3) triazol,4.05 ppm (d,2H,H-3) pyrazole, 3.98 ppm (S,2H,H-2), 4.15 ppm (t,1H ,H-4) pyrazole and 7.77 ppm-6.05 ppm for aromatic hydrogen 8.15 ppm (s ,1H,NH), 8.60 ppm (s ,2H,NH_2) Figure (11).^{13}C-NMR spectrum line of compound (4) show: 39.94 ppm DMSO-d6, 26.81 ppm (C-1,CH_3), 166.71 ppm (C=N),150.94 ppm (C-N) ,153.82-111.36 ppm for (C) aromatic, 39.81 ppm (C-5) triazol, 38.84 ppm (C-4) pyrazole, 36.51 ppm (C-2 ,CH2), 54.47 ppm (C-6) triazol, 36.57ppm (C-3) in the newly synthesized derivative, also the absence of the carbonyl group signal in the spectrum indicates the successful formation of the pyrazole derivative Figure (12).

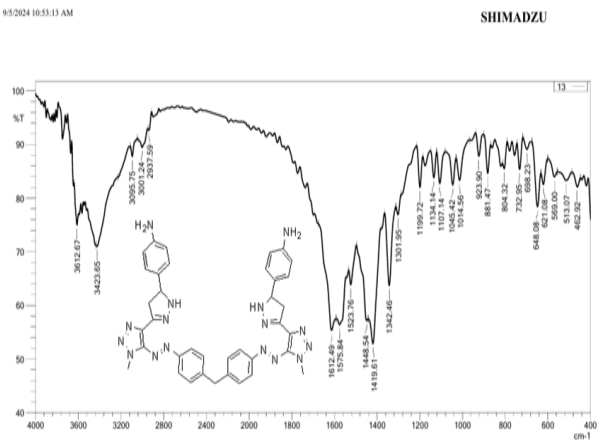

Fig. (10): FT-IR Measurement of (4) Compound.

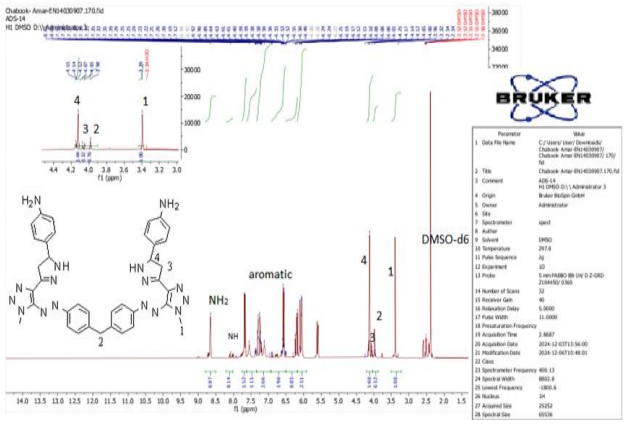

Fig. (11) : ^{1}H-NMR Measurement of (4) Compound.

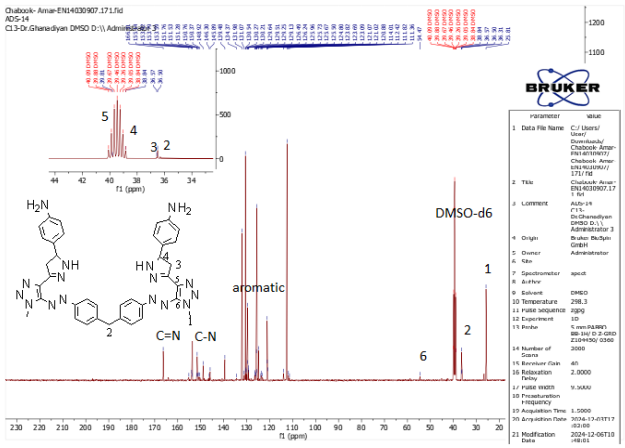

Fig. (12): ^{13}C-NMR Measurement of (4) Compound.

3,3'-((1E,1'E)-(MPDMT)-5,4-diyl))bis(1-phenyl-4,5-dihydro-1H-pyrazole-3,5-diyl))dianiline

The spectrum FT-IR of derivative (5) exhibited a characteristic absorption band at(3292cm^{-1}) for (NH_2), (3090cm-1) for H-aromatic, (2976cm-1) to H- aliphatic, (1591cm^{-1}) to (C=N), (1448cm^{-1}) to N=N we notice the absence of the carbonyl group signal in the spectrum indicates the successful formation of the pyrazole derivative Figure (13). (^{1}H-NMR) data of (5) compound show 2.50 DMSO-d6,3.39 ppm (s,3H ,H-1) triazol,4.21 ppm (d,2H,H-3) pyrazole, 3.59 ppm (S,2H,H-2), 4.21 ppm (t,1H ,H-4) pyrazole and 8.82 ppm-6.50 ppm for aromatic hydrogen 8.89 ppm (s ,1H,NH_2) Figure (14).^{13}C-NMR spectrum data of compound (5) show: 39.94 ppm DMSO-d6, 26.81ppm (C-1,CH_3) , 153.61 ppm (C-N),169.54 ppm (C=N) pyrazole,150.06-109.50 ppm for (C) aromatic, 45.13 ppm (C-5) triazol, 36.96ppm (C-4) pyrazole, 36.50 ppm (C-2 ,CH2), 36.52 ppm(C-3), 51.07 ppm (C-6) triazol we notice the absence of the carbonyl group signal in the spectrum indicates the successful formation of the pyrazole derivative Figure (15).

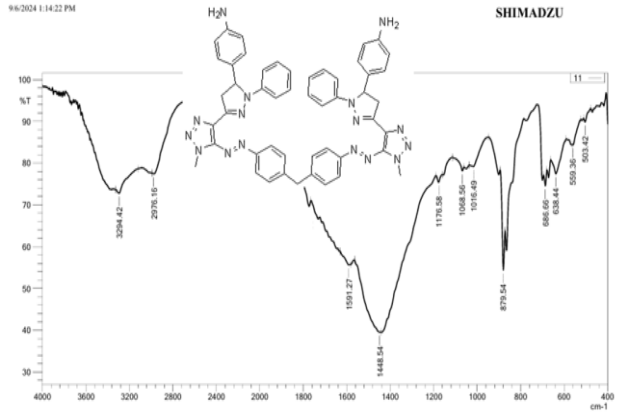

Fig. (13): FT-IR Measurement of (5) Compound.

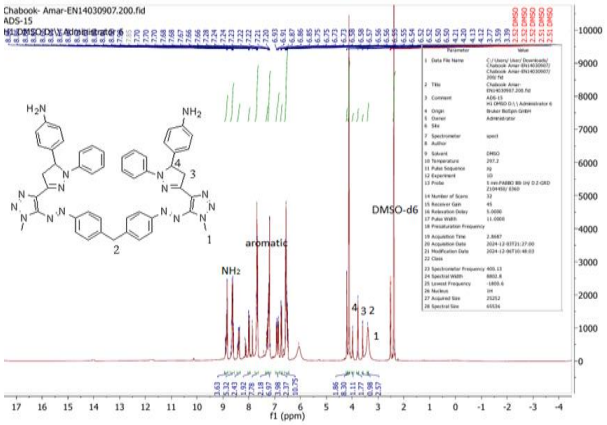

Fig. (14): ^{1}H-NMR Measurement of (5) Compound.

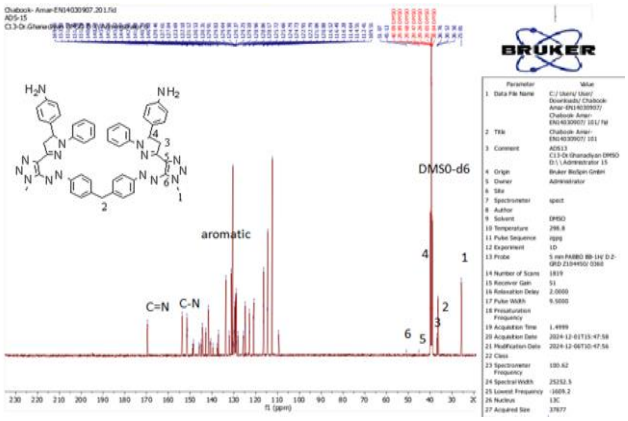

Fig. (15): ^{13}C-NMR Measurement of (5) Compound.

5. Study of Biological Activity Against Bacteria.

Escherichia coli Gram (-ve) bacterial strains were evaluated against the synthesized derivatives and *(Staphylococcus aurous)* (+ve) bacteria . Which are showed greater effectiveness against *E.coli* for compounds (1,3,4) and greater effectiveness against *S. aureus* for compound (3 , 4).

The potency was concentration – dependent with the highest inhibition observed at 10mm, Notably, compound 1,3 had the strongest anti-*E.coli* action. However, compound 3 demonstrated the most anti-*S. aureus* activity at 16 mm as showed in Figure (16) and Figure (17).

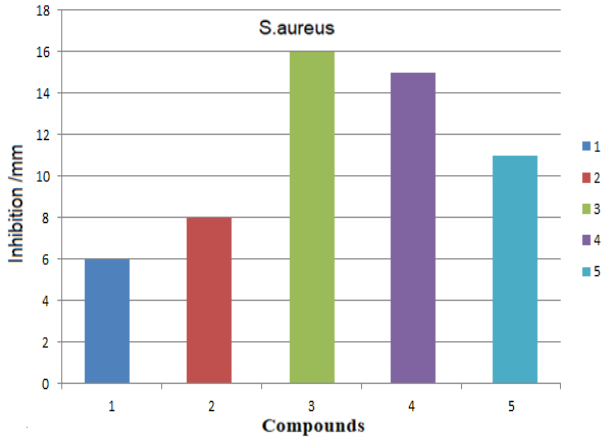

Fig. (16): Antibacterial activity of screened compounds (1,2,3,4,5) against *S.aureus*

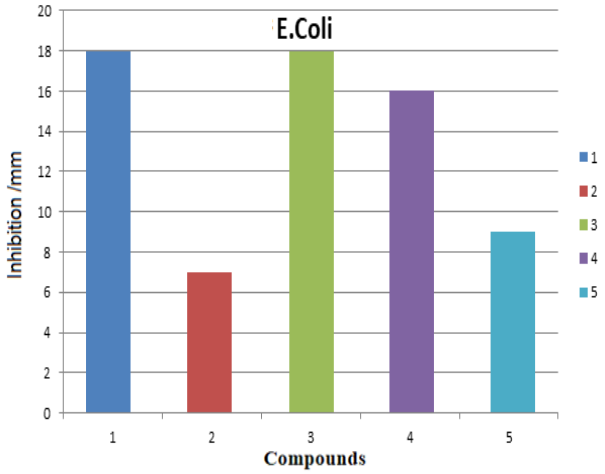

Fig. (17): Antibacterial activity of screened compounds (1,2,3,4,5) against *E.coli*.

6. Conclusions

This study focused on the discovery of novel heterocyclic compounds and the development of efficient synthetic routes for their preparation. The synthesized compounds were fully characterized using various spectroscopic techniques, including NMR and FTIR, along with the assessment of their physical properties. Furthermore, their antibacterial activity was evaluated against both *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative). Notably, compounds 3 and 4 exhibited significant antimicrobial activity against both strains. This enhanced biological performance is likely attributed to the presence of amino groups (–NH_2) in their structures, in addition to the triazole ring, which may play a synergistic role in boosting their efficacy**.**

Table1: The effect of prepared compounds dissolved in DMSO solvent at a concentration of (0.03 mg/ml) on two types of bacteria.

Comp no.	*E.Coli*	Diameter in mm	*Staph aureus*	Diameter in mm

DMSO	**-**	**-**	**-**	**-**
1	**++**	**18**	**+**	**6**
2	**+**	**7**	**+**	**8**
3	**++**	**18**	**++**	**16**
4	**++**	**16**	**++**	**15**
5	**+**	**9**	**++**	**11**

Highly active (+++): inhibition zone greater than 20 mm

Moderately active (++): inhibition zone between 15–20 mm

Weakly active (+): inhibition zone between 10–15 mm

Inactive (−): inhibition zone less than 10 mm

Acknowledgments

The author is grateful the College of Education in University of Al- Qadisiyah Department of Chemistry.

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