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Synthesis of Some New Azo Compounds Derivatives and Study Their Biological Activity

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Abstract

Azo compounds are important class of compounds and have versatile applications in various fields of life. Because of their broad range of applications, it is necessary to have versatile methods of synthesis to have new azo derivatives in good yields. The purpose of this study was to synthesize new azo compounds (Z1-Z4) and verify their structures using 1H & 13C-NMR and FT-IR spectroscopy. Additionally, the study aimed to examine the antibacterial effects of these compounds on medically significant Gram negative (Escherichia coli) and Gram positive (Staphy- lococcus) bacterial strains, as well as fungal strains (Aspergillus Niger, aspergillus fumigatus). Novel azo derivatives had good results when tested with Staphy- lococcus aureus, Escherichia coli as well as against Aspergillus Niger, aspergillus fumigatus fungi.

Keywords: azo compound, tetra ethyl ammonium salts, antibacterial, antifungal.

1.Introduction

Azo compounds are characterized by the superior colouring features due to the chromophore group (-N=N-) connected to heterocyclic or aromatic systems, and their significance could increase in the future. They are essential for the regulation of the dyeing and printing sector. The synthesis of these dyes employs a simple procedure of coupling and diazotization. Several techniques and changes are used to achieve the required colour characteristics, yield, and particle diameters of the dye for enhanced dispersibility [1]. These compounds are very beneficial in pigment manufacturing and are used as colorants in the textile industry, in addition to serving many other uses [2].

Azo dyes are the predominant category of dyes, constituting about 60% of the overall dye market [3]. Around seventy percent of all dyes used in industry are azo dyes [4,5].

Commercial diazo compounds were evaluated for their antibacterial properties, as shown in the paper by Oros et al. It was discovered that, in contrast to the sensitivity for biological effects, the essential chemical structure of synthetic dyes significantly influences the strength of their antibacterial effectiveness [6].

Non-commercial azobenzenes with heteroaromatic moieties may provide antimicrobial compounds, such azo dyes with pyrimidines [7], indoles [8], or nicotinate derivatives [9]. In this context, Aiube et al. showed that azo-based chalcones had remarkable efficacy against Candida albicans and Serratia marcescens in comparison to conventional pharmaceutical anti-biotics and anti-fungal therapies. The findings indicated that azo compounds have activity not only against S. marcescens, a Gram-negative opportunistic bacterium, but also against the yeast C. albicans. Azo moieties, as coloring agents, may exhibit antibacterial properties; nevertheless, the design of functional groups must be executed with precision [10].

For example, Fawzia F. Albelwi, Menier Al-anazi, et al. investigated the in-vitro antibacterial efficacy of oxazolone azo dyes against two gram-negative bacteria (Escherichia coli and Pseudomonas aeruginosa) and two gram-positive bacteria (Bacillus subtilis and Streptococcus pneumoniae) using the agar diffusion method at varying concentrations, with Gentamicin and Ampicillin as controls, and noted their significant potential relative to the reference antibiotics. The study demonstrated that the oxazolone azo chromophore derivatives exhibited moderate to substantial efficacy against gram-negative bacteria, including Escherichia coli, and gram-positive bacteria such as Bacillus subtilis and Streptococcus pneumoniae, but were ineffective against the gram-negative bacterium Pseudomonas aeruginosa [11], Mohammed Bouhdada, Mohamed E.L. et al. have synthesized a metal complex with an azo ligand, which has been assessed for its antibacterial characteristics. The agar disc diffusion method was used to assess the bacterial resistance of the azo dye and its complexes relative to the reference antibiotic, imipenem, against six bacterial strains [12].

In this work, we reported the synthesis and characterizations of organic salts (Z1-Z4) (as shown in Figure 1) and biological activity of these compounds as antibacterial and anti-fungal.

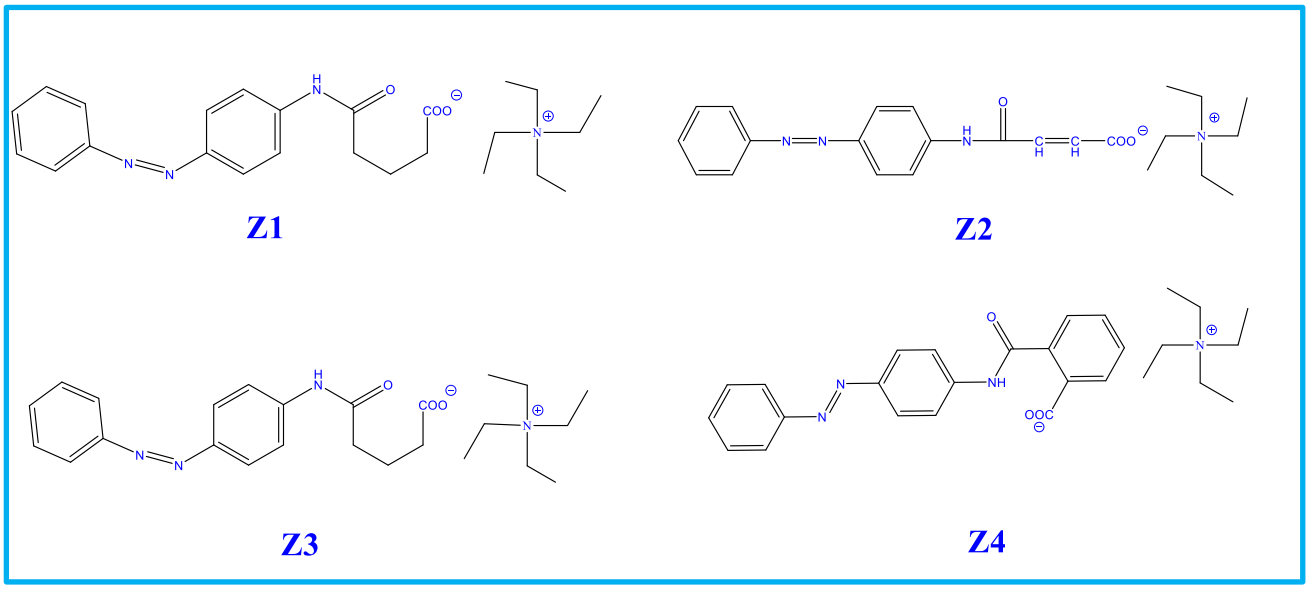


Figure.1. New azo derivatives

2. Experimental part

2.1 Chemicals

All starting components were obtained from Sigma Aldrich and used without further purification.

2. 2. Instrumentation

The melting point was measured by the open capillary technique with a hot stage Gallen Kamp melting point instrument, and the measurement was uncorrected. The structures of the novel organic salts were validated using Brucker FTIR spectrophotometry, with a wave number range of (4000- 400 cm^{-1}). Brucker / 1H-NMR and 13C-NMR were used with DMSO/d6 as the solvent.

2.3. Synthesis of tetraethyl ammonium salts

The synthesis of tetraethyl ammonium salts yielded the following names:4-oxo-4-((4-(phenyl diazenyl) phenyl) amino) butanoate tetraethyl ammonium salts (Z1), 4-oxo-4-((4-(phenyl diazenyl) phenyl) amino) but-2-enoate Tetraethyl ammonium salts (Z2), 5-oxo-5-((4-(phenyldiazenyl) phenyl) amino) pentanoate Tetraethyl ammonium salts (Z3), 2-((4-(phenyldiazenyl) phenyl) carbamoyl) benzoate Tetraethyl ammonium salts (Z4). The synthesis of these organic ammonium salts (Z1-Z4) is detailed below:

The mixture of 0.01 mol of the amine derivative 4-(phenyl) diazenyl aniline with (0.01mol) of (succinic anhydride, maleic anhydride, glutaric anhydride, and phthalic anhydride) in 10 mL of THF as a solvent was stirred for three hours at room temperature and then permitted to stand for 24 hours at the same temperature, resulting in the formation of amic acid compounds (A1-A4). Equal moles of each amic acid (A1-A4) and 35% tetraethyl ammonium hydroxide were thereafter mixed with 10 mL of methanol as a solvent and stirred at room temperature for 3 hours. The mixture was let to dry for 24 hours at 37 °C to produce tetraethyl ammonium salts (Z1-Z4).

A1: 4- oxo-4- ((4-(phenyl diazenyl) phenyl) amino) butanoic acid, m.p: 112-115°C, Yield: 95%, FTIR data (cm-1): -C -H aliphatic :2923,2845, N- H amide :3263, =C -H aromatic: 3023, -C =O amide: 1647, -C=O carboxylic: 1687.

A2: 4 -oxo- 4-((4-(phenyldiazenyl) phenyl) amino) but-2-enoic acid, m.p: 110-113°C, Yield: 96.66%, FTIR data (cm-1): =C- H aromatic: 3017, N- H amide: 3394, -C=O amide: 1625, -C=O carboxylic: 1675.

A3: 5 -oxo- 5-((4-(phenyldiazenyl) phenyl) amino) pentanoic acid, m.p: 111-113°C, Yield: 97%, FT-IR data (cm-1): -C-H aliphatic 2945, 2830, N-H amide 3125, =C-H aromatic 3045, -C=O amide 1650, -C=O carboxylic 1670.

A4: 2-((4 -(phenyldiazenyl) phenyl) carbamoyl) benzoic acid, m.p: 110-112°C, Yield: 87.55 %, FT-IR data (cm-1): =C-H aromatic 3050, N-H amide 3235, -C=O amide 1632, -C=O carboxylic 1660.

Z1: 4- oxo -4-((4-(phenyl diazenyl) phenyl) amino) butanoate tetraethyl ammonium salts, m.p: thick liquid, Yield:91%, FT-IR data (cm-1): -C-H aliphatic 2987, 2890, -N-H amide 3235, =C-H aromatic 3020, -C=O amide 1650, -C=O carboxylic 1675, 1H- NMR data (ppm): 1H of –NH-CO- (s, 13. 4); 9H of aromatic ring (m, 6.2-7.6); 8H of CH2 (m, 3.2); 4H of CH2-CH2 (m, 2.2-2.4); 12H of CH3 (m, 1.2), , 13C-NMR data (ppm): C=O amide (174); C=O acetate (173); aromatic rings (113-155); 4 carbons CH2 (51); 2 carbons CH2 (34, 35); 4 carbons CH3 (7).

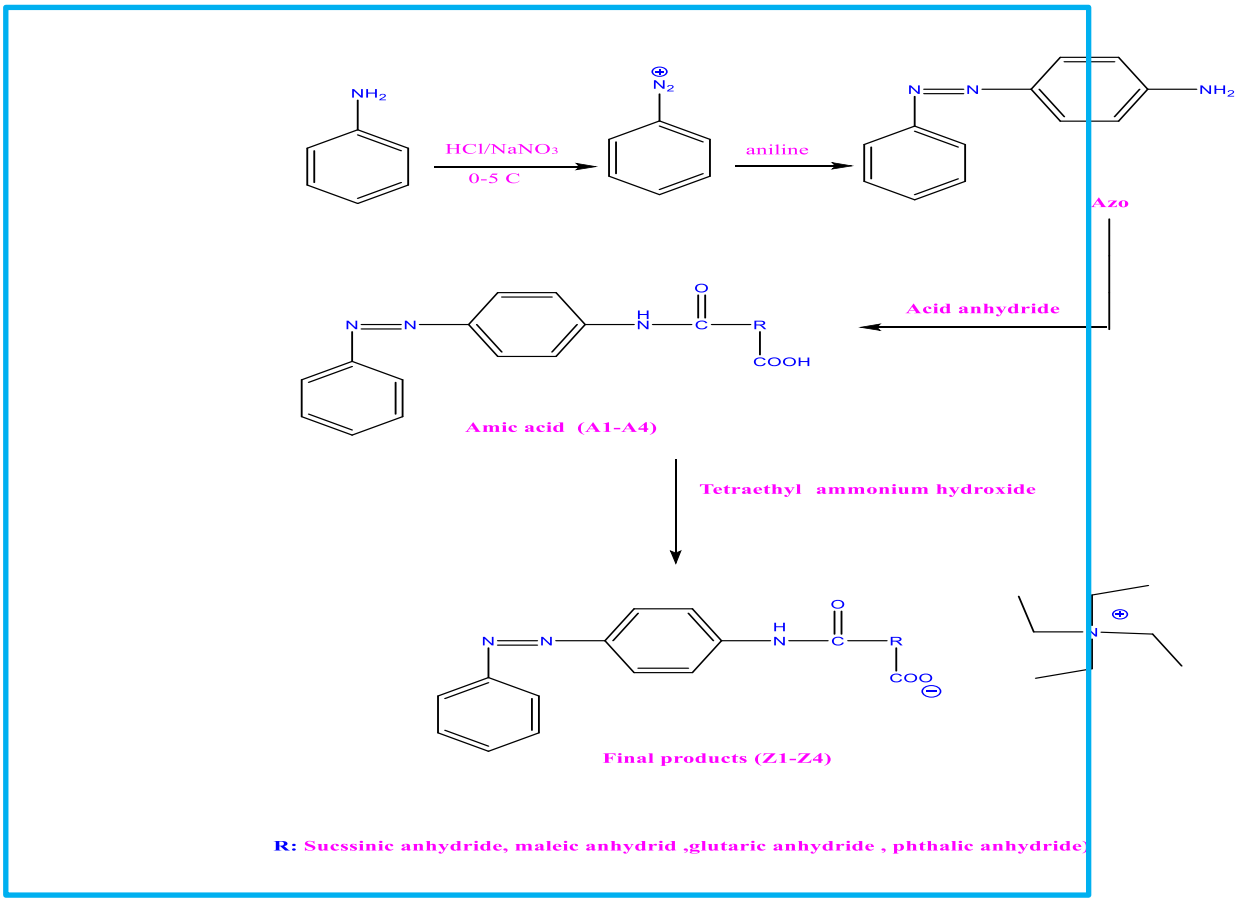
Z2: 4- oxo- 4-((4-(phenyl diazenyl) phenyl) amino) but -2 -enoate Tetraethyl ammonium salts, m.p: thick liquid, Yield:90 %, FT-IR data (cm-1): =C-H aromatic 3245, -N-H amide 3235, -C=O amide 1625, -C=O carboxylate 1680,1H- NMR data (ppm): 1H of –NH-CO- (s, 14.5); 9H of aromatic ring (m, 6.2-7.6); 2H of CH=CH (m, 6.2-6.3); 8H of CH2 (m, 3.2); 12H of CH3 (m, 1.2), 13C-NMR data (ppm): C=O amide (168); C=O acetate (164); aromatic rings (114-153); 4 carbons CH2 (51); 2 carbons CH (122, 142); 4 carbons CH3 (7).

Z3: 5-oxo-5-((4-(phenyldiazenyl) phenyl) amino) pentanoate Tetraethyl ammonium salts, m.p: thick liquid, Yield:92 %, FT-IR data (cm-1): -C-H aliphatic 2987, 2851, -N-H amide 3238, =C-H aromatic 3040, -C=O amide 1660, -C=O carboxylate 1673, 1H- NMR data (ppm): 1H of –NH-CO- (s, 12.4); 9H of aromatic ring (m, 7.2-7.6); 8H of CH2 (m, 4.1); 4H of CH2-CH2 (m, 2.0-2.5); 2H of CH2

(m, 1.6); 12H of CH3 (m, 1.2), 13C-NMR data (ppm): C=O amide (175); C=O acetate (172); aromatic rings (117-147); 4 carbons CH2 (51); 3 carbons CH2 (31,33,35); 4 carbons CH3 (7).

Z4: 2-((4-(phenyldiazenyl)phenyl) carbamoyl) benzoate Tetraethyl ammonium salts, m.p: thick liquid, Yield: 90.30 %, FT-IR data (cm-1): =C-H aromatic 3020, -N-H amide 3238, -C=O carboxylate 1667, -C=O amide 1624, 1H- NMR data (ppm): 1H of –NH-CO- (s, 15.0); 13H of aromatic ring (m, 7.3-8.0); 8H of CH2 (m, 3.2); 12H of CH3 (m, 1.2), 13C-NMR data (ppm): C=O amide (171); C=O acetate (166); aromatic rings (118-152); 4 carbons CH2 (51); 4 carbons CH3 (7)

Scheme.1 show the steps of synthesis of tetraethyl ammonium salts as new azo compounds.



Scheme. 1. synthesis of tetraethyl ammonium salts as new azo compounds (Z1-Z4)

3. Results and Discussion

3.1. FTIR spectra

Chemicals usually show absorption bands of medium to high intensity in the range of 3100-2500 cm $^{-1}$, which may be attributed to the stretching mode of the O-H bonds. Aromatic rings (C=C) have been identified based on their characteristic ring vibrations within the spectral range of 1593-1424 cm^{-1}. The proposed framework of azo dyes is substantiated by the existence of moderately intense peaks, particularly for (N=N) bonds in azo compounds, within the range of 1454-1463 cm^{-1} for the synthesized compounds. The presence of the N-H bond is indicated by the spectral bands in the range of about 3300-3200 cm^{-1} in the spectra of azo compounds. All final products (Z1-Z4) showed C=O stretching bond at

values less 1700 cm^{-1}[13]. Table (1) reveal stretching bands for characterization for compounds (Z1-Z4) [14].

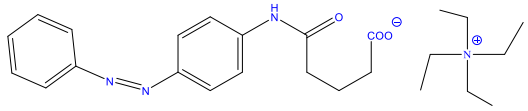
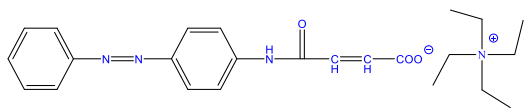
Table (1): Stretching bands (cm^{-1}) for characterization for salts (Z1-Z4)

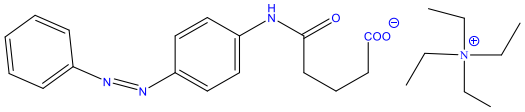
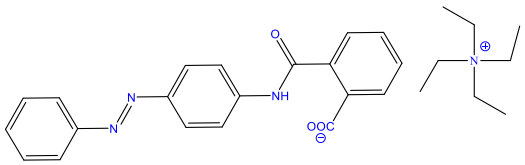
Comp. No.	C-H Aromatic	C-H Aliphatic	C=C Aromatic	C=O Carboxylate and amide	N-H Amide	$-N^{+}-H$
Z1	3027	2984,2895	1563	1677,1630	3196	3665
Z2	3230	2982,2893	1556	1668,1620	3388	3665
Z3	3020	2984,2890	1561	1673,1660	3238	3356
Z4	3027	2983,2894	1550	1670,1630	3394	3664

3.2. ^{1}H & ^{13}C-NMR spectra

To identify the structure of azo compounds (Z1-Z4), The signals seen in the range of 10-14 ppm are singlets and are assigned to the NH of the amide group. On the other hand, the signals in the region of 7-8 ppm are multiples and are credited to the Ar-protons. At higher field ranges, multiple signals are observed, which are related to the aliphatic protons of the ethyl groups, appearing at 1-3 ppm. On the other hand, the structure of azo compounds (Z1-Z4) was confirmed by 13CNMR, the following signals at the range 164-177 for C=O amide and acetate. aromatic rings (112-145) and there are signals attributed to aliphatic protons of ethyl groups which appeared at range (7-51) [15,16]. Table (2) reveal characterization of final products.

Table (2): ^{1}H& ^{13}C-NMR data (ppm) for characterization of azo compounds.

Comp. No.	Compound structure	^{1}H-NMR data	$^{13}CNMR$
Z1		1H of –NH-CO- (s, 12.1); 8H of aromatic ring (m, 6.2-7.6); 8H of CH_2 (m, 3.2); 4H of CH_2-CH_2 (m, 2.2-2.4); 12H of CH_3 (m, 1.2).	C=O amide (167); C=O acetate (164); aromatic rings (118-157); 4 carbons CH_2 (51); 2 carbons CH_2 (34, 36); 4 carbons CH_3 (7)
Z2		1H of –NH-CO- (s, 12.1); 9H of aromatic ring (m, 7.4-8.0); 2H of CH=CH (m, 6.5-6.6); 8H of CH_2 (m, 3.2);	C=O amide (176); C=O acetate (173); aromatic rings (114-152); 4 carbons CH2 (51); 2

		12H of CH_3 (m, 1.2)	carbons CH (124, 143); 4 carbons CH3 (7)
Z3		1H of –NH-CO- (s, 10.4); 8H of aromatic ring (m, 7.2-7.6); 8H of CH2 (m, 4.1); 4H of CH2-CH2 (m, 2.0-2.2); 2H of CH2 (m, 1.6); 12H of CH3 (m, 1.2)	C=O amide (177); C=O acetate (172); aromatic rings (115-144); 4 carbons CH_2 (51); 3 carbons CH_2 (22,36,37); 4 carbons CH_3 (7)
Z4		1H of –NH-CO- (s, 14.4); 12H of aromatic ring (m, 7.2-8.2); 8H of CH2 (m, 3.2); 12H of CH3 (m, 1.1)	C=O amide (172); C=O acetate (171); aromatic rings (119-145); 4 carbons CH2 (51); 4 carbons CH3 (7)

3.3. Biological activity

-Bacterial and Fungal Strains

The experiment used *Escherichia coli* and *Staphylococcus aureus* as the bacteria, and *Aspergillus Niger* and *Aspergillus fumigatus* as the fungus. The bacterial strains were stored on nutritional agar medium at a temperature of 37∘C, while the fungus strains were preserved on (PDA) potato dextrose agar at a temperature of 25∘C.

- Antifungal and Antibacterial Activities

The antibacterial efficacy of recently developed substances was assessed using the agar disc diffusion test [16,17]. In summary, a culture of bacteria that was 24 hours old and a culture of fungus that was 48 hours old were combined with sterile physiological saline (0.9%). Petri plates, each comprising 20mL of Sabouraud-dextrose agar and Mueller Hinton agar, were used to assess antifungal and antibacterial activity (see to Figure 2 and 3). The inoculums were evenly distributed over the surface of the solidified medium. Filter paper discs, specifically Whatman No. 1, with a diameter of 5mm, were soaked with the test compound (20μL per disc) and deposited on the solidified media. The diameter of the zone of inhibition was determined by measuring it in millimeters after incubating bacterial strains at a temperature of 37∘C for a duration of 24 hours, and fungal strains at a temperature 25∘C for 72 hours, in comparison to the controls of antibacterial Amoxicillin and antifungal fluconazole. The compounds were dissolved in dimethyl sulfoxide under the specified test conditions, at a concentration of 500 µg/mL. An empty test demonstrated that the test organisms were not influenced by the DMSO used to create the test solutions [18]. Antibacterial and antifungal activities of compounds (Z1-Z4) are shown in Figures 2 and 3 respectively.

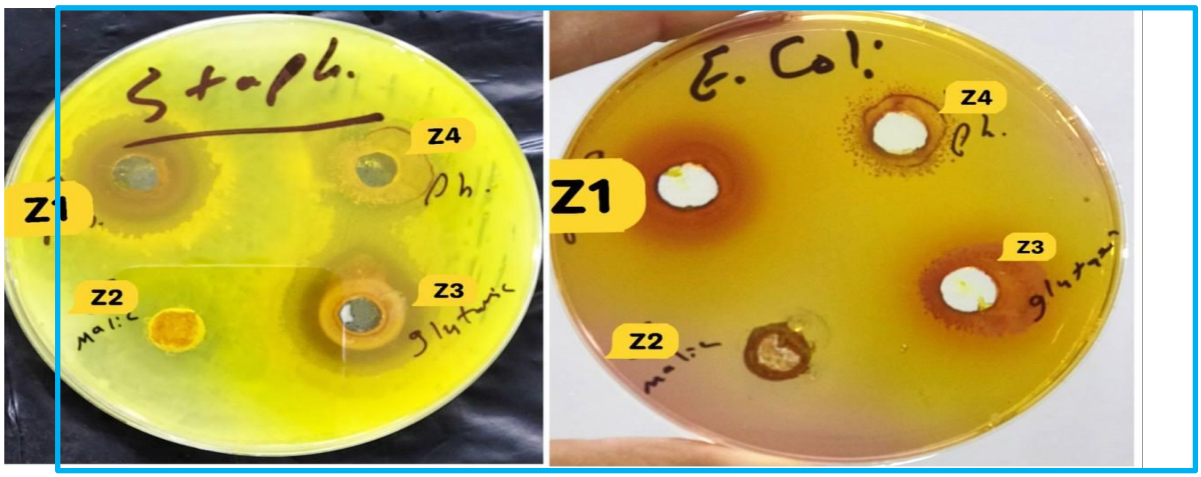


Figure.2. Anti-bacterial activities of compounds (Z1-Z4)

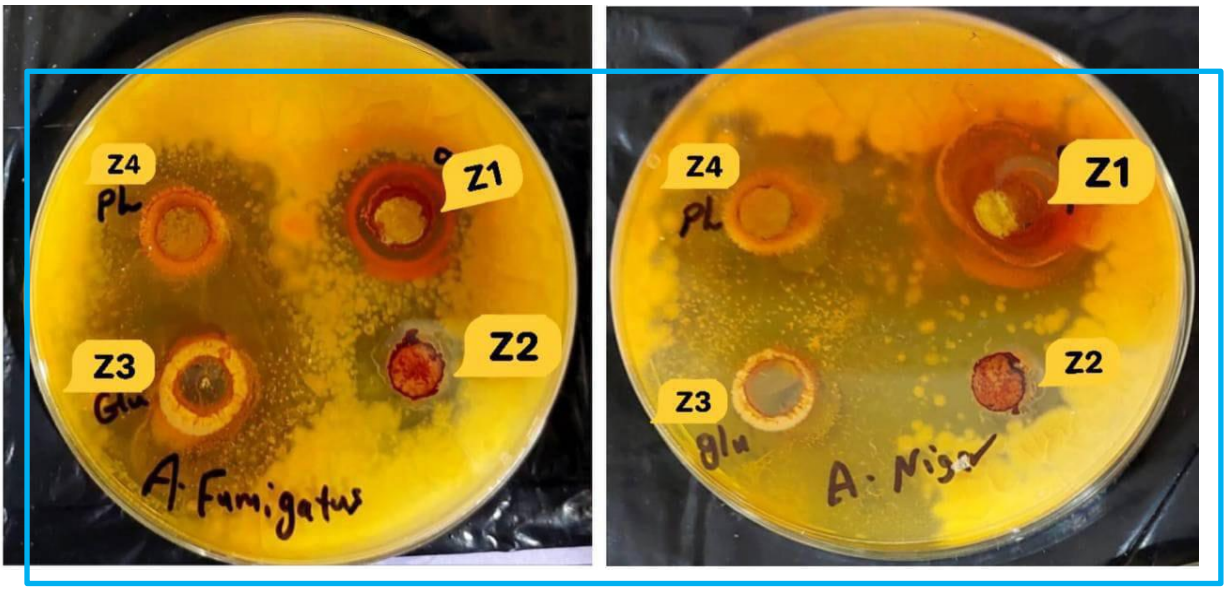


Figure.3. Anti-fungal activities of compounds (Z1-Z4)

Table.3. antifungal and Antibacterial activities of compounds (Z1-Z4)

Compounds No.	Diameter of zone of inhibition (mm)			
	E. coli	*Staphylococcus aureus*	*Aspergillus Niger*	*Aspergillus fumigatus*
Z1	20	24	21	23
Z2	10	14	24	14
Z3	18	22	26	33
Z4	14	20	24	27
Amoxicill	19	21	-	-

in				
Fluconazole	**-**	**-**	**14**	**20**
DMSO	**0**	**0**	**0**	**0**

The new azo compounds (Z1-Z4) had antibacterial activity that ranged from moderate to excellent when tested against various strains, in comparison to the antibacterial drug Amoxicillin for comparison (See Table 3). Among Gram-positive bacteria, the Staphylococcus aureus strain showed the most pronounced suppression of growth. Regarding antifungal activity, the compound showed a better anti-fungal effect in Aspergillus fumigatus and Aspergillus Niger, compared to antifungal fluconazole used as controls, as shown in Table 3, as well as, Figures (4,5).

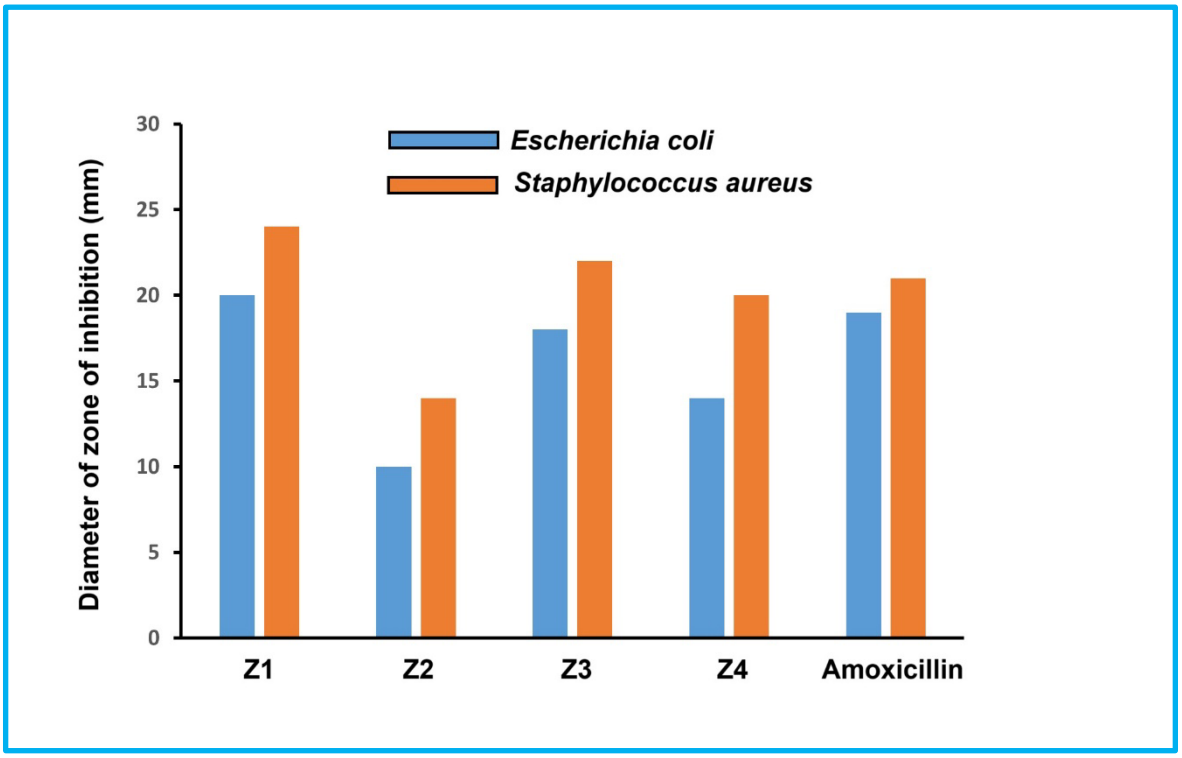


Figure. 4. Comparison between amoxicillin with azo compounds as anti-bacterial

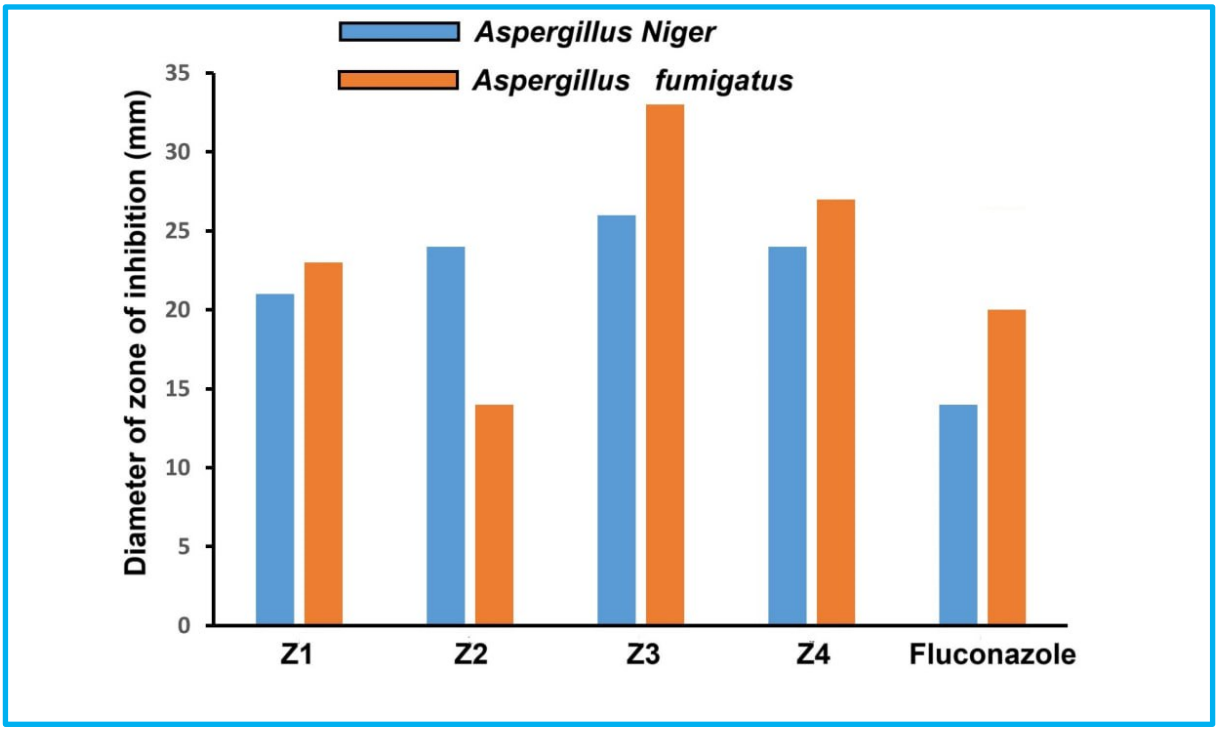


Figure. 5. Comparison between fluconazole with azo compounds as anti-fungi

4. Conclusion

In conclusion, synthesis of four azo compounds. Their physico-chemical properties were determined. The synthesized azo compounds may be classified as direct dyes, based on their coloristic and application qualities. The antimicrobial assessment findings indicated that the azo compounds (Z1-Z4) shown significant activity against both bacteria and fungi, surpassing the effectiveness of the control antibiotics Amoxicillin and fluconazole, which were utilized as benchmarks for antibacterial and antifungal activity, respectively.

Disclosure Statement

The authors indicated that no potential conflicts of interest were present.

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Authors' Contributions

Every author contributed to the information analysis, first draft, and additional modifications of the article. They unanimously decided to accept accountability for each aspect of this endeavor.

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