Journals Pub

International Journal of
Environmental Chemistry

ISSN: 2456-5245
Volume 12, Issue 1, 2026
January–June
DOI (Journal): 10.37628/IJEC

https://chemical.journalspub.info/index.php?journal=JCPDS

Research IJEC

Synthesis, Characterization, Antibacterial Study and Study of Bio Properties for New Analytical Reagents

Nagham Mahmood Aljamali[1,*], Noor Sabah Muttaleb[2], Sara Abdalkareem Moshref[3], Safa Saleem Zaye[4]

Abstract

Due to the widespread pollution of heavy metals in water and environmental samples, the pollution levels have increased. Therefore, researchers have begun working on various environmental solutions to improve the levels of these elements in environmental samples. One such solution is the preparation of analytical reagents as coordination agents and ligands to shield the elements from water and soil, thereby reducing pollution levels. In this study, new analytical reagents were prepared from aniline compounds and others from azo compounds, which are known as analytical reagents with wide-ranging applications in analytical chemistry and biochemistry due to the presence of electron pairs in their hybrid atoms (nitrogen). After preparing the reagents, their precise chemical structures were determined through infrared spectroscopy, resonance spectroscopy, and ultraviolet spectroscopy. Additional studies were conducted, including the evaluation of their biological properties using a chemical analysis software program with Biological Tests.

Keywords: Aldimines, Analytical, Azo, Bacteria, Bio properties, Reagent

INTRODUCTION

Analytical reagents containing active groups are characterized by their ability to react with many elements of the periodic table, especially metallic elements. This property has been exploited by researchers in the field of inorganic chemistry to obtain chelating complexes [1–3], which are widely used in various fields. In analytical chemistry, they have been used for the qualitative and quantitative determination of very small concentrations [4–6]. Analytical reagents that include aniline groups or azo bridge groups are considered to be highly bioactive compounds that have been tested against some types of laboratory animals as antitoxins and antioxidants [7–10], where the hybrid atoms represented by nitrogen (in the azo bridge group) play a major role in the biological activity of penetrating [11–14] the wall of cancer cells and thus killing cancerous cells in cancerous tumors. Some researchers studied evaluation tests for thiazole and imidazole in the field of analytical chemistry [15–19], especially in fluorescence and phosphorylation, and for estimating metal concentrations using coordination complexes [20–22], most of which were in the form of high molecular weight compounds such as coronal ethers. Then the quantity of these elements can be estimated by spectroscopic and physical methods such as atomic absorption [23, 24], as well as estimating some elements in samples taken from patients such as hair samples, body fluids, and tissues., Thiadizole ring derivatives are antioxidants that inhibit free radical activity and protect cells from oxidative damage [25–28]. Thiadiazole derivatives have garnered widespread attention as potent antioxidants, demonstrating the ability to

***Author for Correspondence**
Nagham Mahmood Aljamali
E-mail: dr.nagham_mj@yahoo.com

[1]Professor., Department of Chemistry, College of Education, University of Kufa, Iraq.
[2]Assistant Lecturer, Department of Chemistry, College of Education for Girls, University of Kufa, Iraq.
[3]Assistant Lecturer, Al-Furat Al-Awsat Technical University, Engineering Technical College of Al-Najaf, 31001, Iraq.
[4]Lecturer, Department of Chemistry, College of Education for Girls, University of Kufa, Iraq.

Received date: January 07, 2026
Accepted date: January 27, 2026
Published date: February 10, 2026

Citation: Nagham Mahmood Aljamali, Noor Sabah Muttaleb, Sara Abdalkareem Moshref, Safa Saleem Zaye. Synthesis, Characterization, Antibacterial Study and Study of Bio Properties for New Analytical Reagents. International Journal of Environmental Chemistry. 2026; 12(1): 1–11p.

protect organisms and cells from the damaging effects of oxidative stress. Consequently, scientists across various disciplines are focusing on developing new compounds [29–31], both synthetic and derived from natural sources, that can provide effective components for preventing or mitigating the effects of oxidative stress [32–34]. Thiadiazole derivatives have exhibited broad biological activity through multiple mechanisms of action, including enzymatic activity [35–38]. Their outstanding pharmacological efficacy is attributed to their unique structural properties, such as their moderate polarity [39–41], ability to form hydrogen bonds, and their rigidity and stability under physiological conditions.

MATERIAL AND METHODS

Experimental Part

It is known that azo derivatives are greatly affected by purity levels; therefore, raw material manufacturers with globally recognized origins, known for the precision and purity of their compounds, were selected. Consequently, we observe that the prepared reagents demonstrated high accuracy and clarity in the diagnostic spectra measured.

Synthesis of Reagent {1}

Carboxylic acids are known for their susceptibility to cyclic closure reactions under specific catalysts or through conversion to ester compounds, due to their ease of reactivity in forming closed-ring compounds. We prepared the first reagent from the cyclic closure reaction of benzoic acid (0.02 mol) with (0.02 mol) thiosemicarbazide via reverse sublimation for (28 hours) continuous stirring in (3 ml) of concentrated sulfuric acid to complete the cyclic closure and form an amine-thiadiazole derivative. This derivative was subsequently (0.02 mol) reacted with an aldehyde (0.01 mol) from ortho formal benzaldehyde by using glacial acetic acid in a few drops with ethanol as the solvent for 3 hours concurring to references [10, 17]. After evaporating the solvent and drying the product, it was purified to give a pale orange precipitate with 82% reagent {1}.

Synthesis of Reagent {2}

The second reagent was synthesized from the cyclic closure reaction that was prepared from first step (0.02 mol) reacted with an aldehyde (0.01 mol) from (methoxy formalbenzaldehyde) by using glacial acetic acid in a few drops with ethanol as the solvent for (4 hours). After evaporating the solvent and drying the product concurring to references [10,17], it was purified to give a reddish orange precipitate with 74% reagent {2}.

Synthesis of Reagent {3}

The third reagent was synthesized from the cyclic closure reaction that was prepared from first step (0.02 mol) dissolved in (3 ml) of (HCl) with cold (H_2O) in ice path, then it added to (0.002) of ($NaNO_2$) after that the mixture added to basic ethanolic solution of (0.01 mol) from (4-hydroxy phenol). After (36 hrs) the participation filtered, washed with distilled water, and drying the product concurring to references [10,17], it was purified to give a light orange precipitate with 80% reagent {3}.

Synthesis of Reagent {4}

The fourth reagent was synthesized from the cyclic closure reaction that was prepared from first step (0.02 mol) dissolved in (3 ml) of (HCl) with cold (H_2O) in ice path, then it added to (0.002) of ($NaNO_2$) after that the mixture added to basic ethanolic solution of (0.01 mol) from (4-methyl toluene). After (36 hrs) the participation filtered, washed with distilled water, and drying the product concurring to references [10, 17], it was purified to give a yellowish red precipitate with 78% reagent {4}, (Figure 1).

RESULTS AND DISCUSSION

The (UV-Vis) Assessment

To select the wavelength of the newly prepared aniline and azo reagents, graded standard solutions of the reagents at different concentrations were prepared to determine the optimal ultraviolet spectroscopy using ethanol as a solvent., The violet-visible spectrum of the formed ligands or reagents provides clear evidence of their formation through the formation of azo bridge bonds, which undergo

electron transitions and an electron sequence. Therefore, we observe a more pronounced redshift in the spectra containing azo groups compared to those containing imine groups in other ligands, which exhibited a smaller wavelength shift., as shown in the (Figures 2, 3).

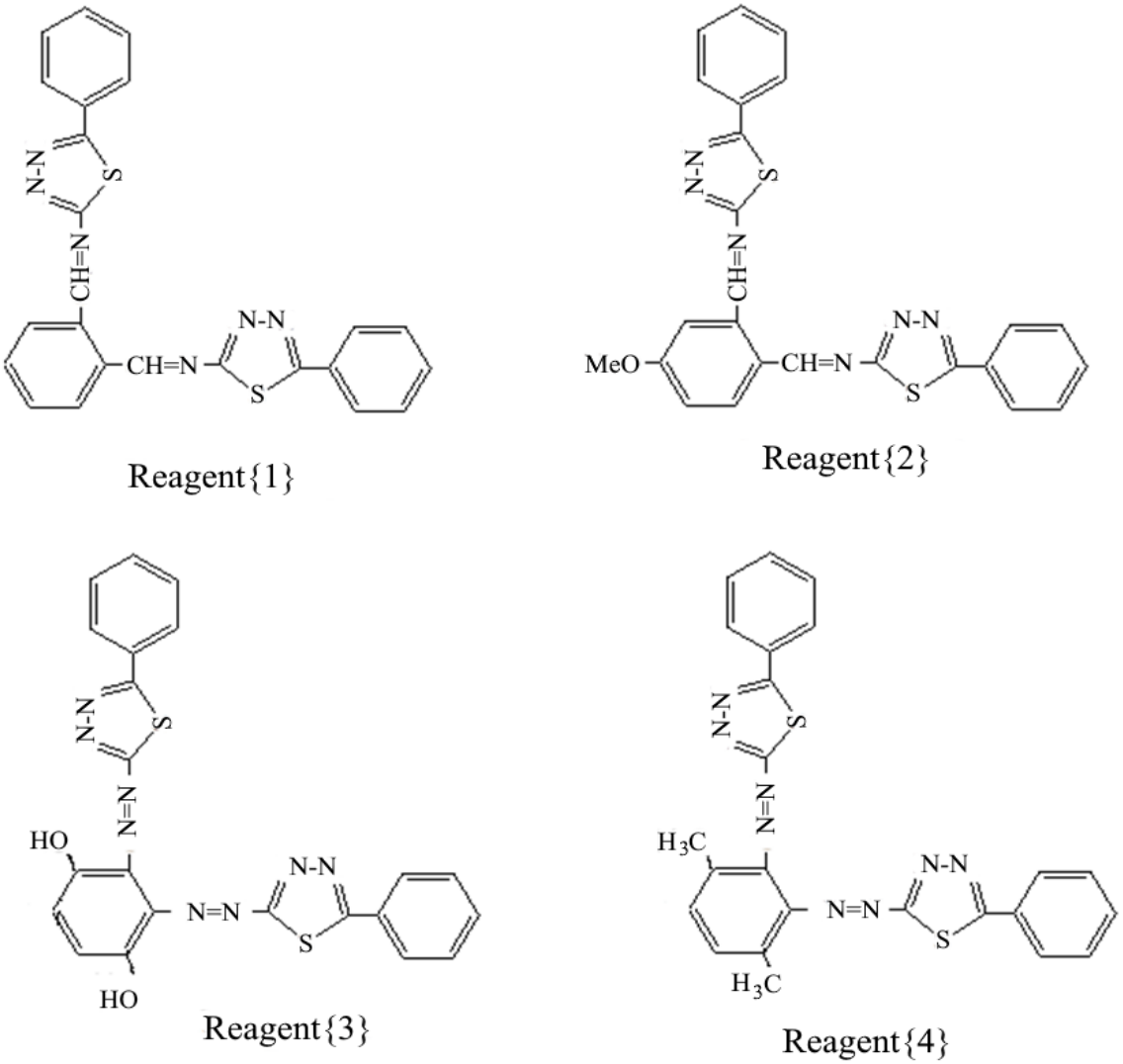

Figure 1. Creation of analytical reagents {1–4}.

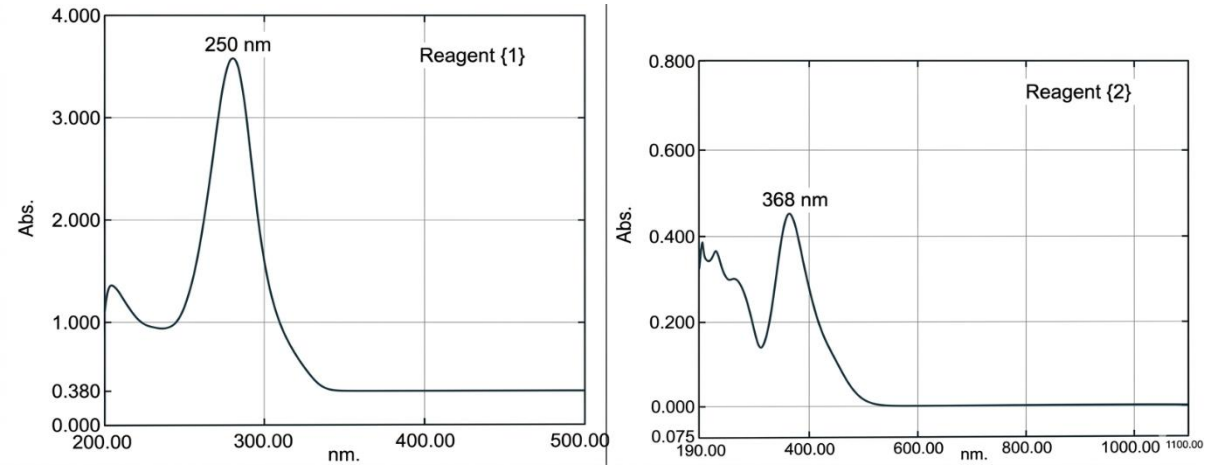

Figure 2. UV-Vis of reagents {1, 2}.

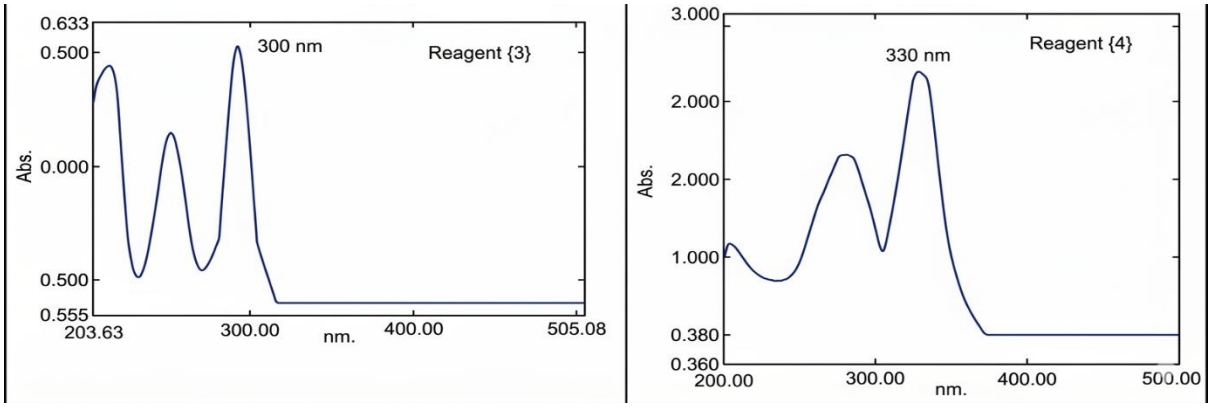

Figure 3. UV-Vis of reagents {3, 4}.

The (IR) Assessment: By observing the spectral figures of the prepared reagents, a band appeared at {1670 ,1680} indicating the formation of the imine group in the reagents {1,2} respectively ,while other bands represented by {(1444, 1498)., (1444,1498)} for azo group in reagents (3, 4}respectively, and it was characterized by being sharp and strong bands, which is one of the known characteristics of the azomethine group, while the band of methoxy (-OCH_3) was observed in the reagent {2} at (1141) cm^{-1} ,and the absorption band at(3446) cm^{-1} indicated and confirmed the presence of the hydroxyl group in the reagent {3)., The infrared spectrum represents the second piece of evidence for ligand formation through the change in the shape and intensity of the spectral bands formed in the detectors as a result of the disappearance of the amine group bands in the ligands, and consequently the formation of the imine group in the first and second ligands, as well as the disappearance of the third and fourth amine groups and the formation of the azo group, which are characterized by being sharp and strong bands. Hence, the other groups and frequencies are revealed in the spectral (Figures 4-7).

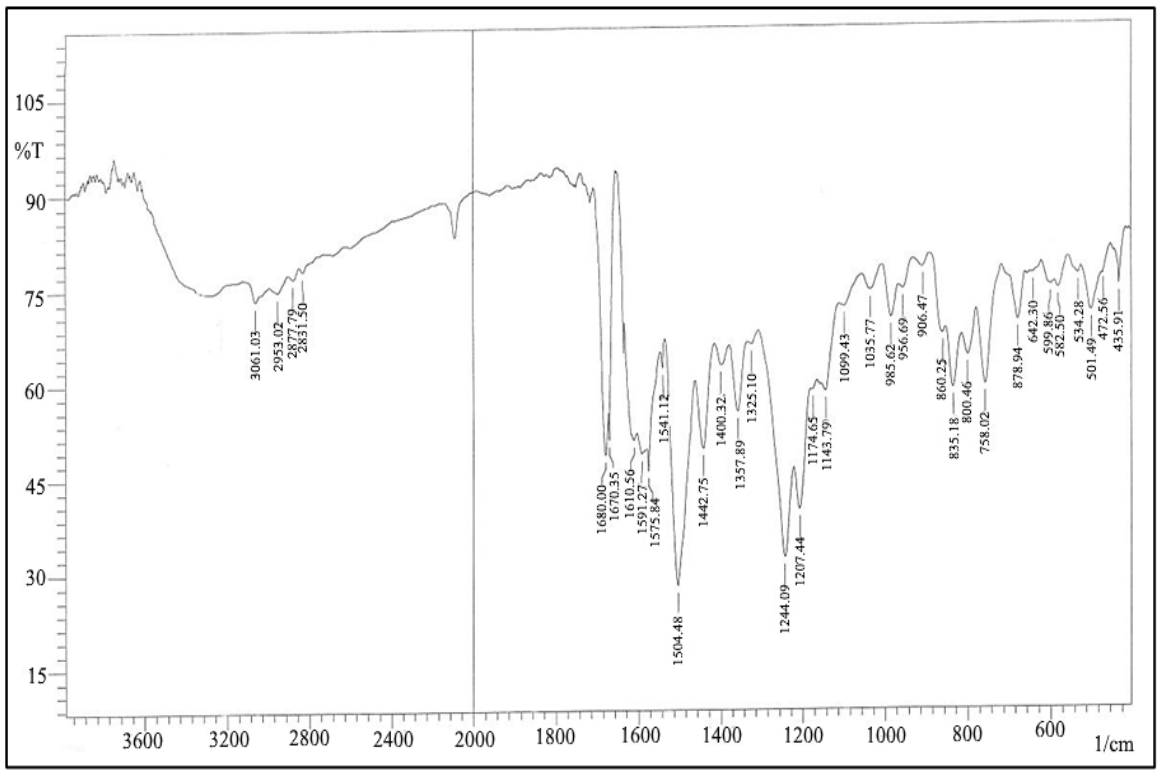

Figure 4. IR for derivative {1}

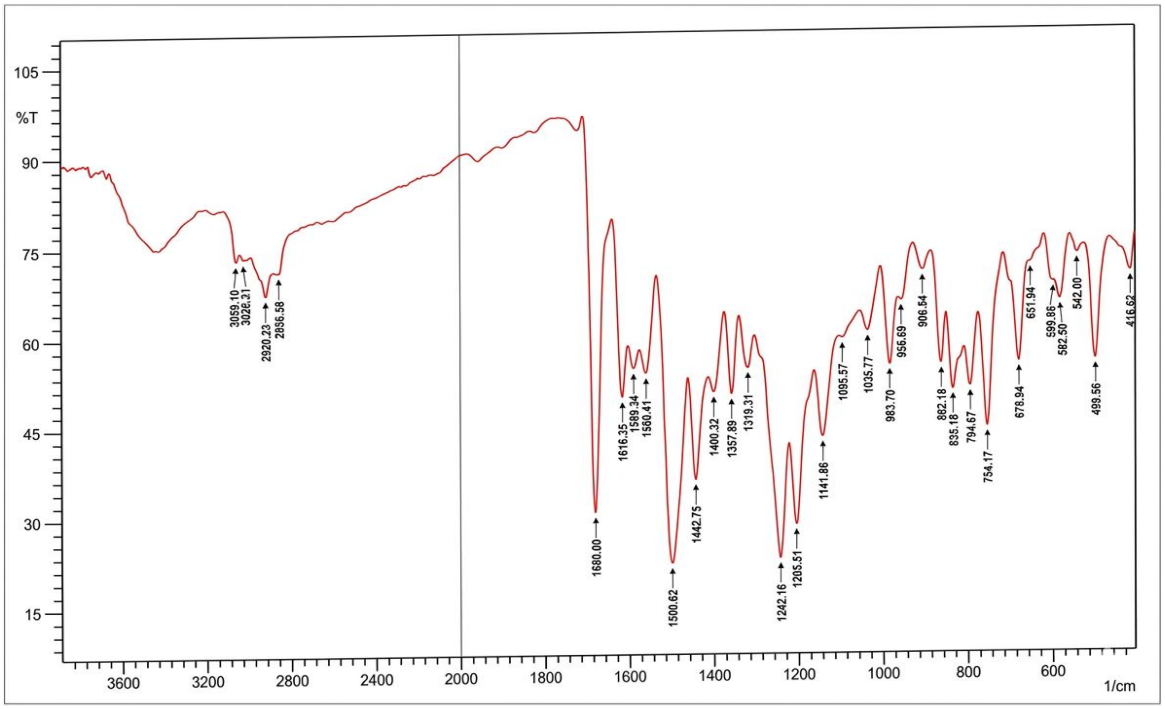

Figure 5. IR for derivative {2}.

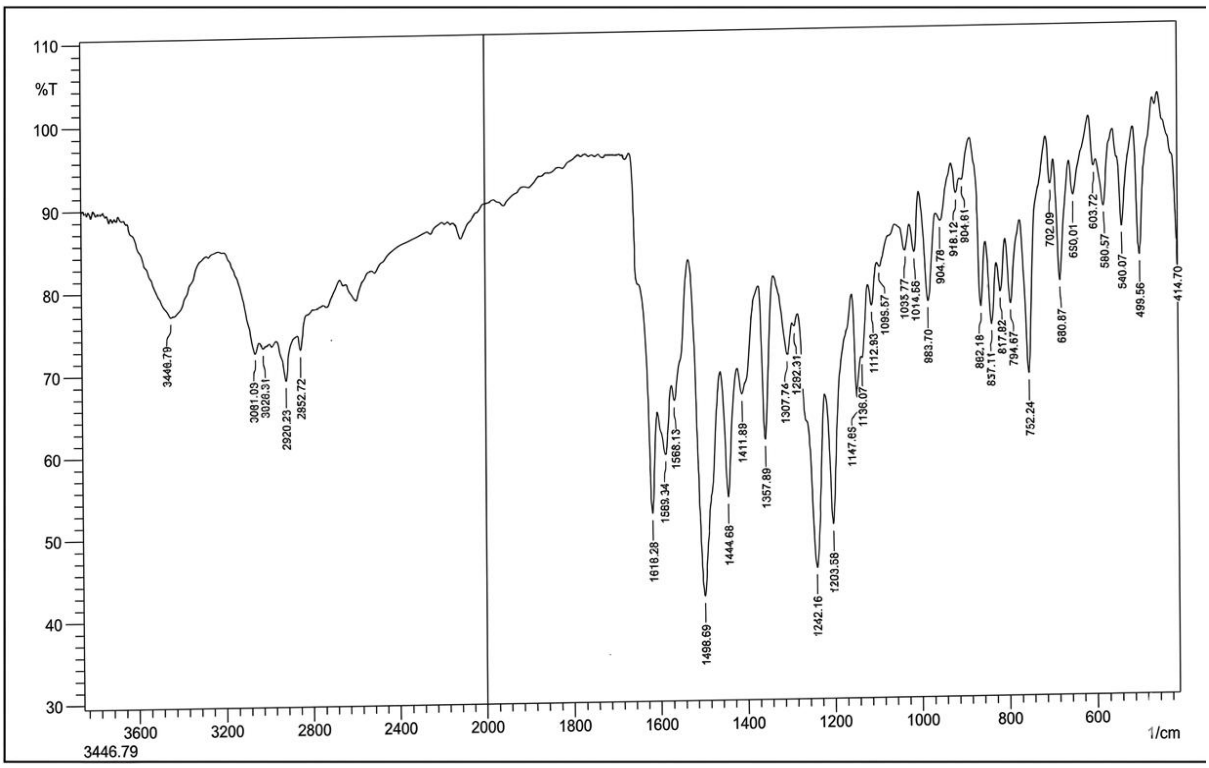

Figure 6. IR for derivative {3}.

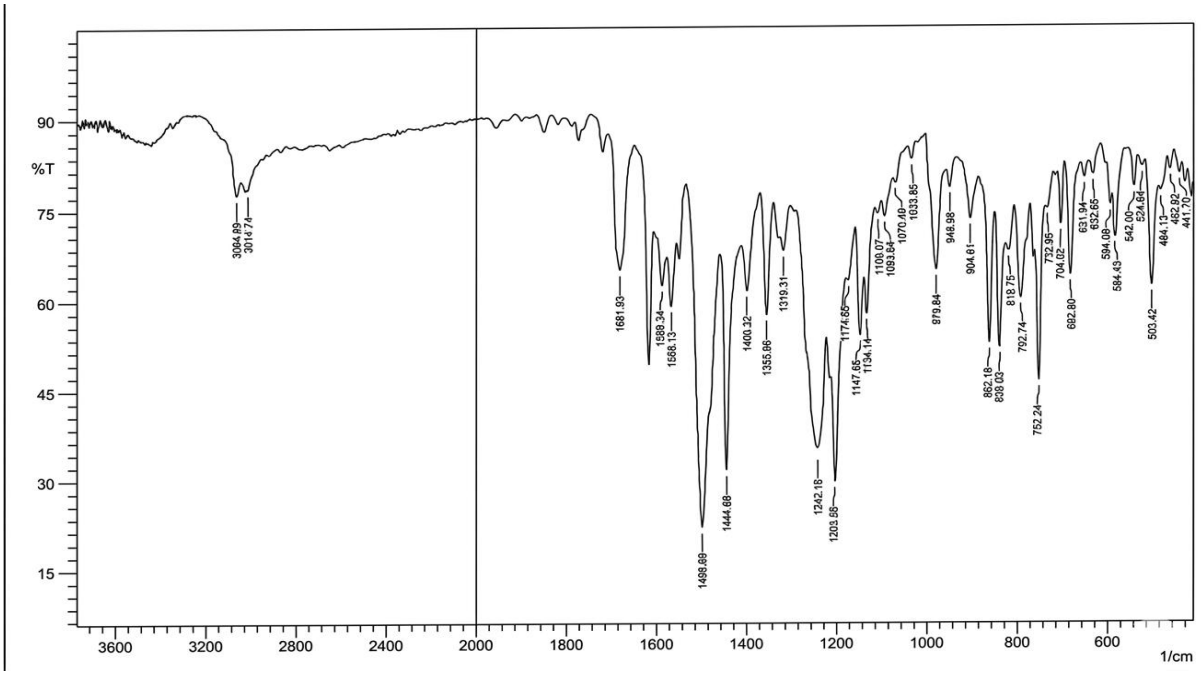

Figure 7. IR for derivative {4}

The (H-NMR) Assessment: By observing the spectral figures of the prepared reagents, a single signal appeared, indicating and confirming the presence of an anile group proton in both reagents {1, 2}at (8.83 , 8.47) and indicating the formation of anile groups in the reagents respectively. In reagent {2}, a clear, distinct signal also appeared for Methoxy group (-OCH_3), while presence of a phenol hydroxyl group (OH) proton in reagent {3} was at (9.08). But in reagent {4}, the signal at (1.24) confirmed the presence of methyl group protons, the other peaks are shown in the spectral (Figures 8-11).

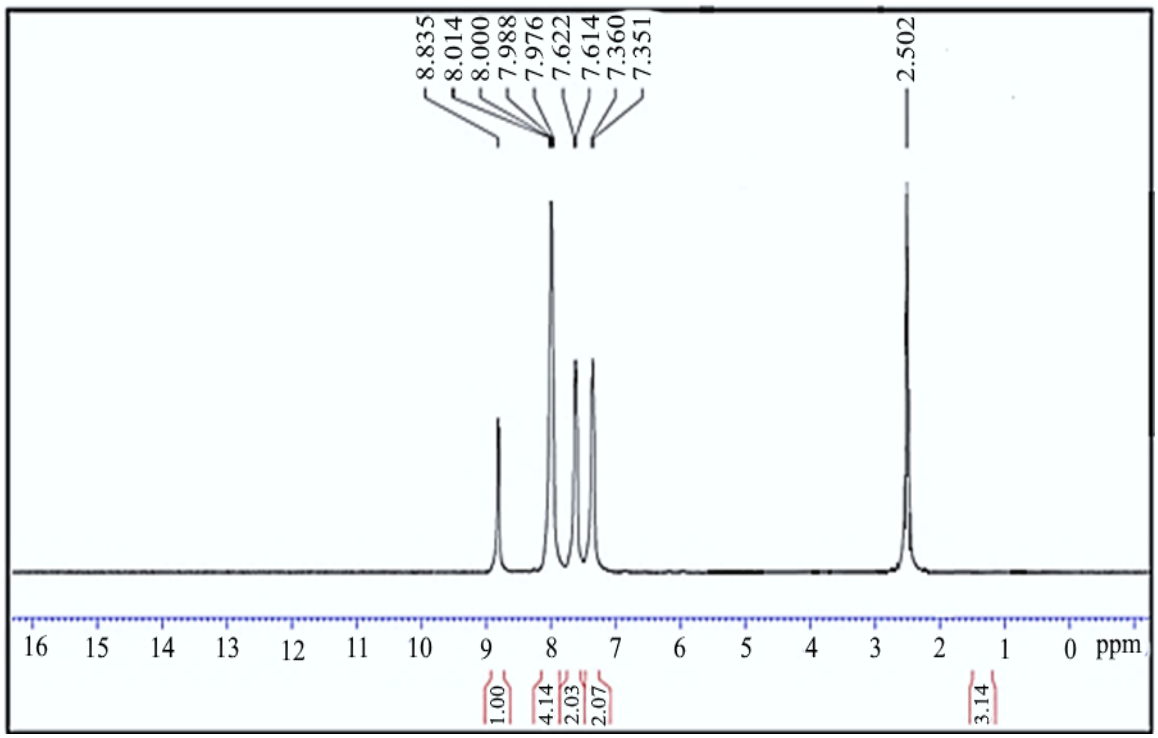

Figure 8. H-NMR for reagent {1}

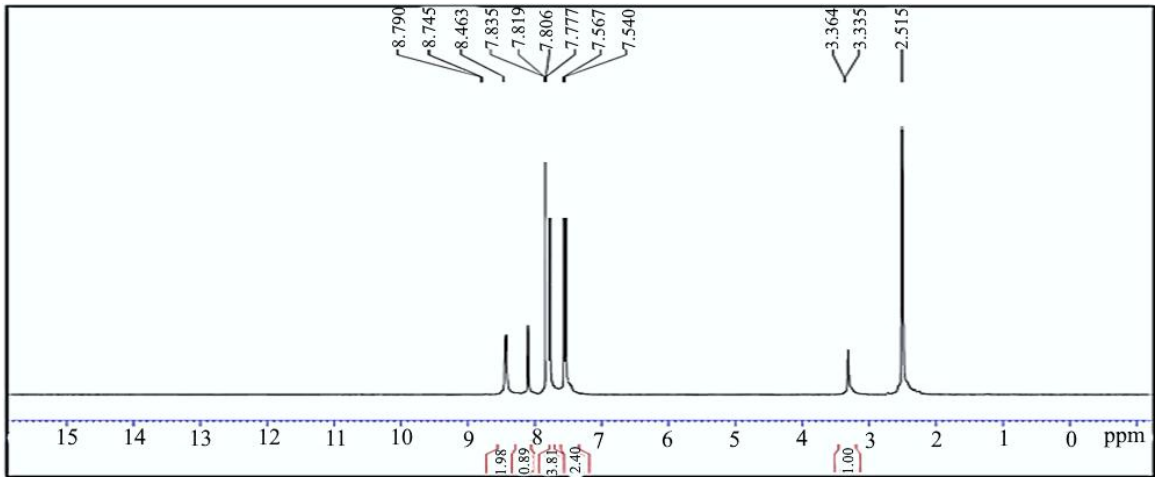

Figure 9. H-NMR for reagent {2}.

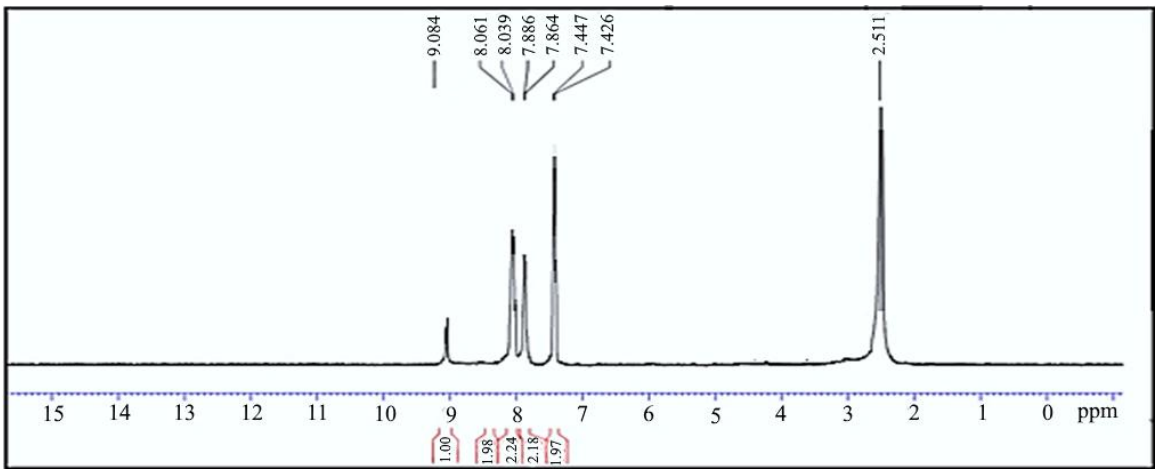

Figure 10. H-NMR for reagent {3}.

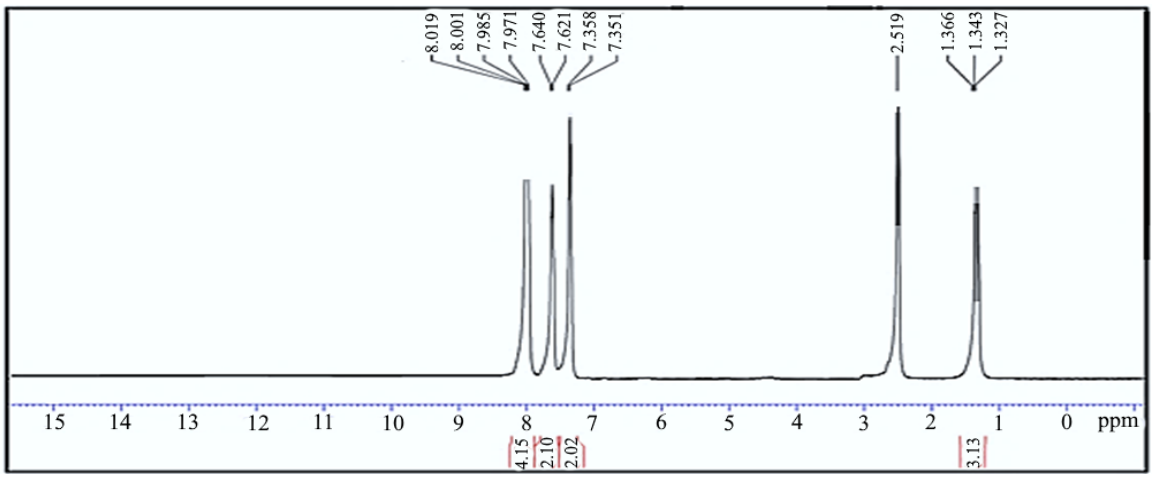

Figure 11. H-NMR for reagent {4}.

Study of Bio Properties via Theoretical Program

The prepared reagents were characterized by their biological activity and demonstrated good efficiency when tested practically in the laboratory, after we studied them theoretically using computer programs to evaluate their efficiency and pharmaceutical properties, which are listed in the Table. (Tables 1, 2).

Table 1. Bio properties of reagent {3}.

Pharmacokinetics	
GI absorption	High
BBB permeant	No
P-gp substrate	Yes
CYP1A2 inhibitor	Yes
CYP2C19 inhibitor	Yes
CYP2C9 inhibitor	Yes
CYP2D6 inhibitor	No
CYP3A4 inhibitor	Yes
Log K_p (skin permeation)	-3.37 cm/s
Drug likeness	
Lipinski	Yes; 1 violation: MW>500
Ghose	No; 3 violations: MW>480, WLOGP>5.6, MR>130
Veber	No; 1 violation: TPSA>140
Egan	No; 2 violations: WLOGP>5.88, TPSA>131.6
Muegge	No; 2 violations: XLOGP3>5, TPSA>150
Bioavailability Score	0.11
Medicinal Chemistry	
PAINS	1 alert: Sulfide_A
Brenk	6 alerts: Hydoxyl – Thiadiazole , Nitrogen, N=N-, N=C, C-S
Lead likeness	No; 2 violations: MW>350, XLOGP3>3.5
Synthetic accessibility	5.90

Table 2. Physicochemical properties and lipophilicity properties of reagents {3}.

Physicochemical Properties	
Formula	$C_{22}H_{14}N_8O_2S_2$
Num. heavy atoms	31
Num. arom. heavy atoms	6
Fraction Csp3	0.04
Num. rotatable bonds	6
Num. H-bond acceptors	2
Num. H-bond donors	2
Molar Refractivity	168.32
TPSA	154.91 Å²
Lipophilicity	
Log $P_{o/w}$ (iLOGP)	0.00
Log $P_{o/w}$ (XLOGP3)	8.49
Log $P_{o/w}$ (WLOGP)	7.81
Log $P_{o/w}$ (MLOGP)	3.81
Log $P_{o/w}$ (SILICOS-IT)	3.69
Consensus Log $P_{o/w}$	4.76
Water Solubility	
Log *S* (ESOL)	-8.08
Solubility	4.18e-06 mg/ml ; 8.23e-09 mol/l
Class	soluble
Log *S* (Ali)	-11.61
Solubility	7.23e-09 mg/ml ;32.43e-12 mol/l
Class	soluble
Log *S* (SILICOS-IT)	-3.37

Solubility	2.19e-01 mg/ml ; 4.32e-04 mol/l
Class	Soluble

Antibacterial Estimation

After conducting an evaluation study using a pharmaceutical software program to determine the medicinal properties of the prepared reagents and their efficacy as antibacterial agents against certain types of bacteria, we selected three types of bacteria from different cases and diverse samples, recording three values for each type. We then averaged the values for each bacterial type to obtain the most accurate values that listed in (Table 3) and (Figures 12, 13), within the different concentrations formatted for the reagents deciding to studies [10, 17] (at concentrations of 10^{-1}, 10^{-3}, 10^{-5} molar). Upon reviewing the results of the bacterial study, we observed that Escherichia coli was the most affected and inhibited bacterium compared to others. This is because its cell wall was more susceptible than those of other bacterial species. Therefore, the electron pairs of the azo bridge groups on the two nitrogen atoms were affected by the bacterial cell wall, enabling it to penetrate and kill the bacteria. Thus, the third compound, which, in addition to the two azo groups, also contains a hydroxyl group known for its antibacterial activity, proved to be the most effective reagent compared to the others.

Table 3. Bio Estimation of reagents {1-4}, reticence diameter (mm) through triplite recordings.

Derivatives	Salmonella	Pseudomonas aeruginosa	E-Coli
Reagent {1}	4	4	6
Reagent {2}	8	12	12
Reagent {3}	10	14	16
Reagent {4}	6	8	8

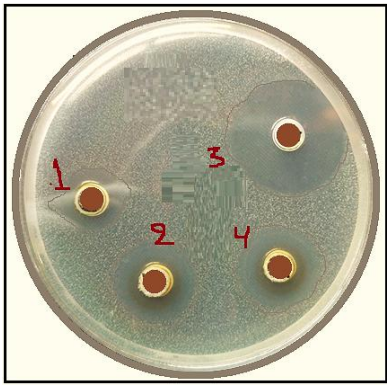

Figure 12. Reticence distance of *E-Coli.*

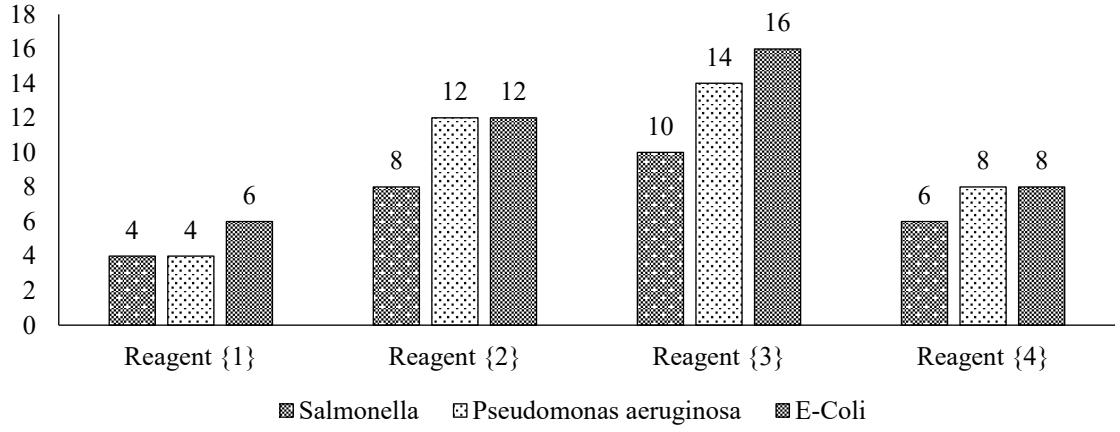

Figure 13. Reticence distance of *all types of selected bacteria.*

CONCLUSIONS

The diagnostic spectral profiles provided clear visual evidence of the composition of the new reagents prepared in our study. Furthermore, the software analysis yielded favorable evaluation values, demonstrating the reagents' antibacterial efficacy. The reagents were tested against three types of bacteria, yielding clear results indicating that the inhibition of bacterial growth resulted from the reagents penetrating the bacterial cell wall and inhibiting its growth. the electron pairs of the azo bridge groups on the two nitrogen atoms were affected by the bacterial cell wall, enabling it to penetrate and kill the bacteria. Thus, the third compound, which, in addition to the two azo groups, also contains a hydroxyl group known for its antibacterial activity, proved to be the most effective reagent compared to the others. The reagent {3} exhibited the highest efficacy.

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