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Synthesis and Characterization of New Formazans from Vanillin Derivatives and Studying Their Biological Activity.

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Abstract

In this study, formazans were synthesized by reacting malic acid with vanillin to produce a diester containing two aldehyde groups. The resulting diester was then reacted with a primary amine (4-bromoaniline) to form Schiff bases. These compounds are widely used in the manufacture of certain pharmaceuticals, insecticides, and other industrial products.

Subsequently, sodium nitrite was reacted with 4-chloroaniline, 2,4-dinitrophenylhydrazone, and 4-nitroaniline in a 1:1 ratio in the presence of hydrochloric acid and water under ice-bath conditions to generate diazonium salts. The freshly prepared diazonium salts were then coupled with the previously synthesized Schiff bases in the presence of pyridine, followed by drying to obtain the desired formazans.

Other formazan derivatives were synthesized using the same procedure. The reactions were monitored by HPLC, and the synthesized compounds were characterized by FT-IR, ^{1}H NMR, ^{13}C NMR, mass spectrometry, and biological activity studies.

Keywords: Biological activity, Formazan, Schiff bases. vanillin.

Introduction

Vanillin is an organic substance containing the formula $C_8H_8O_3$ and its groups [1]. This functional component contains aldehydes, ethers, and phenols that are major constituents of vanilla bean extract. It is also found in roasted coffee and Chinese red pine. Synthetic vanilla extract is sometimes used, rather than natural vanilla extract, as a key factor in foods, beverages, and drugs [1,2].

Vanillin is used extensively as a flavoring agent in pharmaceuticals, food, beverages, and sweets, adding a unique flavor and fragrance. Vanilla is a genus of plants. It finds application in perfumery, as an analytical reagent, and as an intermediate in the preparation of several pharmaceutical preparations [3,4].

The compounds based on the azomethine group have the basic properties as the element of one electron pair on the nitrogen atom is present, and the two bonds of the azomethine group (C-N-). This basicity is not large enough to be stable with ions to form complexes [5,6]. This is

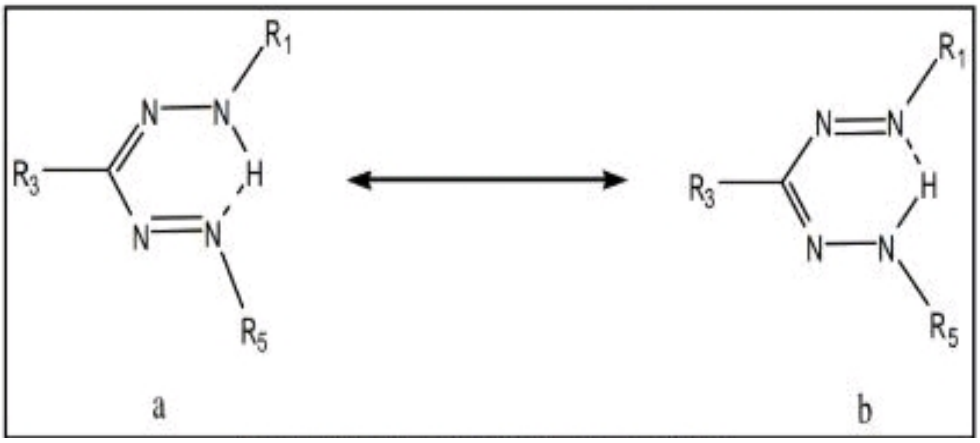
normally achieved by creating five- to six-membered chelating rings bound to a metal cluster. The proton of the phenol group that forms a kelite ring, which has not been sacrificed to a uncharged negative ligand, is generally the one to be sacrificed to a uncharged negative ligand after the salicylate compound. The stability of Schiff bases also relies on whether the carbonyl and amine compounds that are used to make them are aliphatic or aromatic. In the case of Schiff bases prepared from diaryl ketones or aryl-alkyl ketones [7,8]. Water removes molecules from various aldehydes or diaryl ketones in the presence of amine molecules. According to Danilof and Reddlin, azomethine compounds disintegrate fast in the presence of aqueous solutions of metal acids, and remain stable in the presence of aqueous solutions of bases, as shown below[9]:

$R_2C = NR + H_2O = R_2CO + RNH_2$.

The aryl group is more stable when attached to a carbon atom or a nitrogen atom. Thus, imines are commonly called Schiff bases, and this name has come into common use among chemists to refer to organic compounds with an active (-N=C) group [9,10].

Complexes with other metal ions, especially transition metal ions, as well as salts, have been prepared. They have been synthesized as their derivatives, in which electron-donating and electron-withdrawing groups are attached to the 1,3,5-phenyl ring, and the influence of the substitutions on the λmax absorption values has been studied. They also exhibit biological activity, and their biological applications attract considerable interest [11,12]. Due to the biological activity of formazan compounds, it is of paramount importance to understand their oxidation potential and potential mechanisms of action. They are highly colored (between cherry red and deep purple-black) and have a peculiar arrangement of atoms: N-N-C-N-NH-. Formazan compounds are mostly solids with low melting points, despite their large molecular size. Triphenyl formazans can also be highly soluble in chloroform and acetone; their solubility in water appears to be low, with the solvent becoming colored [13,14].

It was Bamberger and von Beckmann who, having agreed to call it formaldehyde compounds, first revealed its structure. In 1933, Bilstein defined the term "German," calling the compound formazan. Three phenyl groups, at (R, R', R), replace the compound, which is known as 1,3,5-triphenylformazan. The formazan structure was quite complicated. The homologous structure of formazan was first reported by Benchmann and Runge, although their findings were inconclusive. In 1941, it was conclusively proved that the persons in each pair of formazans were identical, although they had previously been called homologous. They theorized that formazan is a chelated hydrogen-bridge resonant hybrid. Thus, the researchers postulated an internally coordinated hydrogen-bond structure that may be organized in two mesomeric forms, A and B (**Scheme 1**). So it seems that the formazan molecule is a resonance hybrid of these forms [15,16].



Scheme 1: Formazan mesomeric structure

Experimental part

Synthesis [17]

Synthesis of vanillin esterification (compound k)

(0.06mol) Ethyl acetate was added to a 250 ml round-bottomed flask containing 100 ml dioxane, and then 0.03 mol of malic anhydride was added to the flask. The solution was refluxed with stirring for the next (4-5) hr. at around (90–100Ċ). After completion of the reaction, the solvent was removed under reduced pressure using a rotary evaporator. The obtained product was washed with diethyl ether. The Precipitate product(K) is a brown color.

Synthesis of Schiff Base Ester(K1)

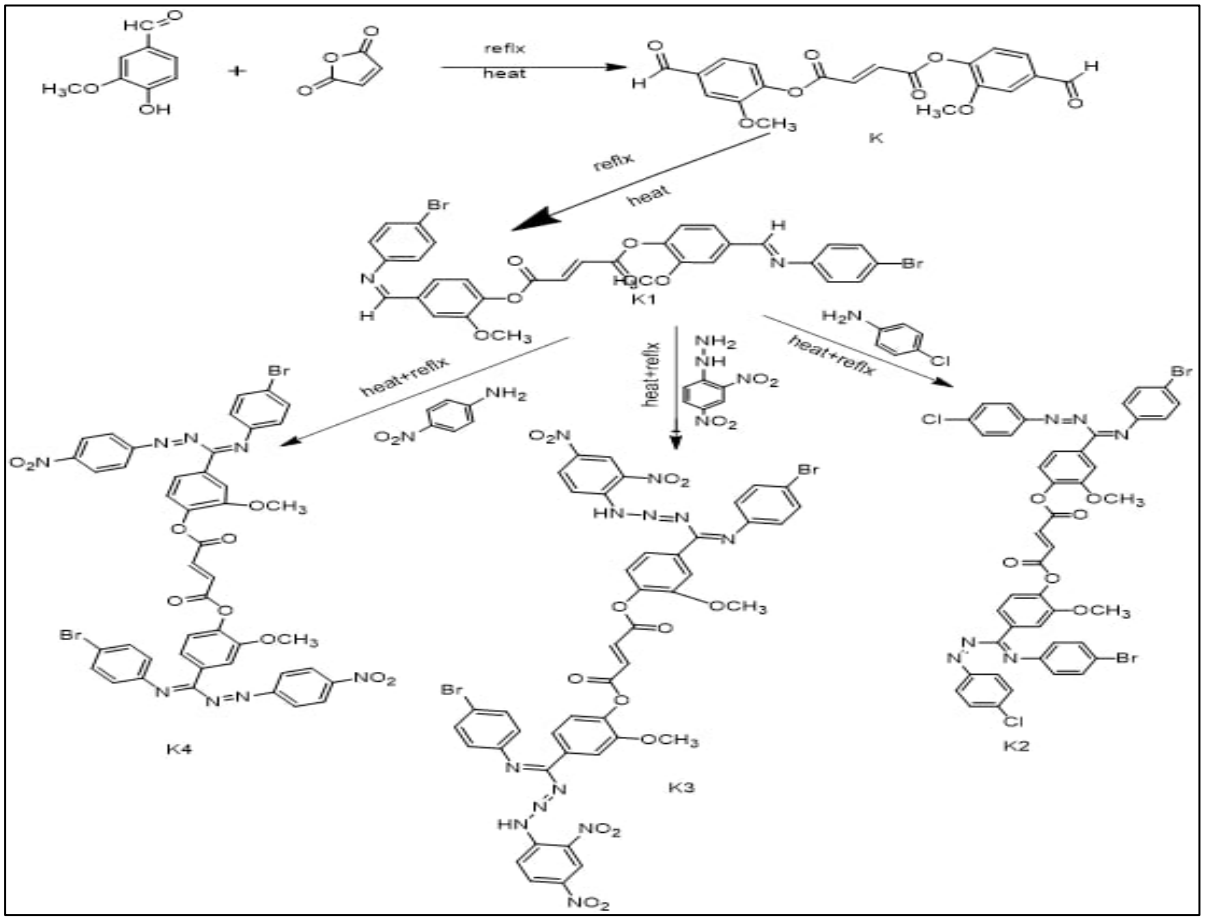
Schiff base (compound ester acetate) was prepared by using(0.008mol)of, 2-bromoaniline, respectively, and mixed with (0.004mol) of ester(compound K)and few drops of glacial acetic acid were added in a 250 ml round-bottomed flask was dissolved with (100 ml) ethanol, mixed solution stirred for(3-4)h, respectively at (90-100° C).the solvent was removed under reduced pressure by a rotatory evaporator.K1 solid product was obtained, washed with ethanol and filtered, dried and melting point was taken. The melting point of compound K1 is 170 ^{0}C.

Synthesis of formazan compounds

Place in a round-bottom flask 0.04 mol (2,4-dinitrophenylhydrazone and 4-bromoanillin) and 4-chloroanillin previously dissolved in aqueous(5ml HCl + 8 mL water)with sodium nitrate 0.8 grams and stirring at 0 º C. This mixture was then added to a cold solution of compound (K1) (0.02 mol) in dry pyridine (10 mL). The mixture was stirred at 0-5 °C for an additional 3 hours. This mixture was then stirred in water. The resulting dark-colored solids were filtered off, washed with water until free of pyridine, dried, and recrystallized from ethanol. Table 1 and Scheme 2 show the physical properties and the synthesis diagram of the K, K1, K2, K3, and K4 compounds.

Table 1: Physical data of derivatives (k-k4)

Compound	Compounds	MW	Color	mp(^{0}C)	RF	Yield %
K	$C_{20}H_{16}O_8$	384	yellow	100-103	0.48	86
K1	$C_{32}H_{24}O_6N_2Br_2$	692	orange	170-175	0.54	90
K2	$C_{44}H_{30}O_6N_6Br_2Cl_2$	968	Dark orang	213-217	0.38	75
K3	$C_{44}H_{24}O_{14}N_{12}Br_2$	1104	nutty	235-240	0.46	78
K4	$C_{44}H_{30}O_{10}N_8Br_2$	990	Dark nutty	219-223	O.42	76



Scheme2: Diagram for synthesis of K.K1,K2,K3 and K4 Compounds

Biological Activity [16][17]

The biological activity of two pathogenic bacteria (Escherichia coli and Staphylococcus aureus) isolated from the urinary tracts of patients at the Maternity and Children Hospital in the Babylon Governorate was measured on Mueller-Hinton agar. The bacteria were grown in a medium

at a concentration of perhaps 0.1 uM and left to stand for half an hour. The medium was then pricked, and 0.05 M of the prepared solution dissolved in dimethyl sulfoxide (DMSO) was added. The other culture plate served as a control to assess the solvent's effect on bacterial growth. The plates were all left to dry for 15 minutes, then put in a container after 24 hours. A ruler was used to measure the diameter of the inhibition zone in millimeters.

RESULTS AND DISCUSSION

FT-IR spectroscopy was used to identify the k compound. There were two distinct FT-IR peaks, including: C=O esterified at 1726.37 cm^{-1} and C=C aromatic at 1661.71 cm^{-1}. We observed that at 3100 cm^{-1} the OH group had disappeared. In addition, a 1H NMR spectrum of the sample was observed in dimethyl Sulfoxide (DMSO) solvent that showed a proton of the aldehyde group at 9.8 ppm, and a CH2 signal at 6.8 ppm, the appearance of the aromatic ring protons at 7.3 ppm, and the methyl group at 3.8 ppm.

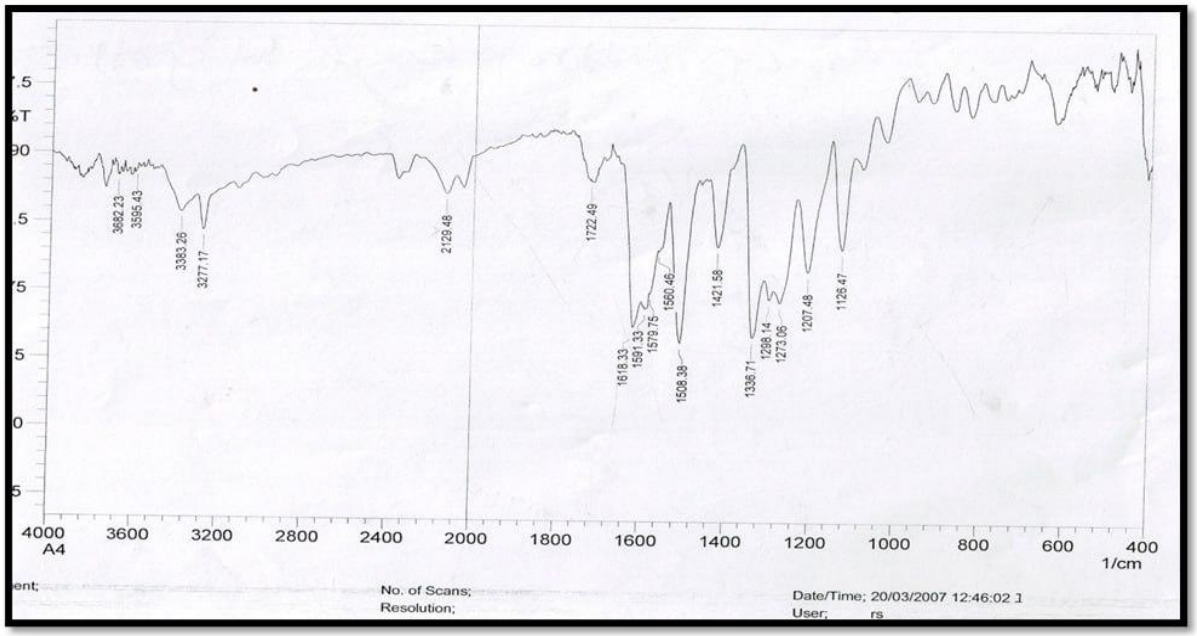


Figure 1: The FT-IR Spectrum for compound K

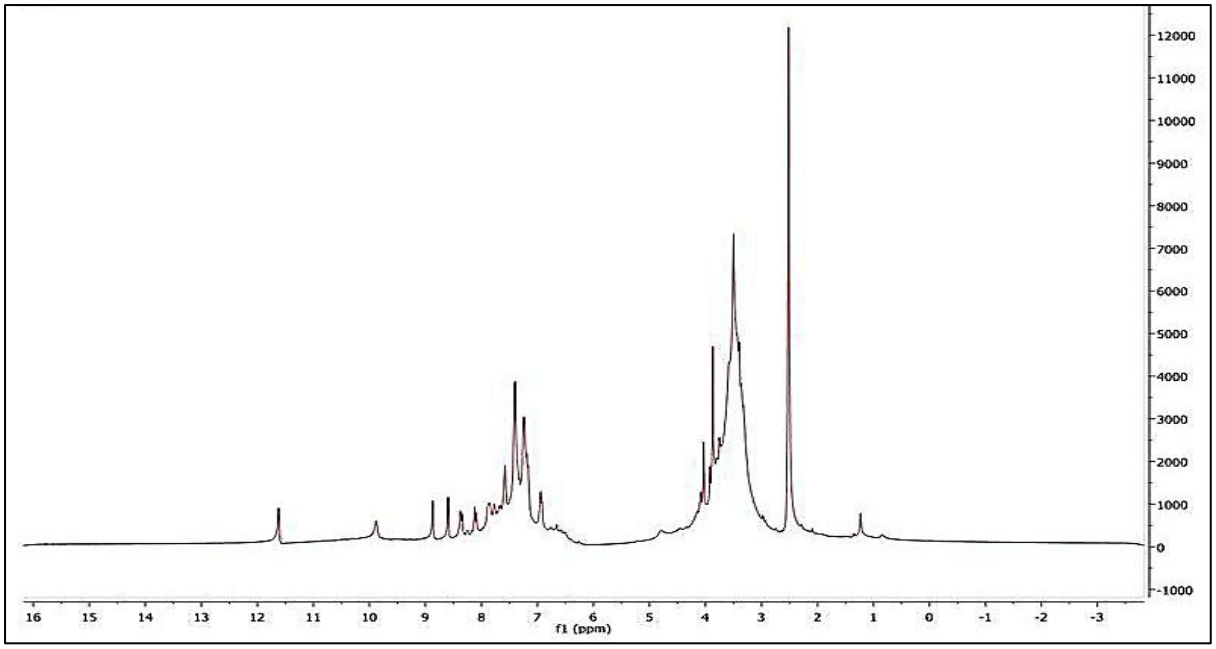


Figure 2: The 1H NMR Spectrum for compound K

When vanillin ester (K) reacted with primary amines such as 4-bromoaniline, a new compound (K1), known as a Schiff base, was produced. Several chemical methods were also used to identify the K1 compound. 1618.33 (C=N, imine), 3277.17 (C-H, aliphatic), 3383.28(C-H, aromatic), 1508.38 (C=C), and the first amine group (NH_2) disappearing at 3595.43-3612.23 cm-1 are all visible in IR (KBr cm^{-1})as in figure1. Additionally, the ^{1}HNMR spectrum(Figure 2) in DMSO solvent showed the appearance of the methyl group band at 3.5 ppm and the aromatic ring proton bands at 7.2 ppm, along with a proton signal of the azomethine group (HC= N) at 8.1 ppm.

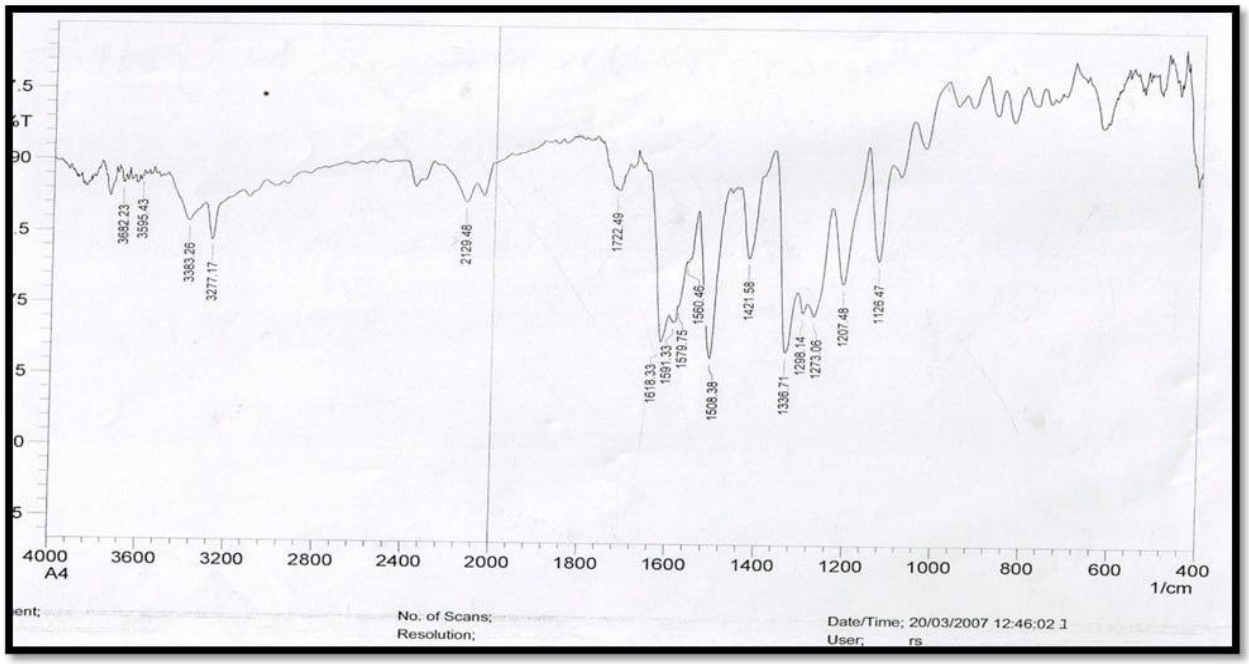


Figure 3: The FT-IR Spectrum for compound K1

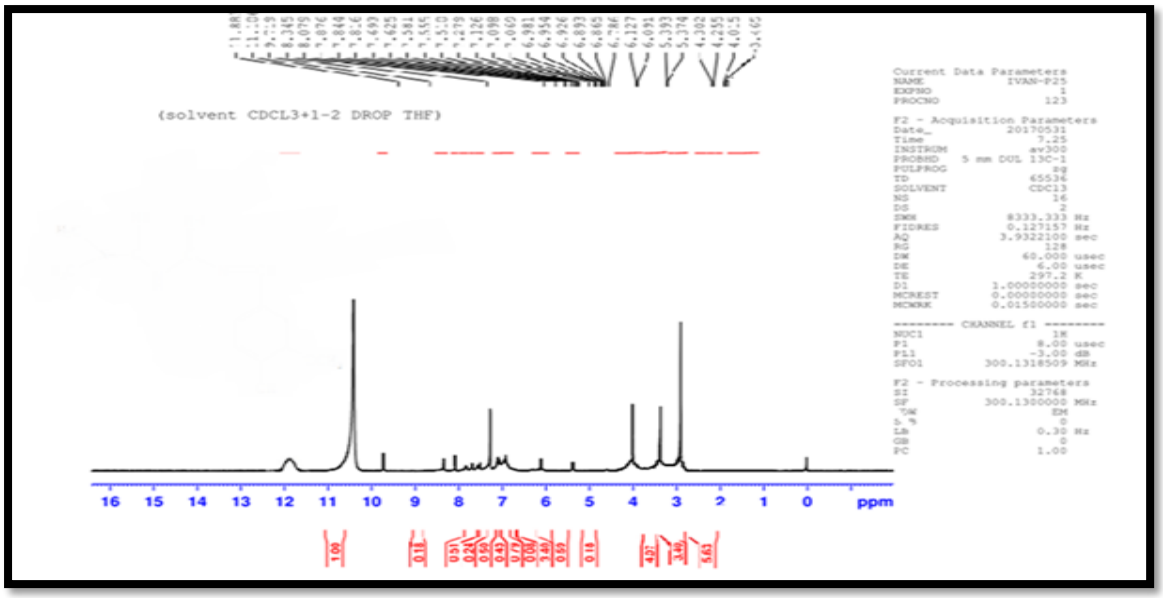


Figure 4: The 1H NMR Spectrum for compound K1

The reaction between the Schiff base compound (K1) and the analyzed dazinium salts with primary amines (4-bromoaniline, 2,4-dinitrophenylhydrazine, and 4-nitroaniline) resulted in the following formazans sequentially: K2, K3, and K4.

Compound K2:

The FT-IR data are shown in the range (1610.61, 2899.11-2963.12, 3081.21, 1763, 1508.38) cm^{-1} to (C=N, C-H aliphatic, C-H aromatic, C=O, N=N) respectively as in Figure 5. The 1 H-NMR (DMSO-d6) data are shown in the range δ: 3.8 (CH3), δ:7.3 (CH aliphatic),7.7 (CH aromatic), 2.5 ppm(DMSO-d^6) as in Figure 6. The ^{13}C-NMR data(figure7) are shown in the (C aromatic)123,(C aliphatic) 60,(C imine) 160,(C CH3) 38.

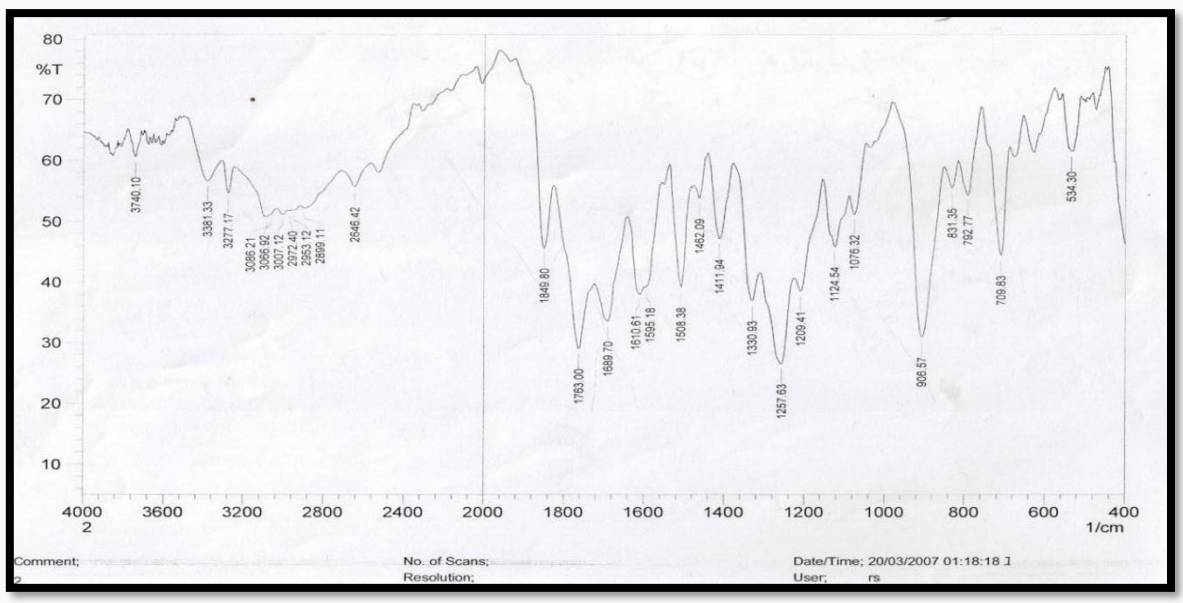


Figure 5: The FT-IR Spectrum for compound K2

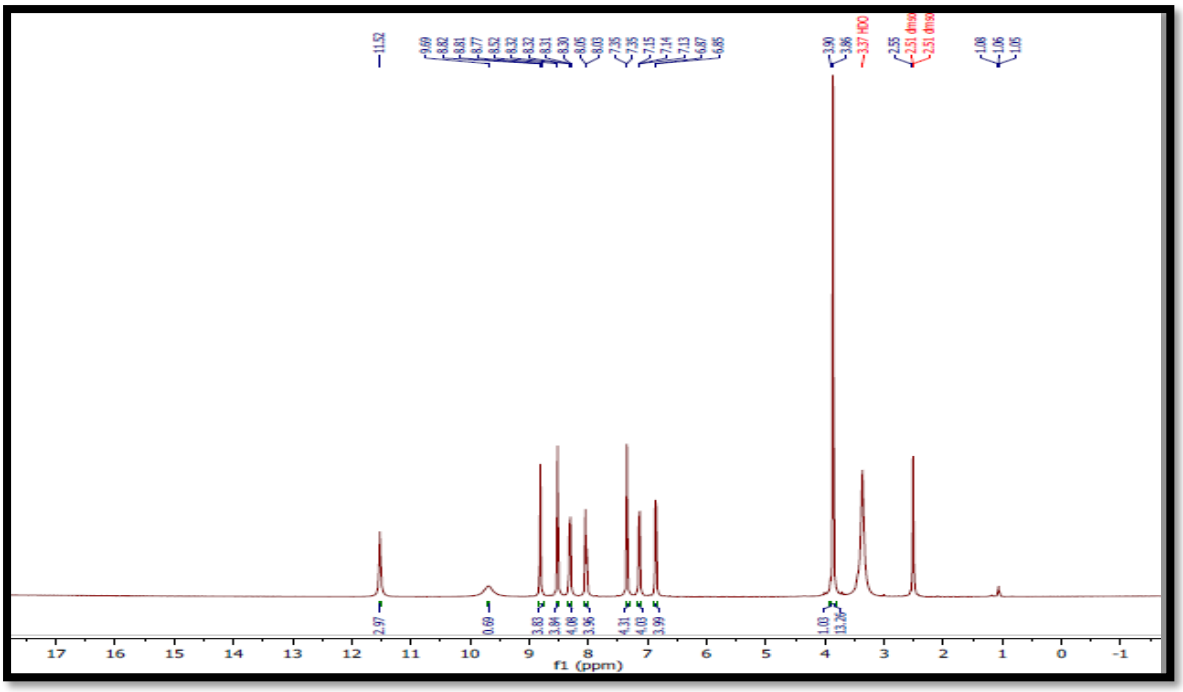


Figure 6: The 1H NMR Spectrum for compound K2

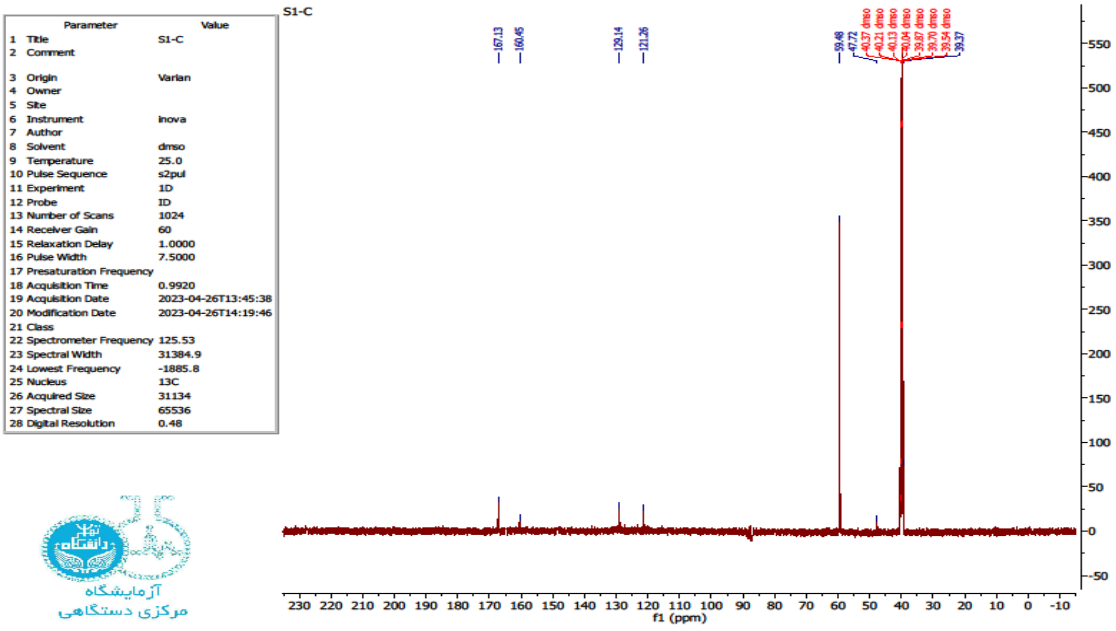


Figure 7:^{13}CNMR spectrum for K2 compound

Compound K3

The FT-IR data in Figure 8 are shown in the range=N (1618.33cm^{-1}), CH aliphatic (2129.48cm^{-1}), CH aromatic (3277.17cm^{-1}), C=O(1722.4cm^{-1}), N=N (1560.45cm^{-1}). The 1 H-NMR (DMSO-d6) data(Figure 9) are shown in the range δ: 3.5 (CH3), δ:6.8 (CH aliphatic),7 (CH aromatic),2.5ppm(DMSO-d^{6}). The C13 data are shown in the (C aromatic)128,(Caliphatic) 122,(C imine) 162. as in Figure 10

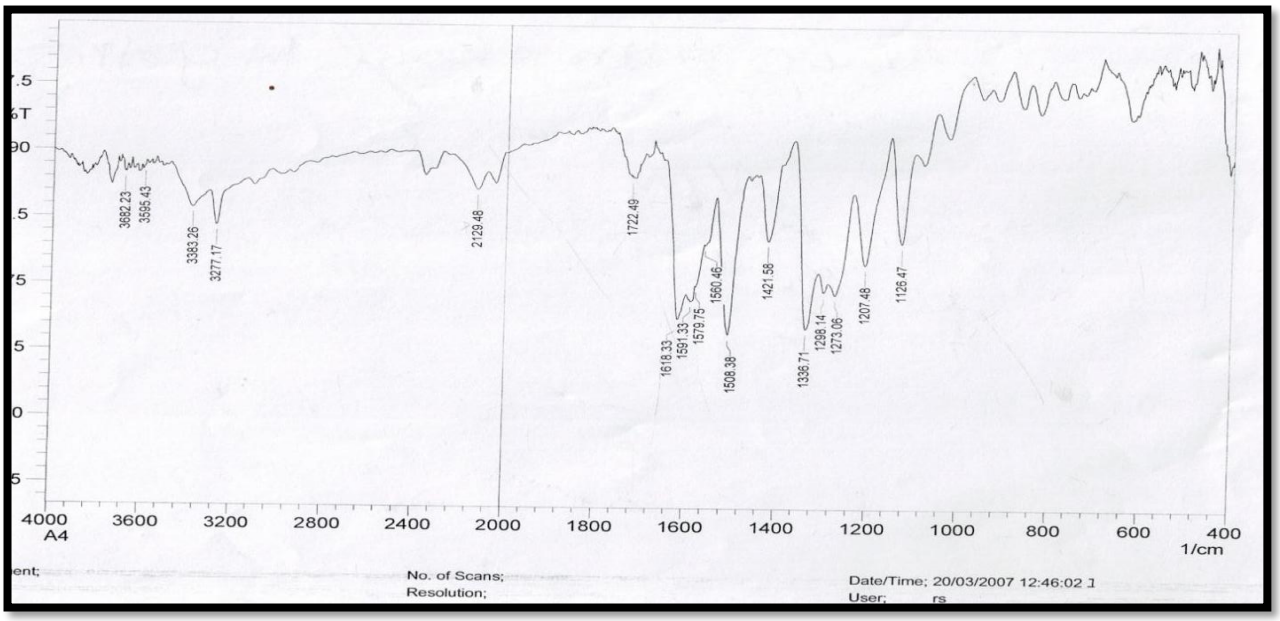


Figure 8: The FT-IR Spectrum for compound K3

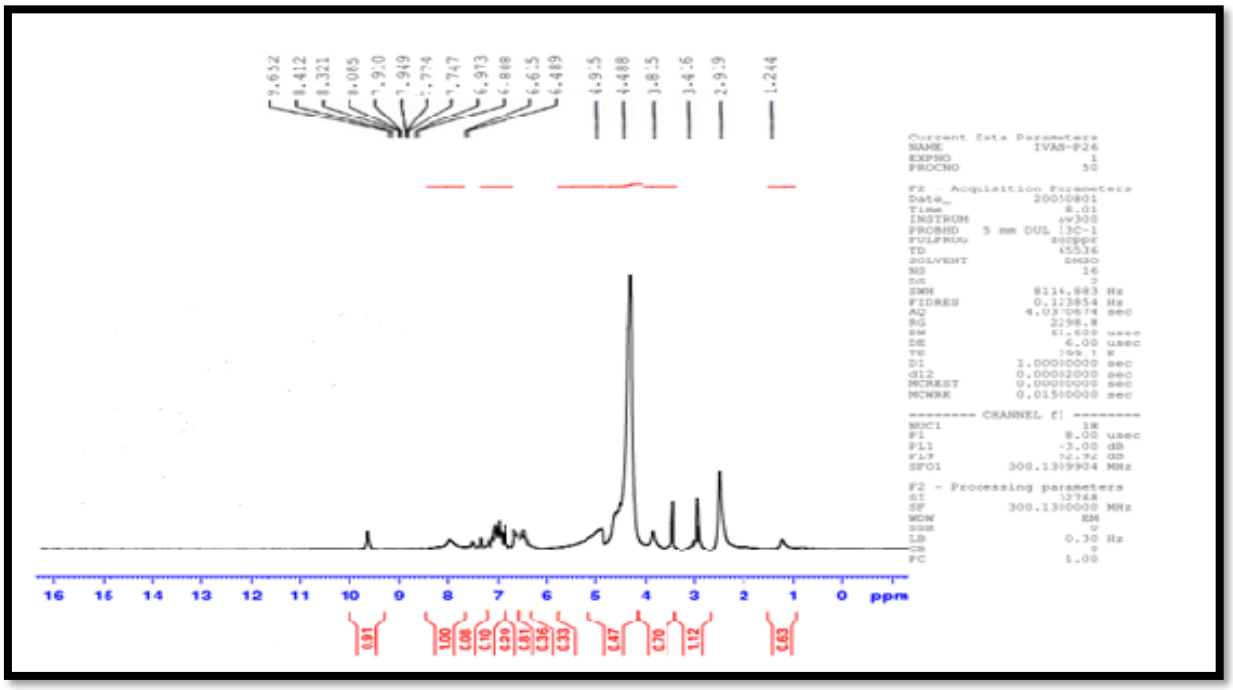


Figure 9: The 1H NMR Spectrum for compound K3

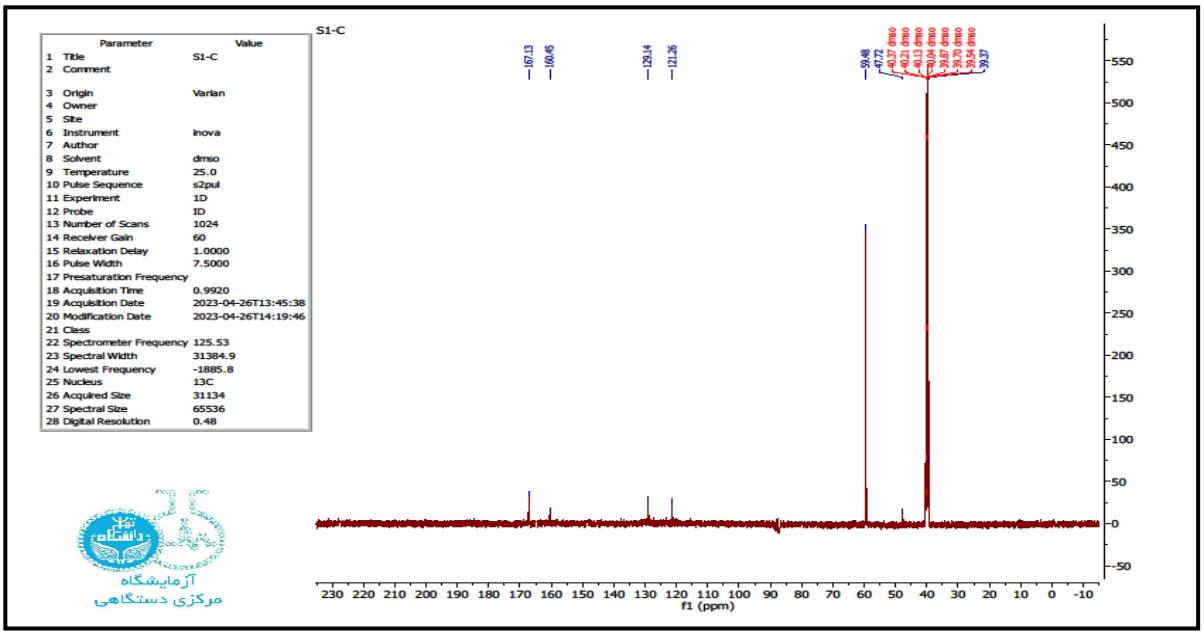


Figure 10: The ^{13}CNMR Spectrum for compound K3

Compound K4

The data is presented in the FT-IR spectrum (Figure 11): CH aliphatic (3051.4cm-1), C=O (1697.41cm-1), CH aromatic (3271.39cm-1), N=N (1566.61cm-1), C=N (1600.97cm^{-1}). The ^{1}H-NMR (DMSO-d6) data are shown in the range δ: 3.7 (CH3),7.4 (CH aromatic), δ:6.9 (CH aliphatic), 2.5 DMSO as in Figure 12. The ^{13}CNMR spectrum(figure13) show the data are shown in ,(C aliphtic) 124,(C aromitic)130,(CH3) 40 ,(C imine) 168 .

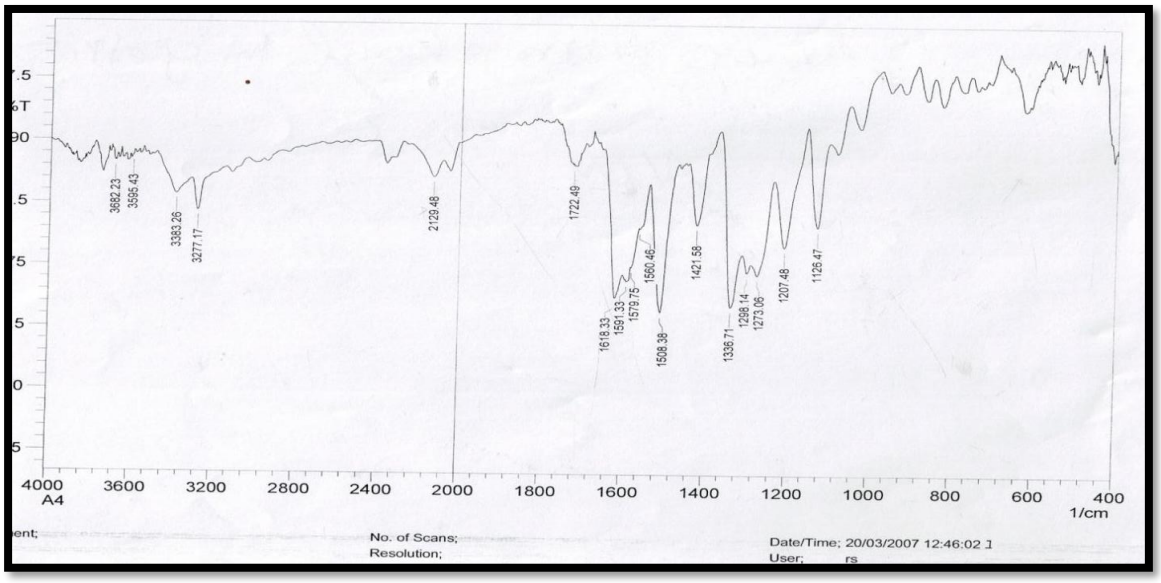


Figure11:FT-IR spectrum for k4 compound

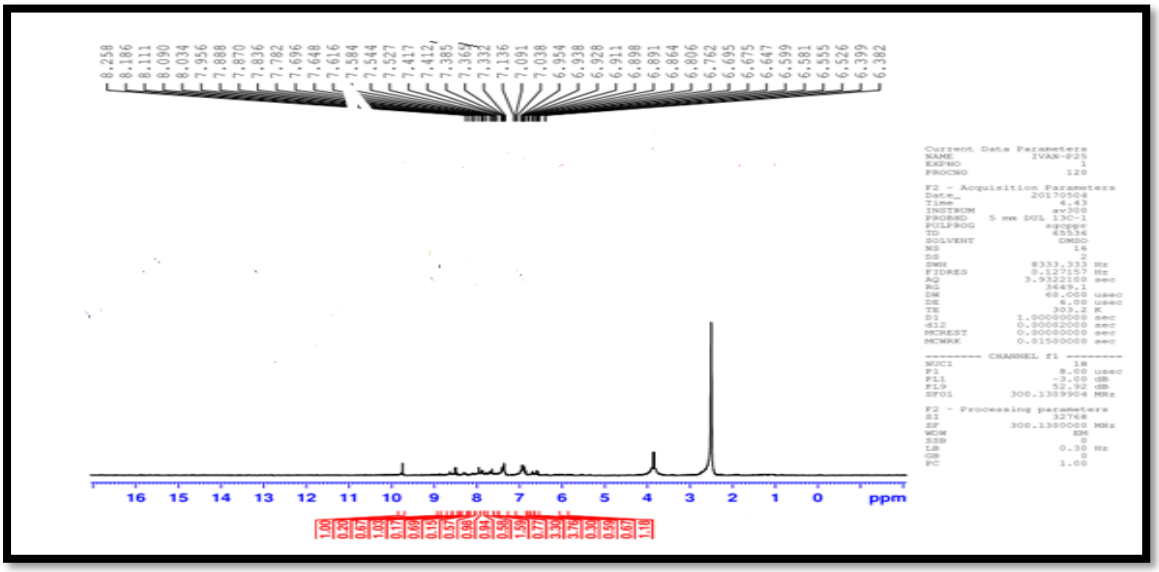


Figure12:1HNMR spectrum for k4 compound

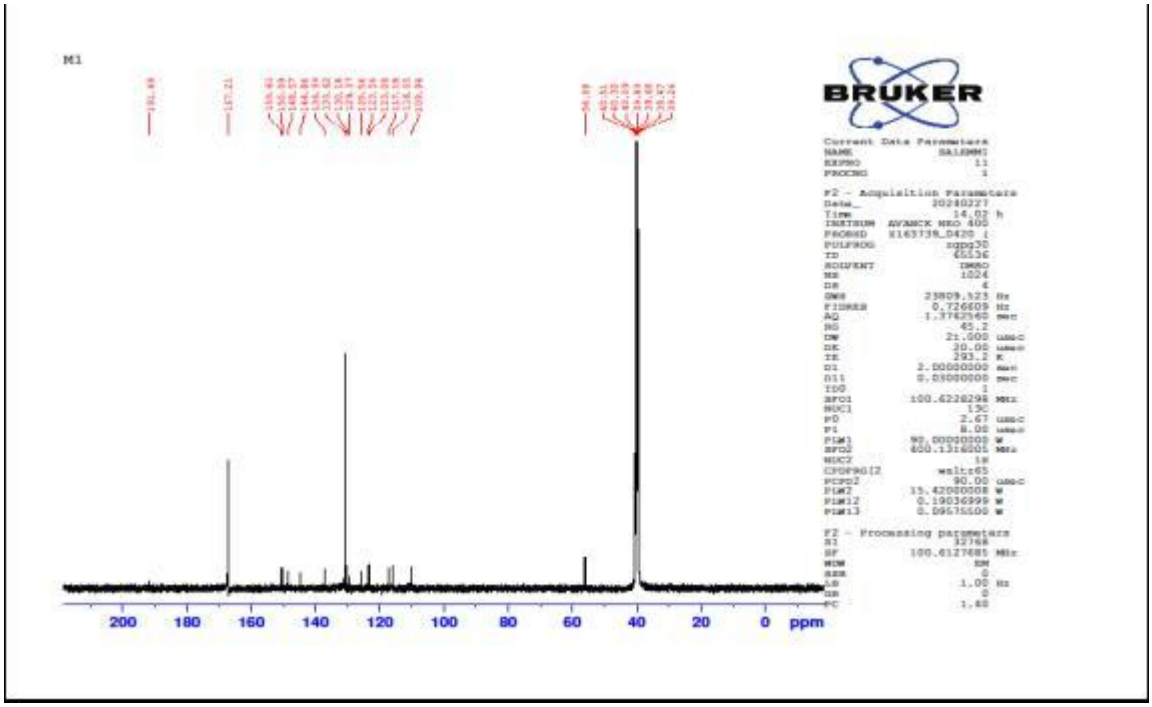


Figure13:^{13}CNMR spectrum for k4 compound

Results of Biological Activity [17]

Bacterial growth was inhibited using organic chemical solutions prepared at a concentration of 0.05 mol. The tables and images below clearly show a zone of inhibition against the growth of all bacterial types. Microorganisms are killed, or their growth is reduced, through the following methods:

1- Cell wall formation damage or prevention.

2- Damage to the permeability of cytoplasmic membranes, as well as the chemical and physical structure of the protein and nucleic acid in the cell.
3- Abnormality of cell enzyme activity.
4- Protein and nucleic acid synthesis inhibition.
Gram-positive and gram-negative bacteria are enclosed by thick cell walls, rendering them resistant to the action of chemical compounds.
This research has turned out to be successful because of the following reasons:
1- Both gram-positive and gram-negative pathogens have a lipid layer in their cell wall. These compounds loosen this layer, causing the breakdown of the cells' secretions.
2- The hydrogen bond between the nitrogen and hydroxyl groups in these compounds and the water molecules in the bacterial cell wall is formed by the certainty of the incorporation of the hydrogen atom in the water molecules, forming a significant percentage of the bacterial cell weight. This results in the loss of important cellular functions and overall health.
3- The formed compounds are capable of forming a coordination complex with the ions that exist in the bacterial cell wall, including potassium, iron, zinc, and calcium. These components are necessary for the normal operation of microorganisms.

CONCLUSION

Three nitrogen- and oxygen-containing formazan compounds were synthesized through Schiff bases prepared by the reaction of a salen-type derivative, obtained from vanillin-based compounds, with the primary amine para-chloroaniline. The synthesized formazan derivatives demonstrated promising biological activity against both Escherichia coli and Staphylococcus aureus by inhibiting their growth or causing bacterial death.

Based on these results, it can be concluded that novel antibacterial formazan compounds can be successfully synthesized from environmentally friendly vanillin-derived materials, with potential applications in pharmaceutical and biological fields.

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