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Molecular Docking, Synthesis of New Schiff base Derivatives, and Study of their Biological Activity

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Abstract

The Schiff bases (E1-E3) are generated by reacting azo derivative (A) with different amine derivatives. The Schiff bases (E1-E3) are obtained via the reaction between azo derivative with 4-methylbenzaldehyde, 4-ethyl-benzaldehyde, and 4-hydroxy-benzaldehyde. The Schiff base E1 namely:2-[(4-methylphenyl)diazenyl]-4-{(E)-[(4-methylphenyl)imino]-methyl}phenol; E2: 4-{(E)-[(4-ethylphenyl)imino]-methyl}-2-[(4-methyl-phenyl)diazenyl]phenol; E3: 4-{(E)-[(4-hydroxyphenyl)imino]methyl}-2-[(4-methylphenyl)diazenyl]phenol. All synthesized derivatives are characterized based on physical characteristics and spectroscopy methods, such as FT-IR and ^{1}H-NMR. Schiff bases derivatives exhibit notable activity against *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis*, and the derivative (E3) was particularly effective against all tested bacterial strains due to a hydroxy group in its structure. *In silico* results are the derivative E1 is exhibiting multiple interactions with the glucosamine-6-phosphate (GP6) receptor most notably a hydrogen bonding between GLU488 and the azo group and others.

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1. Introduction

The field of medicinal chemistry originated from the isolation and purification of active compounds found in plants, animals, microorganisms, and their fermentation products by chemists, pharmacists, and clinicians [1]. Certain substances have been linked to medicinal characteristics. Medicinal chemistry relies heavily on traditional branches of chemistry, particularly organic chemistry, as well as Known as pharmacophores [4].Heterocyclic chemicals are organic molecules with cyclic structures that include at least the presence of heteroatom [5]. The most prevalent heteroatoms in these molecules are nitrogen, oxygen, and sulfur, although heterocyclic rings with additional heteroatoms are additionally well-known. A carbocyclic molecule is an organic substance with a biology and certain aspects of physics [2]. Only a small selection of natural and synthetic compounds can be used directly as medicinal agents. Nevertheless, the lack of specificity sometimes restricts their usage in human and veterinary medicines, similar insecticides, and other agricultural applications [3]. Through chemical analysis, one can identify the therapeutically important molecular segments of these products, cyclic structure where all the elements in the ring are carbon atoms [6]. Heterocyclic molecules are regarded as essential organic chemicals that find application in various biological disciplines owing to their efficacy against numerous diseases [7]. Biological compounds, including DNA, RNA, chlorophyll, hemoglobin, vitamins, and others, possess a heterocyclic ring within their primary

structure [8]. Heterocyclic chemicals have several uses for common ailments. For instance, Schiff base compounds were previously utilized as antibacterial herbicides, urinary antibiotics, or anti-inflammatory in-nature medicines. Schiff base compounds have been documented to exhibit many biological activities, including antibacterial, antifungal, antiviral, and anthelmintic properties [9, 10]. Schiff bases are a widely studied group of chemicals known for their intriguing biological characteristics [11]. Schiff bases are highly adaptable ligands that can form coordination complexes with a wide range of d-block metals and lanthanides [12]. Schiff bases and their metal complexes have been found to have various therapeutic properties, including antibacterial effects against mycobacteria, antifungal activity, antiviral activity, antimalarial effects, and other uses in medicine [13, 14]. This study synthesized a new azo-Schiff base derivative E1-E3 and characterization by FTIR and ^{1}H-NMR. Some of these derivatives are used as antibacterial and antifungal and are studied *in silico* results by using molecular docking. These derivatives were novel compounds and used a simple procedure.

2. Materials and Methods

4-hydroxybenzaldehyde, 4-ethylaniline, 4-methylaniline, 4-hydroxyaniline, glacial acetic acid, ethanol, HCl, $NaNO_2$, and the chemicals used in this study were obtained from BDH and Merck Company.

2.1. Synthesis of 4-hydroxy-3-(p-tolyldiazenyl) benzaldehyde (A)

In a cooling bath (0-5 ℃), prepare 10% HCl solution and add it to 4-methylaniline (0.01 mol). Add (2-3) HCl Conc drops to this solution. Prepare $NaNO_2$ (0.01 mol) in distilled water (5 ml). Add 10% NaOH solution to the solution of 4-Hydroxybenzadehyde (0.01 mol, 1.22 g). After filtration, the residue was collected with a yield of 65%, and the melting point was 162°C [13].

2.2. Synthesis of Schiff bases (E1-E3)

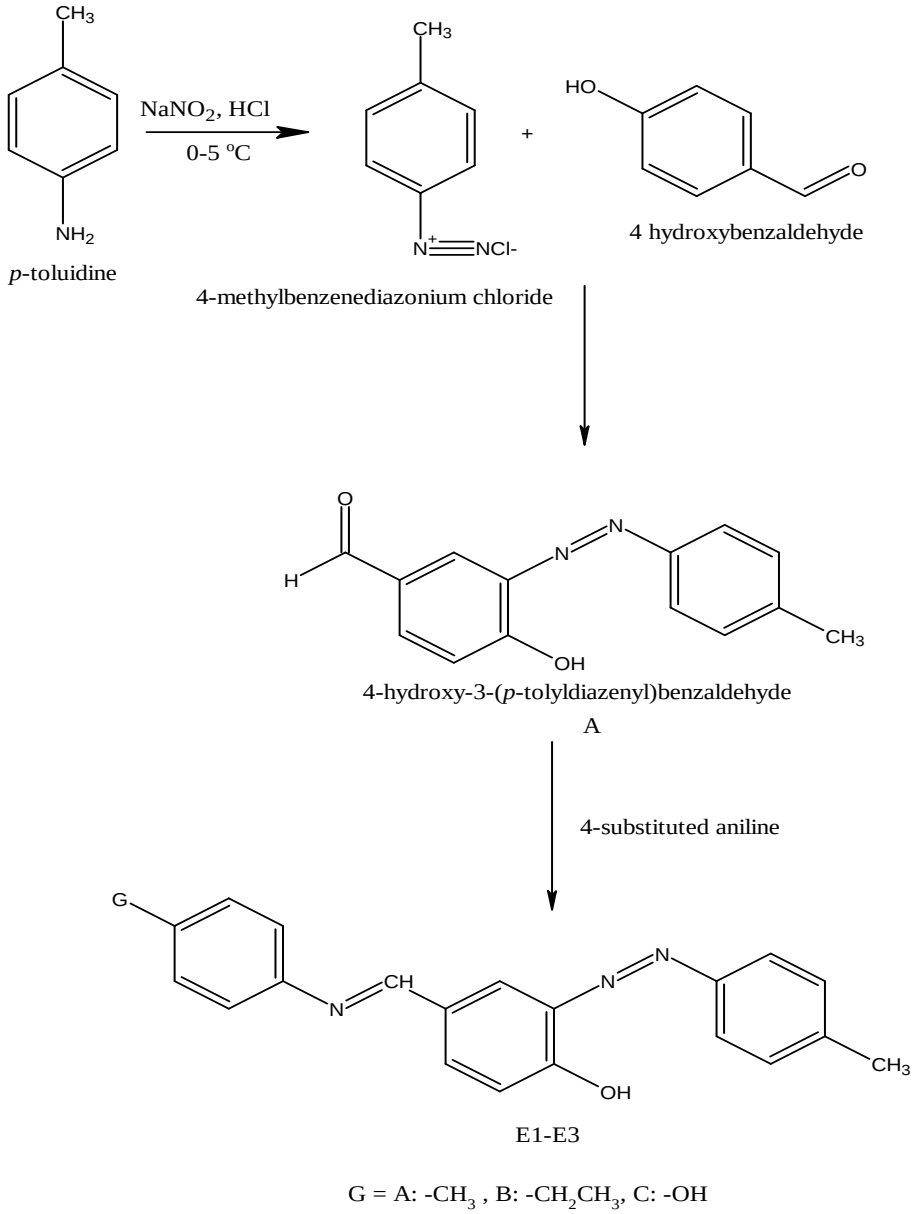
Dissolve the 0.01 mol of azo derivative (A) in 15 ml of ethanol and add 2-3 drops of glacial acetic acid to this solution. The different aniline derivatives (4-methylaniline, 4-ethylaniline, 4-hydroxyaniline) are added separately, respectively, with a solution in three conical flasks. The precipitates were filtered to product derivatives recrystallized from ethanol to give the dark yellow and yellow colors, and the melting points were 162 215, and 203 °C and yielded 71, 75, and 68 for E1-E3 respectively [15, 16].

2.3. Anti-microbial

The Baghdad laboratory in Baghdad, Iraq provided the bacterial cultures of *Bacillus subtilis, Escherichia coli,* and *Staphylococcus aureus*. By placing the cultures of bacteria on nutritional agar, they were cultured for 24 hours at 30 ± 0.1 °C. The Schiff base derivatives (E1-E3) were dissolved at a concentration of 25 mg/ml in dimethyl sulfoxide (DMSO) and kept in a dry condition at room temperature. Each chemical's antibacterial activity was evaluated through the use of the agar disc-diffusion method. After being put onto Petri plates, the Mueller Hinton Agar Media (15 cm^3) was allowed to solidify at 45 °C. 50 µL of normal saline solution with a culture media containing 105 –106 bacteria per milliliter was applied to placed Petri plates (9 cm). The prepared Schiff bases (50 µL) were firmly pushed into the solid agar medium by injecting them into discs. For twenty-four hours, the Petri plates were incubated at 37 degrees centigrade. Using a zone reader, the inhibitory zones that had formed on the medium were measured at the end of the time and recorded in millimeters [17, 18].

2.4. Anti-fungal

Pathogenic strains of *Aspergillus Niger* and *Chalara corda* were donated by the Department of Microbiology at the Baghdad Laboratory, Baghdad, Iraq. The Schiff base derivatives (E1-E3) were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 50 mg/mL and kept in a dry atmosphere at room temperature. The agar disc-diffusion method was used to evaluate each medication's antifungal activity. After being placed into the Petri dishes, the 15 cm^3 Sabarod agar medium was let to solidify at 45 °C. 10 mm-diameter filter paper discs were soaked in 50 µL of the Schiff base derivatives (E1-E3), and then placed onto the substrate that had been seeded with fungi. Following that, the plates were incubated for one to seven days at 27 degrees Celsius. Using a zone reader, the inhibitory zones that formed on the medium were measured at the end of the time and the results were recorded in millimeters [19].



Scheme 1 Synthesis of new azo-Schiff base derivative E1-E3

3. Results and Discussion

In FT-IR, the result of derivative (A) appeared as azo group N=N at 1511 cm^{-1}. The diazonium salt is employed as an electrophile and undergoes methyl, ethyl, and hydroxyl group and meta to the carbonyl group of aldehydes. Aside from this, derivatives (E1-E3) resulted in the disappearance of the C=O and C-H groups of aldehyde and the appearance of the azomethine group C=N of Schiff base [20, 21]. The FTIR (cm^{-1}) in figures (2-4), for electrophilic substitution of aromatic compounds with an electron-rich coupling portion, such as benzaldehyde derivatives that are used. The aryl diazonium ion is oriented ortho to the E1-E3, respectively, shows the imine group of Schiff base at (1628, 1629, and 1632) and the carbonyl group of the aldehyde group disappeared, while the C-H aromatic ring appeared at (3033, 3010, and 3029) and C-H of the aliphatic group at (2981, 2932, 2971). The C=C of the aromatic ring appeared in

(1595, 1592, and 1600). The azo group appeared in (1506, 1514, and 1524) [21].

^{1}H-NMR (ppm) spectroscopy of derivative E1 in Figure 5 showed a singlet signal of proton of the hydroxyl group (1H) at 9.31 and singlet signal of proton of imine group (1H) at 8.30. Multiple signals for protons of the aromatic ring (11H) at 6.92-7.93, while the protons of methyl group (3H) showed at 2.18 as doublet singles [20]. ^{1}H-NMR (ppm) spectroscopy of derivative E2 in Figure 6 showed a singlet signal of proton of the hydroxyl group at 9.39 and singlet signal of proton of imine group at 8.81. Multiple signals for protons of the aromatic ring (11H) at 7.01-8.01, while the protons of methyl group (3H) showed at 1.23 as triplet singles and protons of CH2-CH3 (2H) at 2.70 [21, 22]. ^{1}H-NMR (ppm) spectroscopy of derivative E3 in Figure 7 showed a singlet signal of proton of the hydroxyl group (1H) at 9.39 and singlet signal of proton of imine group (1H) at 8.62. Multiple signals for protons of the aromatic ring (11H) at 7.16-8.15, while the protons of methyl group (3H) showed at 2.00 as doublet singles [23].

3.1. Molecular Docking

Docking simulations play an essential function in investigating the ways in which ligands attach to a target compound. Glucosamine-6-Phosphate synthase (GP6 synthase) has garnered the attention of many scientists because of its significance in the synthesis of microbial cell walls [24]. The enzyme facilitates the initial stage of hexosamine biosynthesis by transforming Fructose-6-Phosphate into GlcN-6-P (Glucosamine-6-Phosphate). This compound is recognized as a precursor to Uridine Diphosphate N-acetyl glucosamine (UDP-NAG), a vital constituent of the peptidoglycan layer found in the cell wall of microorganisms [25]. In silico results, shown in figures (8 and 9), the ligand (derivative E1) is exhibiting multiple interactions with the GP6 receptor most notably a hydrogen bonding between GLU488 and the azo group in addition to others with binding affinity -7.3 kcal/mol that obtained automatic molecular docking.

3.2. Antibacterial activity

addition to other interactions with a binding affinity of -7.3 kcal/mol. We expect that the molecules will not cause any side effects when used as a drug.

The antibacterial evaluation of the Schiff base at a dosage of 50 mg/ml has yielded data indicating their effectiveness against all microorganisms. The diameter of the inhibitory zones was measured in millimeters, and the corresponding findings are shown in Table 1. The antimicrobial testing results demonstrate that Schiff base derivatives exhibit notable activity against Staphylococcus aureus, Escherichia coli, and Bacillus subtilis. Derivative (E3) was particularly effective against all tested bacterial strains due to a hydroxy group in its structure, which possesses inherent antimicrobial properties. The antibacterial activity of these chemicals demonstrates an increasing order. As concentration increases, the region of growth inhibition likewise increases as shown in table 1.

3.3. Anti-fungal activities

The results of the study of the antifungal activity demonstrate that the Schiff base derivatives (E3) exhibit greater potency against all tested fungi compared to the Schiff base derivative (E1). The findings of the antifungal activity are shown in Table 2.

4. Conclusions

New Schiff base E1-E3 derivatives were synthesized in this investigation. Using ^{1}H-NMR and infrared spectrometry, the structures of the compounds were verified, and their antibacterial, antifungal, and enzyme activities were evaluated. The synthesized compounds had antibacterial activity against these Schiff base derivatives. exhibited marked activity against Staphylococcus aureus, Escherichia coli, and Bacillus subtilis, and showed significant activity against all microorganisms tested. G6P enzyme with affinity chromatography. The effect of synthesized compounds on the enzymes was examined with in-silico and in-vitro studies. According to the results of these studies, the enzymes that play an important role in preventing many metabolic pathways were not inhibited by the compounds that have antibacterial activity. In silico results, the E1 derivative showed multiple interactions with the GP6 receptor, most notably a hydrogen bond between GLU488 and the AZO group, in

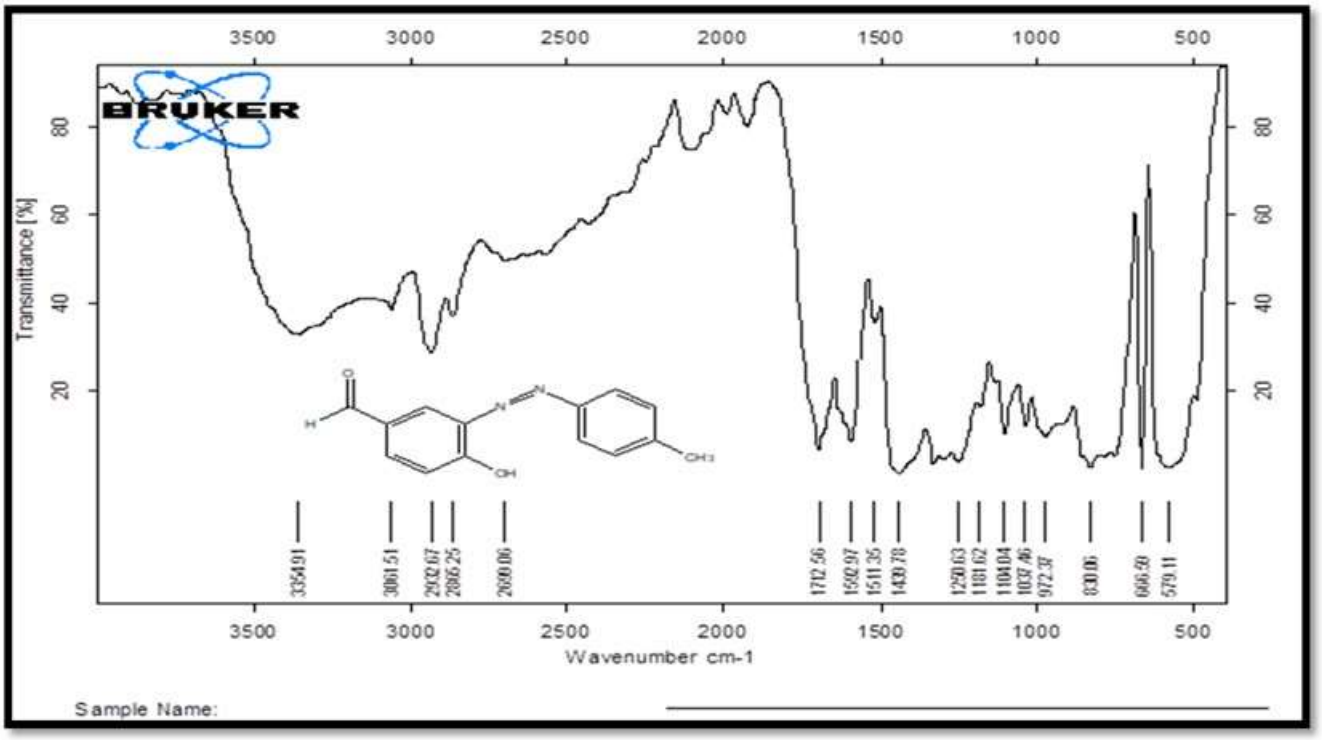


Figure 1. FTIR for azo derivative (A).

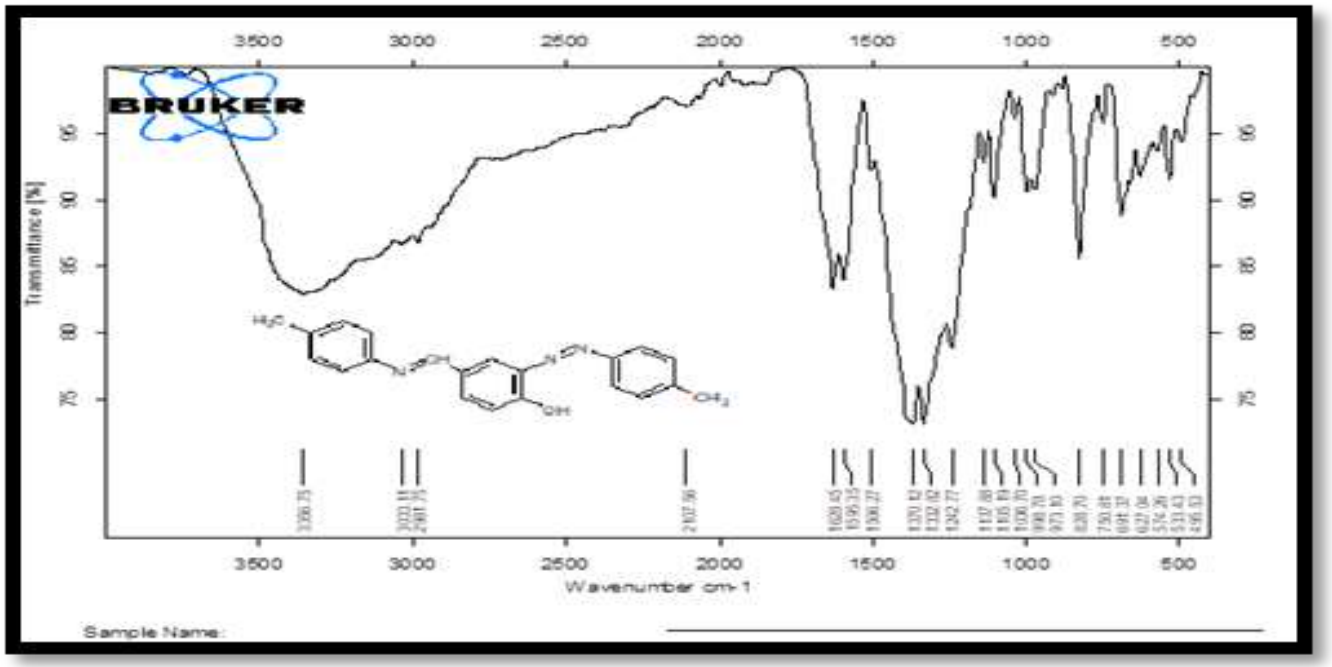


Figure 2. FTIR for schiff base derivative (E1).

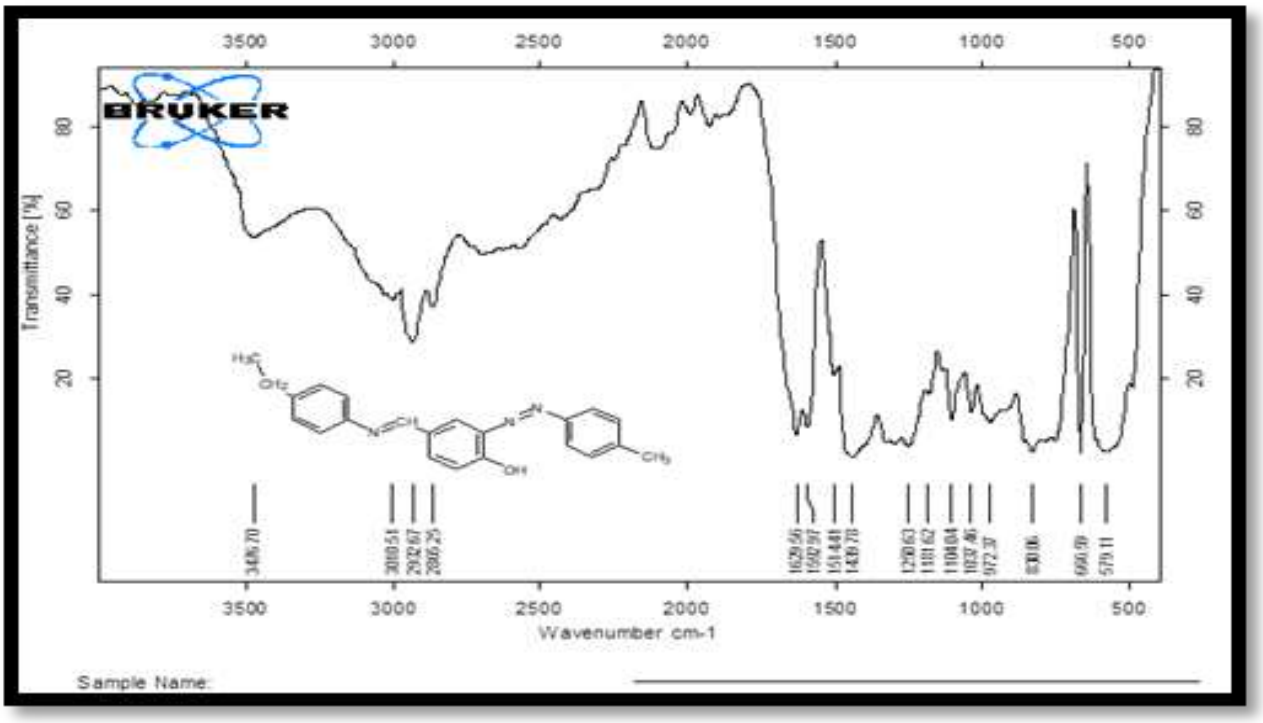


Figure 3. FTIR for schiff base derivative (E2).

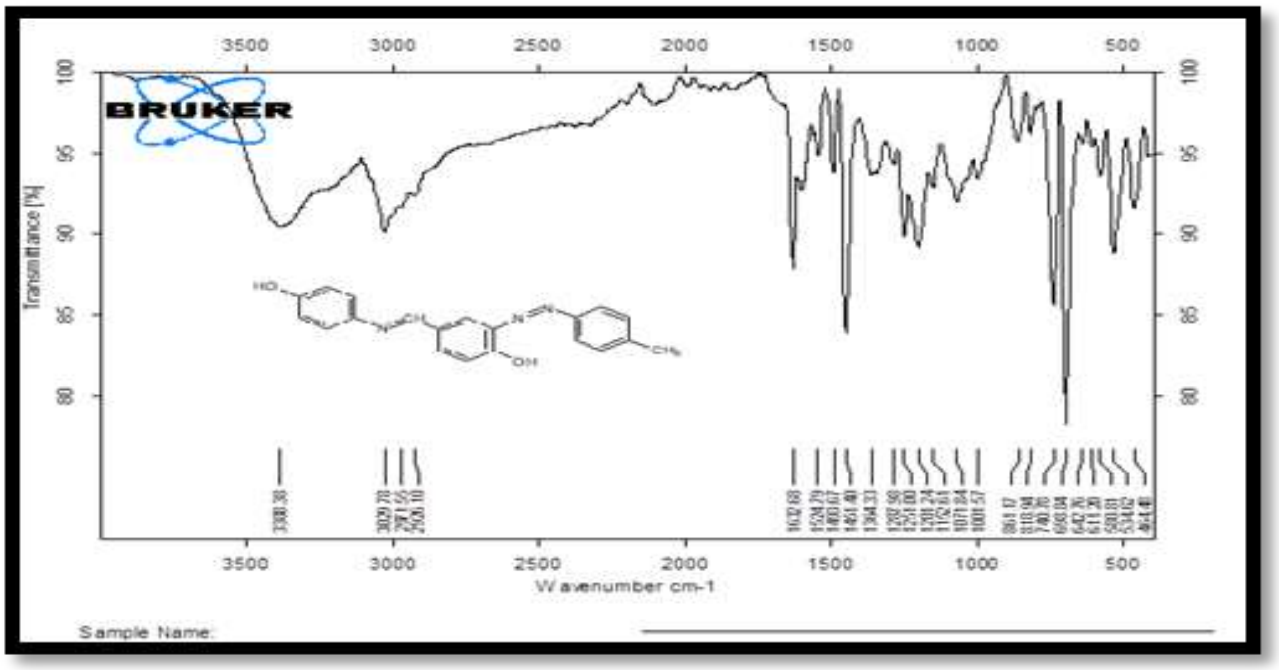


Figure 4. FT-IR for schiff base derivative (E3).

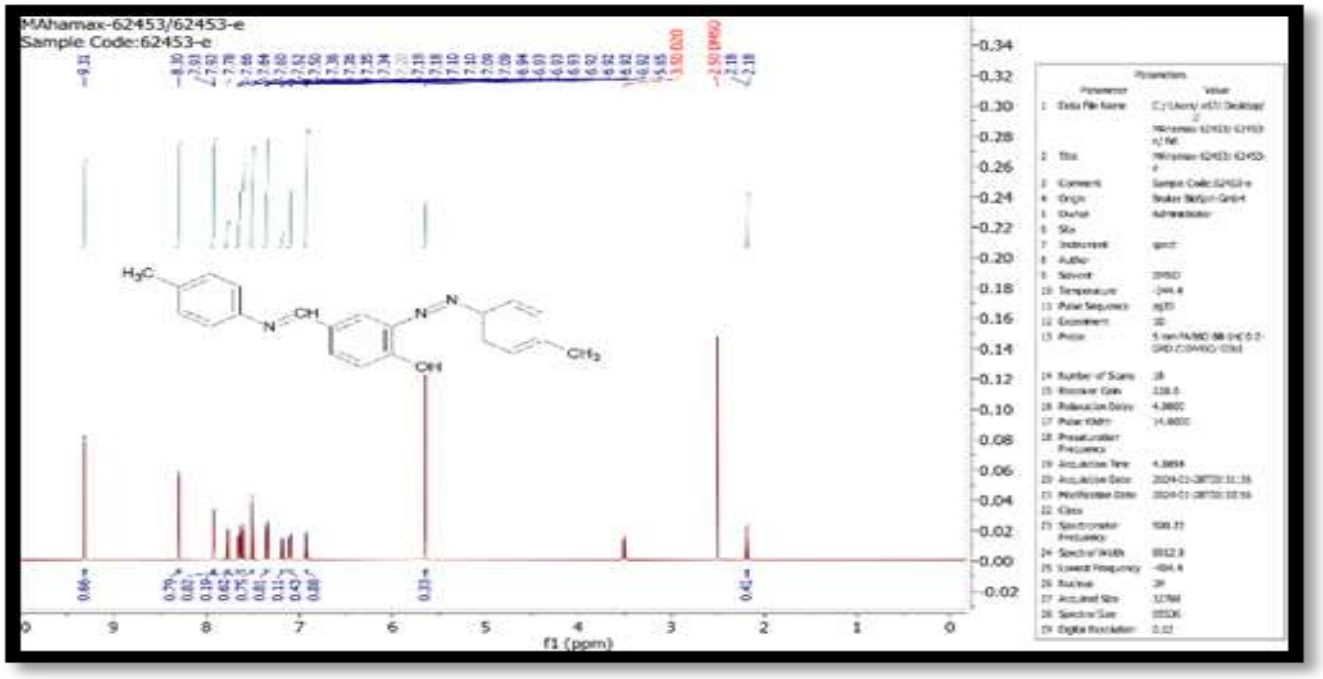


Figure 5. 1H-NMR for Schiff base derivative (E1).

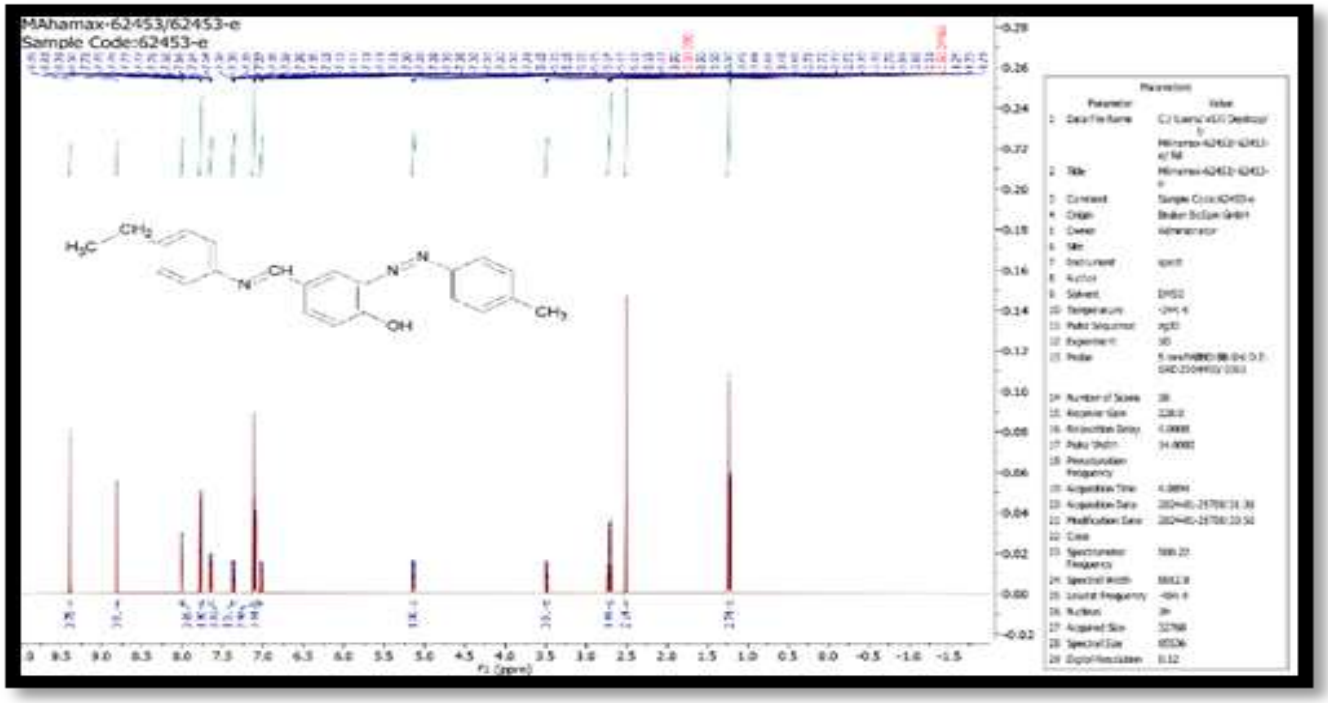


Figure 6. 1H-NMR for Schiff base derivative (E2).

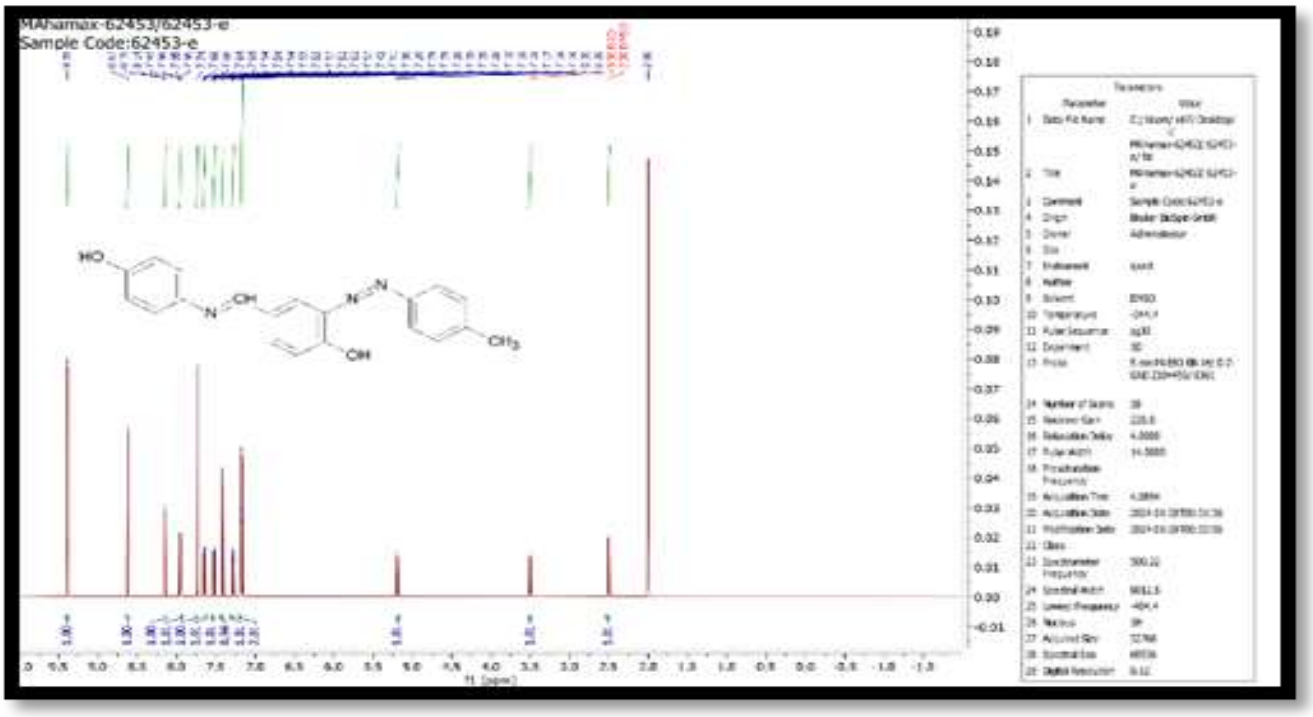


Figure 7. 1H-NMR for schiff base derivative (E3).

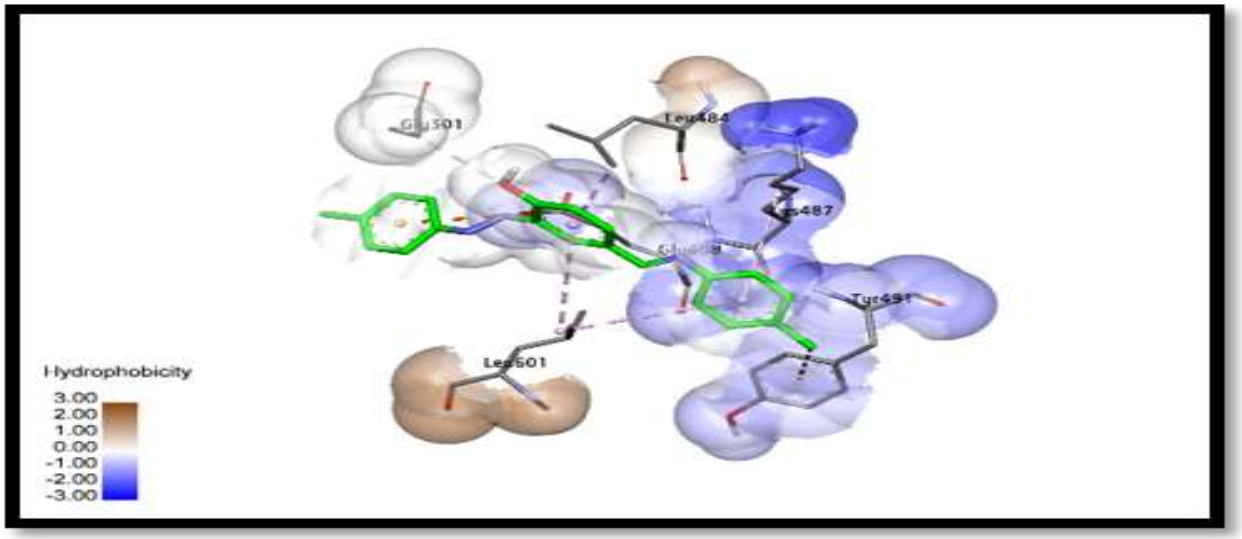


Figure 8. 3D-Molecular Docking of derivative E1.

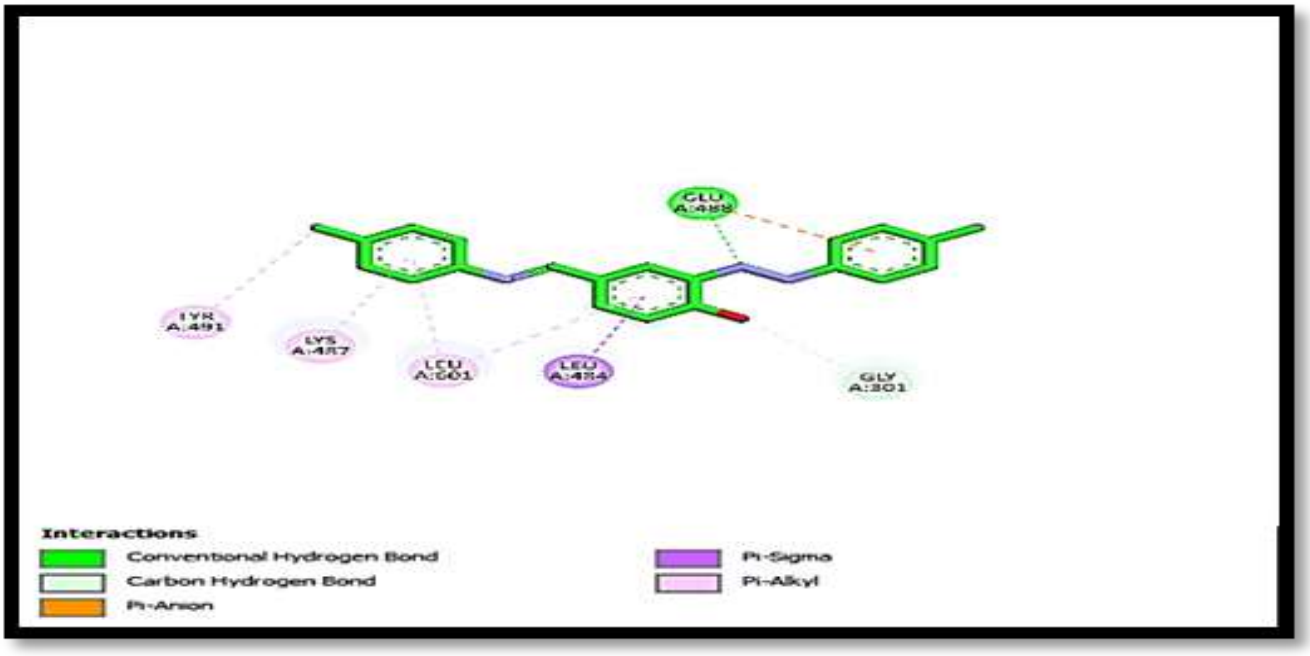


Figure 9. 2D-Molecular Docking of derivative E1.

Table 1. Antibacterial activity of Schiff base derivatives (E1-E3).

Compound	*Staphylococcus aureus*	Zone inhibition *Bacillus subtilis*	*Escherichia coli*
E1	18 mm	21 mm	24 mm
E2	14 mm	16 mm	21 mm
E3	26 mm	21 mm	23 mm

Table 2. Antifungal activity of Schiff base derivatives (E1 and E3).

Compound	Zone inhibition	
	Chalara Corda	*Aspergillus Niger*
E1	17 mm	11 mm
E3	20 mm	16 mm

Conflicts of Interest: The authors declare no conflict of interest.

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