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Preparation and characterization of novel imidazole compounds derived from (4,4',4''-(1,3,5-triazine-2,4,6-triyl)trianiline and evaluation of their bactericidal activity

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Abstract :

In this study, the compound 4,4',4''-(1,3,5-triazine-2,4,6-triyl)trianiline was used as the core material for Schiff base preparation using a modern and environmentally friendly microwave irradiation method. This compound was reacted with benzaldehyde derivatives to obtain a tertiary Schiff base, which was subsequently reacted with the amino acid alanine to form several imidazole-derived cyclic compounds. This method proved to be highly efficient and environmentally friendly, yielding good product quality with short reaction times. The validity and accuracy of the results were confirmed using spectroscopic techniques (FT-IR, ^{1}H, ^{13}C-NMR), which showed the disappearance of the characteristic azomethine (C=N) group of Schiff bases and the appearance of characteristic imidazole ring groups. The pharmacological efficacy of the prepared compounds was also evaluated against two types of bacteria, Escherichia coli and Staphylococcus aureus. The results showed that compound [E_1] possessed the highest inhibitory activity against both types, with the diameter of the inhibition zone reaching approximately 3.0 cm for Gram-negative bacteria and 3.1 cm for Gram-positive bacteria. This high activity may be attributed to the presence of a high molecular weight bromine substitute, which improved membrane permeability in the bacteria.

Keywords: Schiff base, imidazole, Biological activity, *Staphylococcus aureus* , Escherichia coli .

تحضير وتشخيص مركبات إيميدازول جديدة مشتقة من (4،4،4''-(1،3،5-تريازين-2،4،6-تريل) ثلاثي انيلين وتقييم فعاليتها كمضادات للبكتيريا

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مستخلص:

في هذه الدراسة، استُخدم المركب 4,4',4"-(1,3,5-تريازين-2,4,6-تريل) ثلاثي انيلين كنواة أساسية في تحضير قواعد شف باستخدام طريقة حديثة وصديقة للبيئة تعتمد على التشعيع بالمايكروويف. جرى تفاعل هذا المركب مع مشتقات البنزالديهايد للحصول على قاعدة شف ثلاثية، والتي تفاعلت لاحقًا مع الحامض الأميني الألانين لتكوين عدد من المركبات الحلقية المشتقة من الإيميدازول، فقد اثبتت هذه الطريقة كفاءتها العالية ومراعتها للبيئة حيث ان نسبة المنتوج جيدة مع أوقات تفاعل قصيرة .

تم تأكيد صحة ودقة النتائج باستخدام التقنيات الطيفية (FT-IR, ^{1}H,^{13}C-NMR)، حيث أظهرت النتائج اختفاء مجموعة الأزوميثين (C=N) المميزة لقواعد شف وظهور مجاميع حلقة الايميدازول المميزة. كما تم تقييم الفعالية الدوائية للمركبات المحضّرة ضد نوعين من البكتيريا هما Escherichia coli و Staphylococcus aureus وأظهرت النتائج أن المركب [E_1] يمتلك أعلى فعالية تثبيطية ضد كلا النوعين، إذ بلغ قطر منطقة التثبيط نحو 3.0 سم للبكتيريا سالبة الغرام و3.1 سم للبكتيريا موجبة الغرام، يمكن ان يعود هذه الفعالية العالية الى وجود معوض البروم ذو الوزن الجزيئي العالي التي حسنت من نفاذية الاغشية في البكتيريا .

الكلمات المفتاحية: قواعد شف، الإيميدازول، الفعالية الحيوية، المكورات العنقودية الذهبية، الإشريكية القولونية.

1. Introduction

Over the past century, nitrogen-based heterocyclic rings have attracted a lot of attention due to their fundamental structure, which exhibits fascinating pharmacological properties and therapeutic potential in a range of disease domains. The diversity of nitrogen-based heterocyclic rings in medicinal chemistry has led to the development of several medications, including antibacterial agents. Because these rings may create hydrogen connections with biological targets, they are also helpful building blocks for developing novel drug candidates [1]. Imidazole derivatives are compounds with two nitrogen atoms and a five-membered ring that are widely used in a variety of fields, including medicine. Because of their diverse pharmacological properties, imidazole derivatives have attracted a lot of attention among the various chemical substances explored in medicinal chemistry [2].Furthermore, the electron-rich nitrogenous heterocyclic ring may readily donate or absorb protons and create a variety of weak reactions [3]. Imidazole derivatives, such as imidazole-4-one, are widely used to treat a variety of diseases, which attests to their versatility and importance in medicinal chemistry. Cancer, diabetes, Alzheimer's disease, viral disorders, bacterial infections, fungal infections, and inflammation are among the diseases they combat. The broad range of biological effects of imidazole compounds highlights their importance in the development of drugs and therapeutic treatments for a variety of disease areas [4]. For example, cyclic imidazole compounds have shown potential antibacterial, anti-inflammatory, and anticancer effects [5]. Imidazole compounds have demonstrated the ability to inhibit the growth of several bacterial strains, including Candida albicans and both Gram-positive and Gram-negative bacteria, due to their antibacterial and antifungal characteristics [6]. It functions by interfering with the reproduction of bacterial DNA, rupturing cell membranes, and creating cell walls [7]. Therefore, the objective of this work is to develop novel imidazole derivatives and assess their antibacterial efficacy against *Staphylococcus aureus* and *Escherich-*

ia coli. The objective of this endeavor is to get a deeper knowledge of the potential of these novel compounds in therapeutic and bactericidal contexts.

2. Experimental:

2.1. Material: All of the chemicals used in this project were bought from Fluka, Aldrich, and BDH and weren't further purified.

2.2. Devices used: The 9300 thermoelectric melting apparatus was used to determine the melting points of the materials. A Shimadzu KBr spectrometer (400-4000 cm^{-1}) was used as a reference spectrometer in the FT-IR spectrometer (8400S) from Shimadzu. Bruker spectrometers operating at 400 MHz were used to measure the ^{1}H-NMR and ^{13}C-NMR spectra.

2.3. Preparation of Schiff base [E_1-E_4] [8]

In a ceramic beaker, (0.009 mol) of benzaldehyde derivatives and (0.003 mol, 1 g) of (4,4',4''-(1,3,5-triazine-2,4,6-triyl)trianiline were combined. Two drops of glacial acetic acid and twenty milliliters of DMF solvent were added to the mixture. After that, the beaker was microwaved for two to five minutes at 150°C while covered with aluminum foil with tiny holes to enable vapor to escape. TLC verified the response. Tetrahydrofuran (THF) was used to recrystallize the precipitate after it had been collected and cleaned with distilled water. As in Table 1 .

Table 1 lists a few physicochemical characteristics and the proportion of Schiff base [E_1-E_4].

Comp No.	R	Molecular Formula	M.Wt g/ mol	Color	Time (mint)	M.P (^{0}C)	R*f*	Yield (%)
E_1	Br	$C_{42}H_{27}Br_3N_6$	855.43	Yellow	5	230-232	0.57	74
E_2	Cl	$C_{42}H_{27}Cl_3N_6$	722.07	Light ellow	3	220-222	0.46	75
E_3	F	$C_{42}H_{27}F_3N_6$	672.72	Yellow	3	211-213	0.86	60
E_4	CH_3	$C_{45}H_{36}N_6$	660.83	Yellow	2	200-202	0.73	78

2.3. Preparation of imidazole derivatives [E_9-E_{12}] [9,10]

In a ceramic beaker, (0.001 mol) of Schiff base derivatives [E_1-E_4] were combined with (0.003 mol, 0.27 g) of the amino acid alanine, and then (15 ml) of DMF solvent was added. After that, the beaker was placed in a microwave oven set at 150°C for three to eight minutes while covered with aluminum foil with tiny holes to enable vapor to escape. TLC was used to check that the reaction had finished. The precipitate was collected, cleaned with distilled water, and then recrystallized using the same solvent (DMF). As in Table 2 .

Table 2. lists a few physicochemical characteristics and the proportion of imidazole [E_9-E_{12}].

Comp No.	R	Molecular Formula	M.Wt g/mol	Color	Time (mint)	M.P (^{0}C)	R*f*	Yield (%)
E_9	Br	$C_{51}H_{42}Br_3N_9O_3$	1068.67	Green	7	282-284	0.72	51
E_{10}	Cl	$C_{51}H_{42}Cl_3N_9O_3$	935.31	Light brown	8	269-271	0.82	53
E_{11}	F	$C_{51}H_{42}F_3N_9O_3$	885.95	Brown	5	241-243	0.84	58
E_{12}	CH_3	$C_{54}H_{51}N_9O_3$	874.06	Darkbrown	3	254-256	0.76	52

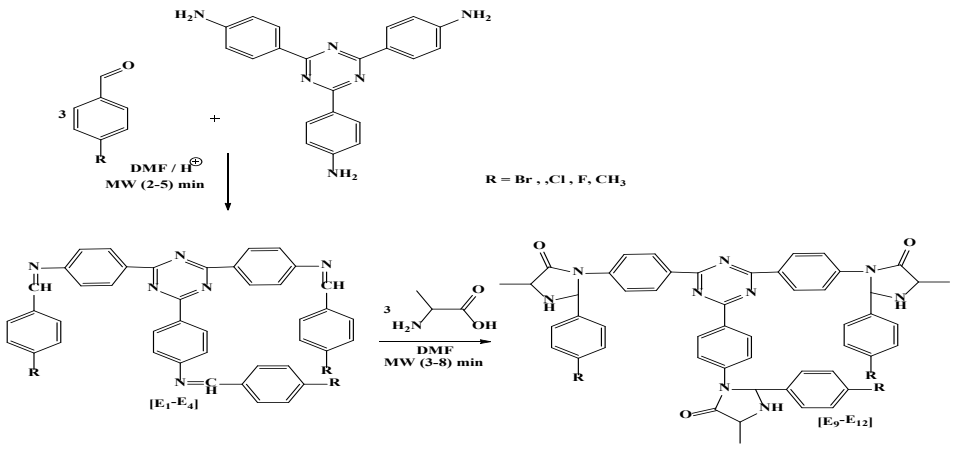
2.4. Biological activity study [20, 21]

Gram-positive (***Staphylococcus aureus***) and Gram-negative (***Escherichia coli***) bacteria were identified in the Department of Biology laboratory. These are among the most common types of bacteria. Mueller-Hunter agar was prepared by dissolving 40 g of bacteria in 1 liter of distilled water and sterilizing it in a steam generator at 1.5 bar for 15 minutes. The mixture was then transferred to Petri dishes and allowed to cool [11, 12]. To ensure bacterial dispersal, the bacterial cells were spread on the dishes in three different directions. After polarization, the resulting compounds [E5, E8] were diluted with dimethyl sulfoxide (DMSO) to three concentrations (0.1, 0.01, and 0.001 mg/ml). To prepare an initial dilution of 0.1 mg/ml, 0.1 g of the compounds

were dissolved in 10 mL of the solvent. One milliliter of this diluted solution was transferred to another tube, and 9 milliliters of solvent were added [13, 14]. A third diluted solution of 0.01 mg/mL was then prepared by further dilution. Subsequently, a third diluted solution of 0.001 mg/mL was prepared by transferring one milliliter of this diluted solution to a new tube and adding 9 milliliters of solvent. The results of the resulting compounds were compared with those of antibiotics such as ***ciprofloxacin and imikaicin***. The antibiotics were chosen because of their broad spectrum of activity and proven efficacy against these bacteria, making them suitable benchmarks for determining the antibacterial activity of the prepared compounds. A centimeter ruler was used to read the results after the plates had been left to stand for a full day [15, 16].

3. Results and discussion

As seen in Scheme 1, one mole of Schiff base derivatives and three moles of the amino acid alanine were reacted to create five-membered imidazole derivatives. The completion of the reaction was confirmed by TLC, which was used to monitor the reaction flow. The difference in ***Rf*** values in Tables 1 and 2 can be attributed to the difference in the polarity of the compounds and the nature of the substituents attached to the benzene ring, reflecting a reaction with the stationary phase, silica gel. Therefore, ***Rf*** is considered a key factor in the success of the reactions.



Scheme (1): The produced chemicals' route

3.1. Characterization of Schiff base [E_1-E_4]

In the FT-IR spectrum of the produced Schiff bases [E_1-E_4], the removal of the (C=O) group expansion band and the appearance of an absorption band at the (3080-3052) cm^{-1} band are attributed to the (C-H) aromatic bond expansion. Between the two spectra, two absorption bands appeared at the frequency bands (1484-1475) cm^{-1} and (1577-1561) cm^{-1}, attributed to the (C=C) aromatic bond expansion, and an intermediate expansion band at the frequency band (1620-1614) cm^{-1}, attributed to the (C=N) group [17,18]. As in Table 3 and Figure 1,2 .

Table 3. FTIR spectral values for compounds [E1-E4]

Comp. No.	R	IR (KBr) cm-1			
		νC-H Arom.	νC=N	νC=CArom.	Others
E_1	Br	3059	1620	1577 1481 ,	ν (C-Br) 540
E_2	Cl	3052	1617	1565 1476 ,	ν (C-Cl) 687
E_3	F	3071	1616	1561 1484 ,	ν (C-F) 864
E_4	CH_3	3080	1614	1566 1475 ,	ν (C-H) *asy.* (2920), *sym.* (2852)

When examining the ^{1}H-NMR nuclear magnetic resonance spectrum of compound [E_1] using solvent (DMSO-d^6), the protons of the (HC=N) groups were identified as the source of a single signal at the chemical shift of (8.72) ppm, the protons of the aromatic rings were identified as the source of a multiple signal in the range of (7.08-7.94) ppm, and the protons of the solvent (DMSO-d^6) at the chemical shift of (2.51) ppm. As in Figure 3

When examining the ^{13}C-NMR nuclear magnetic resonance spectrum of compound [E_1] with the solvent (DMSO-d^6), signals were detected at the following chemical shifts (179.14) ppm for the carbon of the triazine ring; (161.19) ppm for the carbon of the azomethine groups (C=N); (154.39-

121.89) ppm for the carbons of the aromatic ring; and (39.34-40.59) ppm for the carbon of the solvent (DMSO-d^6). As in Figure 4 .

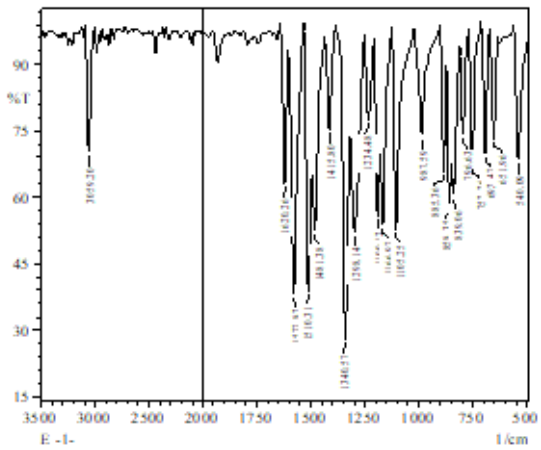


Figure 1. FT-IR for [E_1]

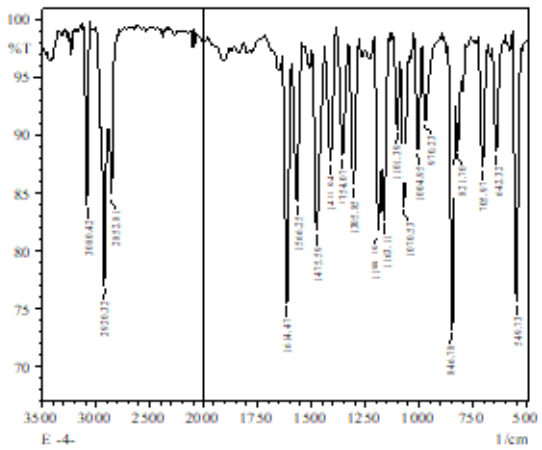


Figure 2. FT-IR for [E_4]

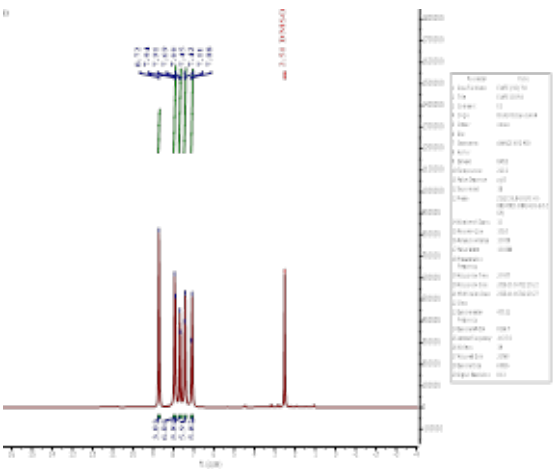


Figure 3. ^{1}H-NMR for [E_1]

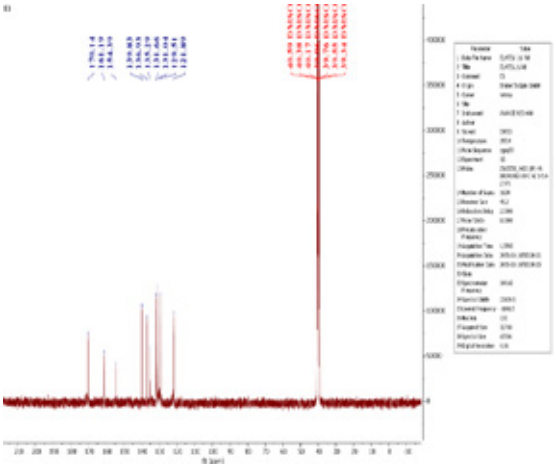


Figure 4. ^{13}C-NMR for [E_4]

3.2. Characterization of imidazole [E_9-E_{12}]

The azomethine (C=N) stretch band, which appeared in the (1614–1620) cm^{-1} range in the synthesized Schiff base compounds [E_1–E_4], disappeared when examining the infrared spectra of the prepared compounds [E_9–E_{12}]. The stretching of the (N–H) group in the imidazole ring was responsible for

the appearance of an intermediate band in the (3138–3267) cm^{-1} range. The stretching of the aromatic (C–H) bond was responsible for the appearance of an absorption band in the (3047–3066) cm^{-1} region. In addition, the stretching of the aliphatic (CH) bond was responsible for the appearance of symmetric and asymmetric stretch absorption bands in the (2852–2871) cm^{-1} and (2916–2942) cm^{-1} ranges, respectively. The stretching of the (C=O) group in the imidazole ring is responsible for the appearance of an intermediate band in the range (1680-1697) cm^{-1}. Two other bands were also found. The appearance of the bands at (1477-1467) cm^{-1} and (1577-1552) cm^{-1} is attributed to the vibration of the aromatic (C=C) bond, while the appearance of the bands at (1232-1199) cm^{-1} is attributed to (C-N) [19,20]. As in Table 4 and Figure 5,6 .

Table 4. FTIR spectral values for compounds [E_9-E_{12}]

Comp. No.	R	IR (KBr) cm^{-1}						
		νN-H	νC-H Arom.	νC-H Aliph.	νC=O	νC=C Arom.	νC-N	Others
E_9	Br	3267	3066	2925 2865	1686	1561, 1474	1232	ν (C-Br) 597
E_{10}	Cl	3138	3053	2916 2854	1680	1552, 1477	1251	ν (C-Cl) 669
E_{11}	F	3221	3047	2922 2852	1697	1577, 1467	1199	ν (C-F) 852
E_{12}	CH_3	3232	3048	2942 2871	1684	1567, 1472	1220	ν (C-H) *asy*. (2942) *sym*. (2871)

When examining the proton ^{1}H-NMR nuclear magnetic resonance spectrum of compound [E_{11}], a single signal at chemical shift (8.44) ppm was identified as the (NH) proton, a multiple signal in the range (7.32-8.18) ppm as the aromatic ring protons, a single signal at chemical shift (5.99) ppm as the (CH) proton, a quadrupole signal in the range (3.33-3.96) ppm as the (CH_3) proton, and a signal at chemical shift (DMSO-d^6) ppm as the solvent pro-

tons. As in Figure 7

When examining the carbon-^{13}C-NMR nuclear magnetic resonance spectrum of compound [E_{11}], it was found that the carbon of the carbonyl groups (C=O) was responsible for a signal at the chemical shift of (175.65) ppm, the carbon of the triazine ring was responsible for a signal at the chemical shift of (173.58) ppm, the aromatic ring carbons were responsible for multiple signals at the chemical shifts of (155.60, 65.21) parts per million, the carbon of (CH), the carbon of (25.72) ppm, and the solvent (DMSO-d^6) was responsible for signals at the chemical shift of (39.31-40.56) ppm. As in Figure 8 .

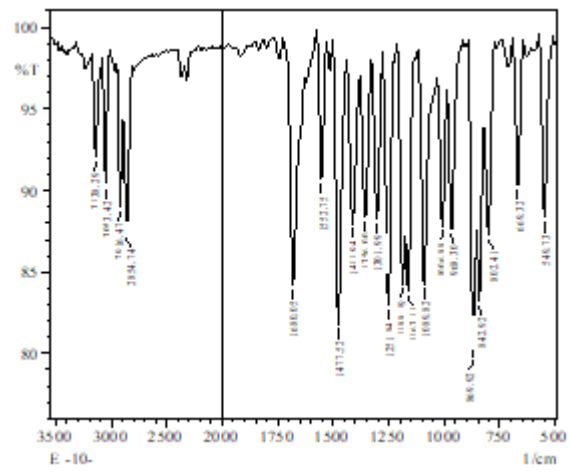


Figure 5. FT-IR for [E_{10}]

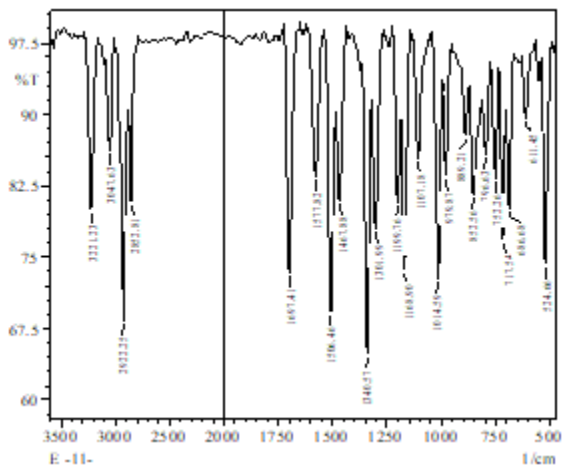


Figure 6. FT-IR for [E_{11}]

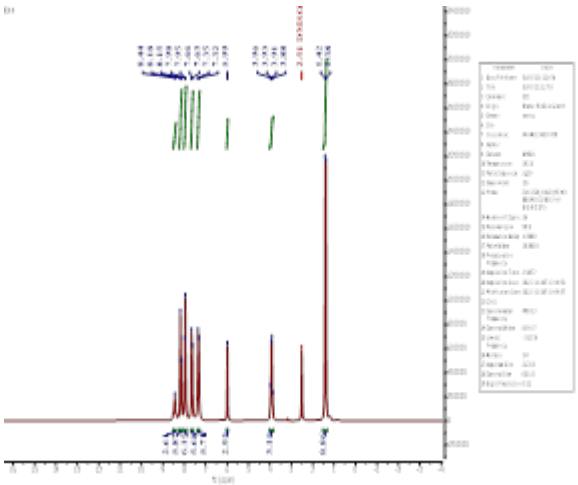


Figure 7. ^{1}H-NMR for [E_{11}]

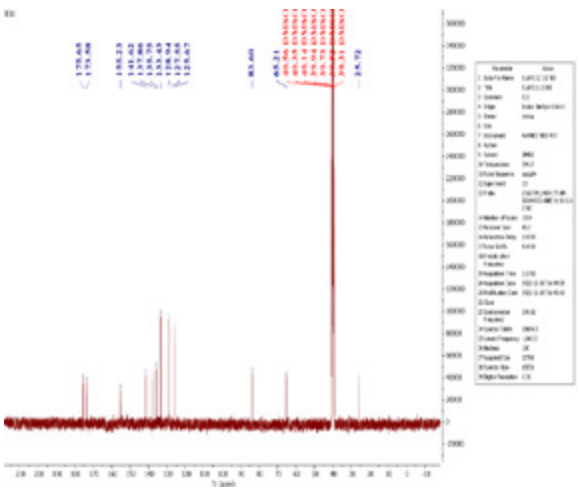


Figure 8. ^{13}C-NMR for [E_{11}]

3.2. Evaluation of the Biological Activity of Prepared Compounds

Table 5 shows the results of measuring the efficacy of the prepared compounds against two types of bacteria, one Gram-positive and the other Gram-negative. Three concentrations were used: 0.1, 0.01, and 0.001 mg/ml. The results were read 24 hours after the concentrations were placed in the dishes. The results showed that compound [E_1] was the most effective against both types of bacteria used, with an inhibition diameter of approximately 3.0 cm in Gram-negative Escherichia coli. The inhibition percentage was good at the other concentrations, indicating its ability to penetrate the cell membrane [21]. The inhibition diameter reached approximately 3.1 cm in Gram-positive Staphylococcus aureus bacteria, but this inhibition decreased rapidly at other concentrations, indicating that the inhibitory ability of this compound is concentration-dependent. The effectiveness of compound [E_1]may be attributed to the presence of bromine as a substituent, which enhanced membrane penetration through the cell membrane, particularly in Gram-negative bacteria. These bacteria are known for possessing an outer membrane that restricts drug passage. Additionally, its large size contributes to electron distribution compared to fluorine and chlorine, leading to the formation of halogenated bonds with enzymes or nucleic acids within the bacterial cell, thus retaining it inside the cell for a longer period [22]. The results of the other compounds were mixed. Compound [E_2]demonstrated high activity against Gram-negative bacteria, with a diameter similar to compound [E_1](3.0 cm). Compound E_{10} showed good activity at the highest concentration against bacteria, with an inhibition diameter of approximately 2.8 cm in Gram-negative bacteria. As for compound [E_9] against Gram-positive bacteria, its activity was higher at moderate nitrate concentrations compared to high concentrations, where growth was inhibited at a moderate concentration of 2.8 cm^{-1} and at a high concentration of 2.6 cm^{-1} [23, 24]. These results indicate the promising potential of some of the prepared compounds as antibacterial agents, particularly compound [E_1], which demonstrated activity exceeding standard levels in some cases [25], as illustrated in Figures 9 and 10.

Table (5): Biological efficacy of produced compounds and control treatments (inhibition in cm).

Comp. No.	*Escherichia coil*			*Staphylococcus aureus*		
Conc. mg/ml	0.001	0.01	0.1	0.001	0.01	0.1
E_1	2.3	2.1	3.0	1.6	1.6	3.1
E_2	0.7	1.8	3.0	0.2	0.0	1.0
E_9	0.3	1.3	0.9	0.8	2.8	2.6
E_{10}	0.3	0.7	2.8	0.2	0.4	1.0
Ciprofloxacin 10 mg	1.5			0.8		
*Amikasin*10 mg	0.5			0.8		

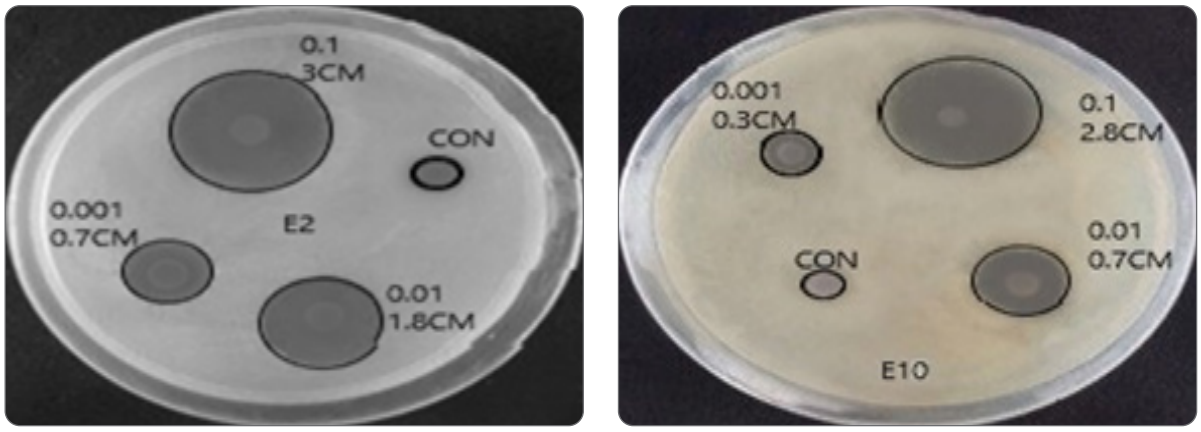


Figure 9. Inhibitory ability of [E_2,E_{10}] of the mercaptoides against *Escherichia coli* bacteria

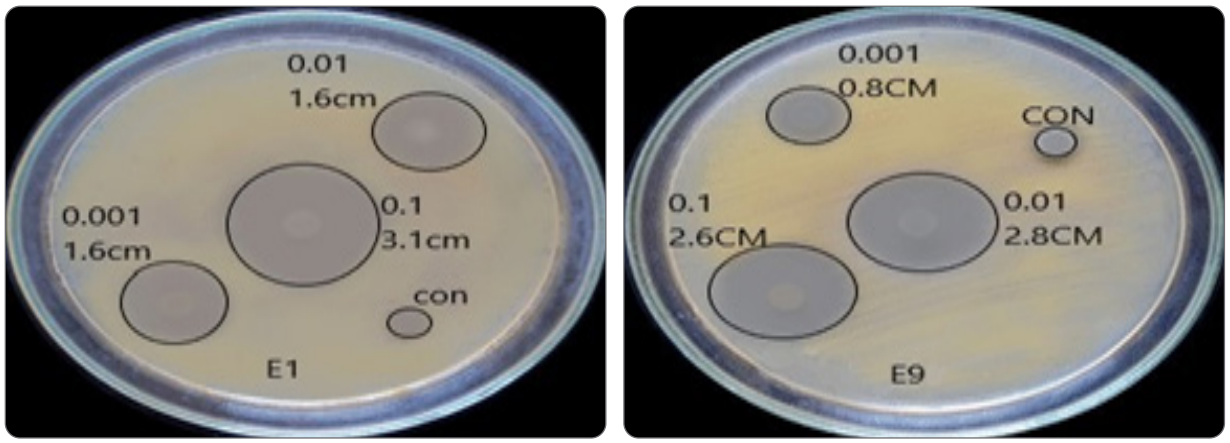


Figure 10. Inhibitory ability of [E_1,E_9] of the mercaptoides against *Staphylococcus aureus* bacteria

4. Conclusions

The results demonstrate that the adopted microwave-assisted technique for synthesizing heterocyclic imidazole derivatives is an efficient, rapid, and clean method, yielding products with good purity and high structural accuracy. This is clearly confirmed by the FT-IR and $^{1}H^{13}$&C-NMR spectral analyses, which showed distinct spectral transformations corresponding to the formation of the imidazole ring. The study further revealed promising antibacterial activity among the synthesized compounds. Compound [E_1] exhibited the highest inhibitory effect at various concentrations and, in some cases, surpassed the activity of standard antibiotics, suggesting its strong potential as an effective agent against Gram-negative and Gram-positive bacteria.

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