SYNTHESIS AND CHARACTERIZATION OF SOME HETEROCYCLIC COMPOUNDS (AZETIDINE, OXAZEPINE) DERIVED FROM SCHIFF BASES AND EVALUATION OF THEIR BIOLOGICAL ACTIVITY.

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Abstract:

In this study, Some new Compounds have been Synthesized including the preparation of some Schiff bases form the reaction terphthadehyde compound with two moles of aniline derivatives (2-amino-5-methyl benzoic acid ,3-amino phenol) ,also. The stage include preparation of new heterocyclic compounds four-membered (β-Lactam) using the traditional methods and seven membered (Oxazepine, Oxazepane) using the microwave methods. The prepared compounds were characterized by FT-IR spectra, 1H-NMR, and 13C-NMR spectra, in addition to their melting points and TLC (Thin Layer Chromatography).finally the prepared compounds are tested against Gram-positive and Gram-negative bacteria to study their biological activity

Keywords : Terphthaldehyde, Schiff bases, β-Lactam, (Oxazepine, Oxazepane), anti-bacterial activity.

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1. INTRODUCTION:

Schiff bases are formed by the condensation of primary (aromatic) amines with aldehydes or ketones that contain the azomethine (imine) moiety (-CR=N-)[1,2] They are regarded as versatile pharmacophores for a variety of pharmacological activities[3] in which the azomethine group has been shown to be critical to bioactivity [4]. Schiff bases, for example, whether natural or synthetic have shown promising antibacterial, antitubercular, antifungal, antiparasitic, antiviral, antioxidant, and anticancer properties [5].

Often classified as β-lactam, the Azetidin-2-ones compounds have been used as building blocks in the synthesis of essential biological compounds [6, 7] . The lactam is an amide ring in which the nitrogen atom is linked to the carbon atom with a beta position relative to the total carbonyl group, and its compounds are heterocyclic compounds with a heterocyclic (nitrogen) atom, and are classified within the ring addition reactions [2+2] and among the most famous chemical reactions for their preparation. Beta-lactams are considered the basis of many medicines and medical drugs because of their vital efficacy, especially after the discovery of penicillin and its ability to eliminate disease-causing bacteria by the scientist Alexander Fleming (1928) Beta-lactams are also used in the treatment of neurological disorders and other antibiotics that Beta-lactam rings include Cephalosporins, nocardiosis, and monobactams that have been widely used to treat microbial diseases and have interesting biological activities [8], according to recent research and studies. They are often prescribed as antibiotics to treat bacterial infections.

Oxazepine is a heterocyclic unsaturated ring consisting of one oxygen atom and one nitrogen atom in addition to five carbon atoms [9]. There are three isomers (1, 2), (1, 3), and (1,4) [10]. This numbering depends on the position of the oxygen and nitrogen atoms in the seven-ring [11]. Derivatives of 1,3-0xazepine were prepared by reaction of Schiff bases with anhydrides. Oxazepine compounds are important because they have a wide range of biological and pharmacological activity[12] including antibacterial[13] antifungal [14] and anticancer, They also have anticorrosion properties[15].

1. Experimental:

1.1 Materials and Instruments :

Reagents and reactants are used as obtained from commercial suppliers without further purification. The solvents were previously purified. The purity of the derivatives and the course of the reaction were monitored using Thin layer chromatography on silica gel G (Merck grade) with a mixture of ethanol and benzene as the mobile phase. Melting points were measured in open capillaries, with the help of a melting point (Stuart) apparatus (SMP30, England) pronounced in°C and uncorrected. The infrared (IR) spectra were recorded on a Shimadzu Prestige-21 spectrophotometer using potassium bromide (KBr pellets) and the values in cm^{-1}, 1 H NMR, and 13 CNMR derivative spectra were recorded on a Bruker (Avance III, Bruker 300MHz NMR Spectrophotometer using TMS as an internal standard and values are expressed in ppm at University of Tehran – Iran.

1.2 Preparations of Schiff base General Synthesis of Schiff base (Z5,Z10)[16]

3,3'-((1,4-phenylenebis(methaneylylidene))bis(azaneylylidene))diphenol {Z5}

6,6'-((1,4-phenylenebis(methaneylylidene))bis(azaneylylidene))bis(3-methylbenzoicacid) {Z10}

Dissolve (0.003mol) of Terphthadehyde was dissolved in ethanol absolute (25 ml)

added (2-3) drops of glacial acetic acid and added (0.006 mol) of different amine (2-amino-5-methyl benzoic acid,3-amino phenol) The mixture was refluxed for (10-16 hours) with stirring. Then the mixture was allowed to cool at room temperature and the solid product was filtered and recrystallized from ethanol. The physical properties were listed in Table (1).

General Synthesis of (oxazepine) compounds (Z6-Z8) (Z11-Z13) [17]

2,2'-(1,4-phenylene)bis(3-(3-hydroxyphenyl)-2,3-dihydro-1,3-oxazepine-4,7-dione) {**Z6**}

3,3'-(1,4-phenylene)bis(4-(3-hydroxyphenyl)-3,4-dihydrobenzo[e][1,3]oxazepine-1,5-dione) {**Z7**}

2,2'-(1,4-phenylene)bis(3-(3-hydroxyphenyl)-1,3-oxazepane-4,7-dione) {**Z8**}

6,6'-(1,4-phenylenebis(4,7-dioxo-4,7-dihydro-1,3-oxazepine-2,3(2H)-diyl))bis(3-methylbenzoic acid) {**Z11**}

6,6'-(1,4-phenylenebis(1,5-dioxo-1,5-dihydrobenzo[e][1,3]oxazepine-3,4(3H)-diyl))bis(3-methylbenzoic acid) {**Z12**}

6,6'-(1,4-phenylenebis(4,7-dioxo-1,3-oxazepane-2,3-diyl))bis(3-methylbenzoic acid) {Z13}

(0.004 mol) Schiff bases were mixed with various anhydrides (0.008 moles) (maleic anhydride & phthalic anhydride & succinic anhydride)The mixture was thoroughly crushed with a mortar until a powder was obtained. The homogenized mixture is then placed in a ceramic eyelid. Insert a ceramic cover into the microwave oven and irradiate it at 120 w for)4 -15 minutes (after which the precipitate is cooled to laboratory temperature. The resultant material was washed with benzene, then the precipitate was separated by filtration and recrystallized from Absolute ethanol, dried, and the reaction was validated using TLC technology; the physical parameters are reported in Table 1.

General Synthesis of substituted Azetidin-2-ones (Z9,Z14)[18]

4,4'-(1,4-phenylene)bis(3-chloro-1-(3-hydroxyphenyl)azetidin-2-one){**Z9**}

6,6'-(1,4-phenylenebis(3-chloro-2-oxoazetidine-4,1-diyl))bis(3-methylbenzoic acid{**Z14**}

Dissolve (0.0005 moles) of the Schiff base compound in (20mL) of dry1,4-dioxane with continuous stirring on a magnetic stirrer until dissolution, then add (0,001 mol) of triethylamine in drops with Continuous stirring in an ice bath at (5-10 °C) with continuous stirring for a period of (30 min), then add drops (0,001 mol) of chloroacetyl chloride, while continuing to stir and maintaining the temperature for a certain period of time. The course of the reaction was followed up by The TLC using (ethanol: benzene) at a ratio of (2:4) and after a period of intermittent stirring for (17-22hr). The product was dried and recrystallized using ethanol absolute as in the equation below and its chemical and physical properties are shown in Table (1)

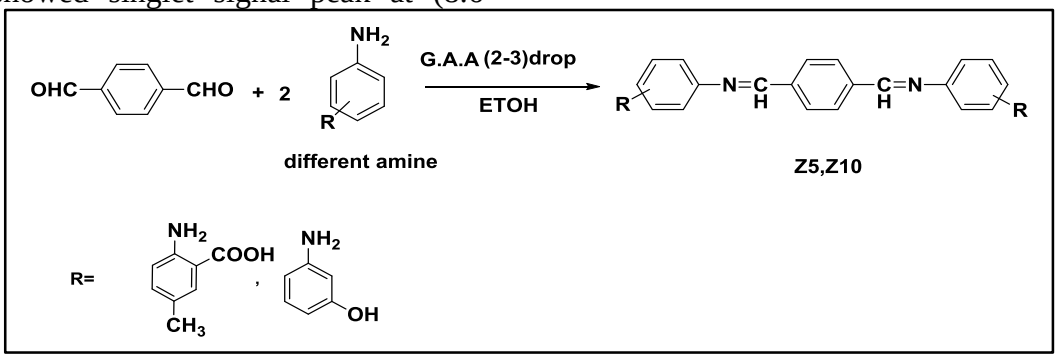
	Table(1): physical properties for synthesis compounds (Z5-Z14)							
NO. COMP	**Name of compound**	**M.F**	**M.W(g\mol)**	**M.P (°C)**	**Rf (2:4) (Ethanol:**	**Color**	**Yield %**	**Time**

					Benzene)			
Z5	**3,3'-((1,4-phenylenebis(methaneylylidene))bis(azaneylylidene))diphenol**	**$C_{20}H_{16}N_2O_2$**	**316.36**	**126-128**	**0.88**	**Orange**	**79**	**13 hr**
Z6	**2,2'-(1,4-phenylene)bis(3-(3-hydroxyphenyl)-2,3-dihydro-1,3-oxazepine-4,7-dione)**	**$C_{28}H_{20}N_2O_8$**	**512.47**	**>310 Dec**	**0.69**	**Red**	**80**	**10 min**
Z7	**3,3'-(1,4-phenylene)bis(4-(3-hydroxyphenyl)-3,4-dihydrobenzo[e][1,3]oxazepine-1,5-dione)**	**$C_{36}H_{24}N_2O_8$**	**612.59**	**136-138**	**0.78**	**Dark red**	**76**	**7 min**
Z8	**2,2'-(1,4-phenylene)bis(3-(3-hydroxyphenyl)-1,3-oxazepane-4,7-dione)**	**$C_{28}H_{24}N_2O_8$**	**516.51**	**140-142**	**0.66**	**Light red**	**80**	**9 min**
Z9	**4,4'-(1,4-phenylene)bis(3-chloro-1-(3-hydroxyphenyl)azetidin-2-one)**	**$C_{24}H_{18}Cl_2N_2O_4$**	**469.32**	**175-177**	**0.84**	**Reddish brown**	**89**	**20 hr**
Z10	**6,6'-((1,4-phenylenebis(methaneylylidene))bis(azaneylylidene))bis(3-methylbenzoic acid)**	**$C_{24}H_{20}N_2O_4$**	**400.43**	**129-131**	**0,83**	**Yellow**	**84 %**	**16 hr.**
Z11	**6,6'-(1,4-phenylenebis(4,7-dioxo-4,7-dihydro-1,3-oxazepine-2,3(2H)-diyl))bis(3-methylbenzoic acid)**	**$C_{32}H_{24}N_2O_{10}$**	**596.55**	**144-146**	**0,75**	**brown**	**87 %**	**11 min**
Z12	**6,6'-(1,4-phenylenebis(1,5-dioxo-1,5-dihydrobenzo[e][1,3]oxazepine-3,4(3H)-diyl))bis(3-methylbenzoic acid)**	**$C_{40}H_{28}N_2O_{10}$**	**696.67**	**154-156**	**0,86**	**Brown**	**71 %**	**12 min**
Z13	**6,6'-(1,4-phenylenebis(4,7-dioxo-1,3-oxazepane-2,3-diyl))bis(3-methylbenzoic acid)**	**$C_{32}H_{28}N_2O_{10}$**	**600.58**	**133-135**	**0,71**	**Dark brown**	**82 %**	**9 min**

Z14	**6,6'-(1,4-phenylenebis(3-chloro-2-oxoazetidine-4,1-diyl))bis(3-methylbenzoic acid)**	**$C_{28}H_{22}Cl_2N_2O_6$**	**553.39**	**180-182**	**0,73**	**Orange**	**78 %**	**21 hr.**

2. RESULTS AND DISCUSSION

The Schiff bases were prepared by reacting terephthaldehyde (1mole) and amines (2mole) within ethanol absolute. The prepared compounds were characterized by FT-IR spectra, 1H-NMR, and 13C-NMR spectra, in addition to their melting points and TLC (Thin Layer Chromatography). The FT-IR spectrum of Schiff bases(Z5, Z10)of new azomethine (C=N)[19] group at (1633-1625cm^{-1}) and(1587-1494cm^{-1}) for (C=C) aromatic while were characterized 1H-NMR spectrum of Schiff bases(Z5, Z10)showed singlet signal peak at (8.6 ppm) due to(s N=C-H) proton of an amine group, the spectrum also showed multiple signals at it (7.0-7.6 ppm) belonging to the protons of the aromatic ring of different environments (Ar-H) while were characterized 13C-NMR spectrum of Schiff bases showed signal appeared at the site (158-153 ppm) belonging to the carbon of the imine group (C=N) as well as the spectrum showed multiple signals at the site (105-130 ppm) belonging to the carbon of the aromatic ring of the different environment (C-Ar).**scheme1**



Scheme1: Synthesis of Schiff base compound

Schiff bases(Z5, Z10)were reacted with acetyl chloride via a [2 + 2] cyclic loading scheme2 in dry dioxane in the presence of triethylamine to give the corresponding beta-lactam derivatives (Z9, Z14). The prepared beta-lactam compound was identified by (the FT-IR) spectrum, where it was observed that an absorption band belonging to the azomethine group of Schiff bases disappeared, and this is evidence of the formation of the prepared beta-lactam compound[20], as an absorption band appeared at the frequency (1703 - 1699 cm^{-1}) It is due to the expansion of the carbonyl group of the beta-lactam(C=O) and the appearance of an absorption band (C-Cl) at a frequency (773 cm^{-1}). Where the compound (Z14) was identified by the (1H-NMR) spectrum a signal appeared at (2.52 ppm) belonging to the solvent used (DMSO-d^6), and a binary signal appeared at (4.2 ppm) due to the proton of the quadruple ring (CH-Cl). Multiple signals were observed at (7.4-8.2 ppm) due to the protons of aromatic rings (Ar-H) with different environments. Also, the compound (Z14) was identified by(13C-NMR) spectrum and a signal appeared at the site (40ppm) belonging to the solvent used (DMSO-d6), and a signal appeared at the site (171ppm) belonging to the carbonyl group (C= O)β-lactam. The spectrum also

showed a signal at the site (163ppm) belonging to carbon (CH-N) belonging to the endo cyclic β-lactam. The spectrum showed a signal at the site (62ppm) belonging to the carbon group (C-Cl). The spectrum also showed multiple signals at The position (116-131 ppm) is attributed to a heterocyclic aromatic carbon (C-Ar)

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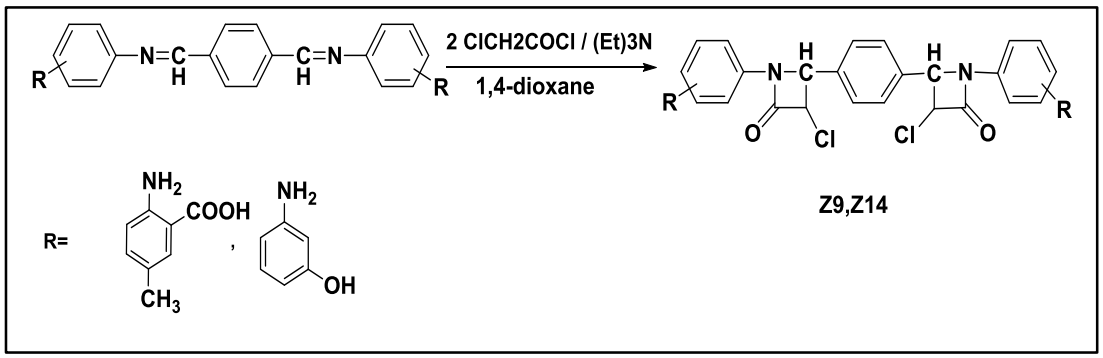
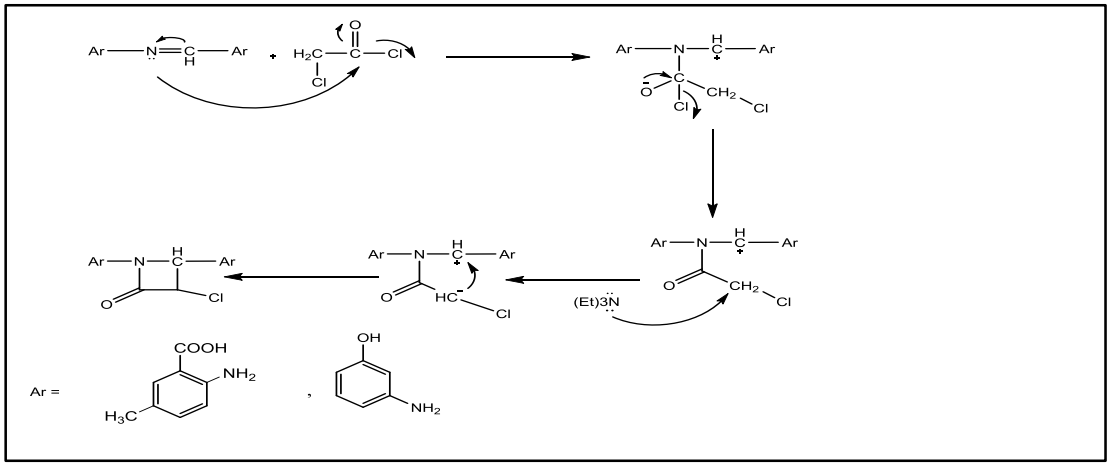


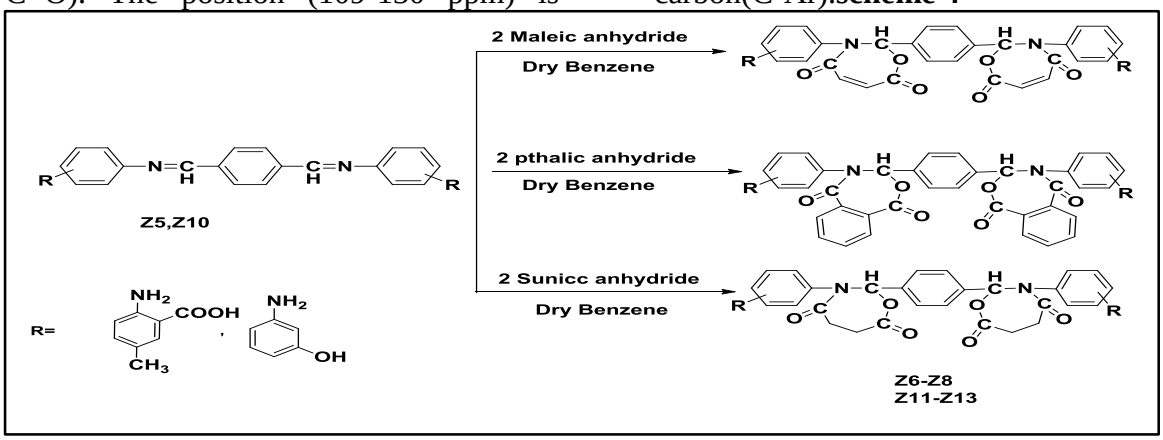
Scheme2: Synthesis of four-membered form Schiff base compound



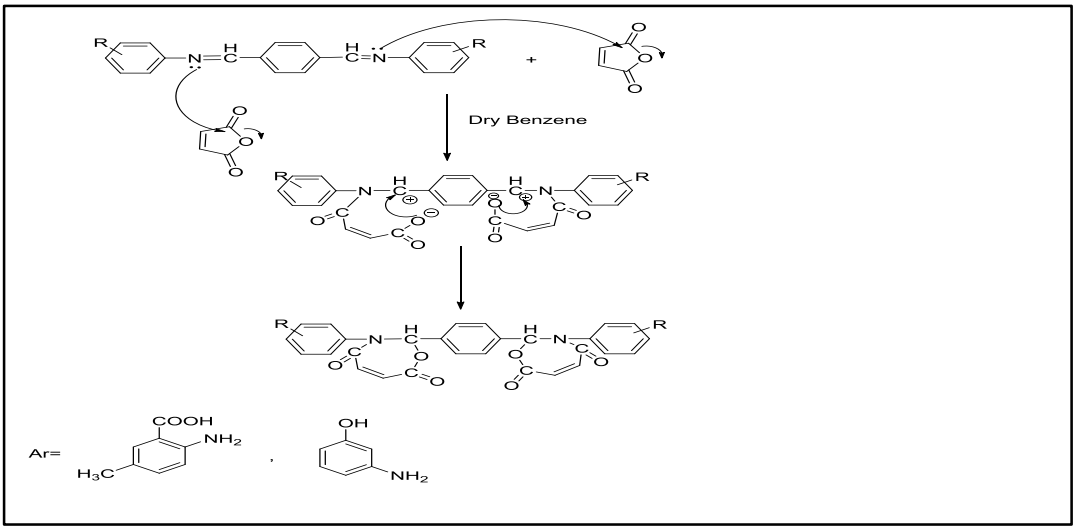
Scheme (3)mechanism of β-lactam

In this study new derivatives of heterocyclic compounds)(Oxazepine, Oxazepane) (Z6-Z8), and (Z11-Z13) were prepared from the reaction of Schiff bases with different anhydrides. The FT-IR spectrum disappearance of an absorption band belonging to the azomethine group of Schiff bases and the appearance of a stretch band of the bond of the lactone carbonyl group (C=O-O) at the frequency (1782-1701cm^{-1}), while the absorption band of the bond of the amide [21] carbonyl group (N-C=O) at the frequency (1625-1591cm^{-1})due to (C=C) at frequency(1589-1502cm^{-1}) Where the compound was identified by (1H-NMR) spectrum, a signal appeared at (2.52 ppm) belonging to the solvent used (DMSO-d6) and a signal appeared at (9.6 ppm) due to the proton of the seven ring group (N-CH). It was observed that multiple signals appear at (7.0 -7.9 ppm) due to the protons of the aromatic rings in different environments (Ar-H). Also, the compound was identified by the (13C-NMR) spectrum, and a signal appeared at the site (40ppm) belonging to the solvent used (DMSO-d6), and a signal appeared at the site (176ppm) belonging to the carbon atom of the lactone carbonyl group (O-C= O) and at the site (172 ppm) it belongs to

the carbon of the amide carbonyl group (N-C=O). The position (109-130 ppm) is attributed to the heterocyclic aromatic carbon(C-Ar).**scheme 4**



Scheme4: Synthesis of Seven membered forms Schiff base compound



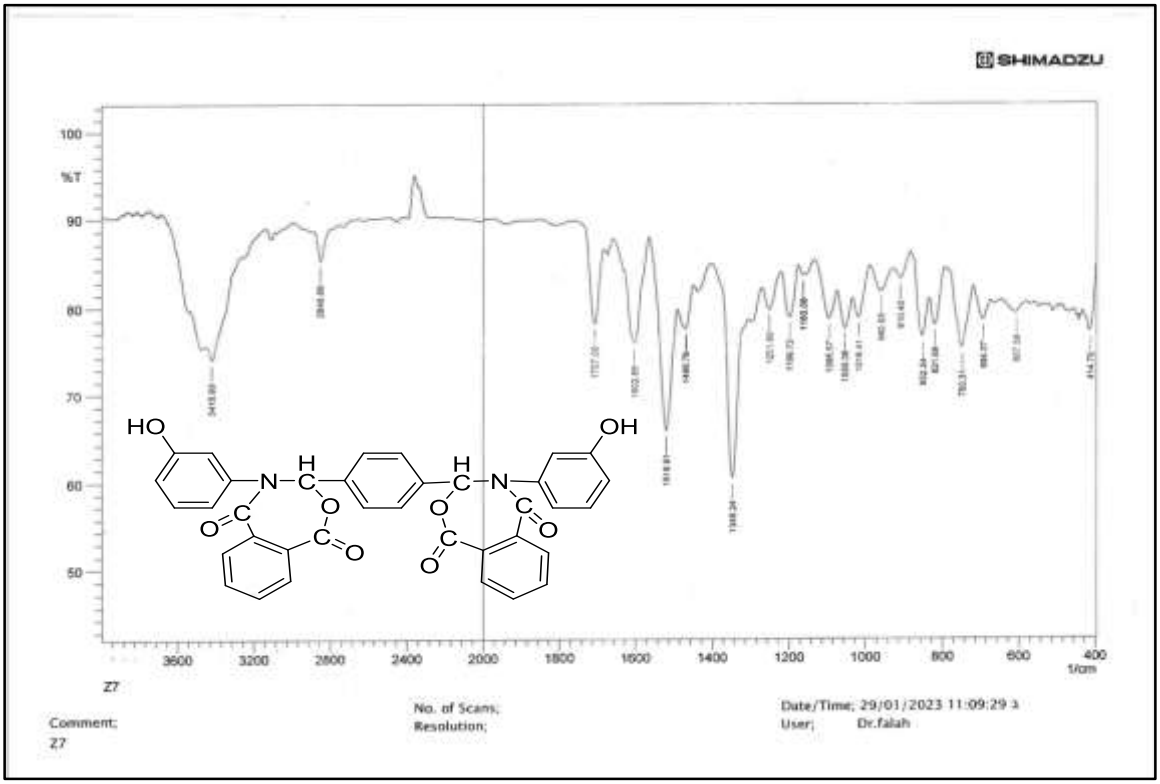


Figure (1):FT-IR spectrum for compound(Z7)

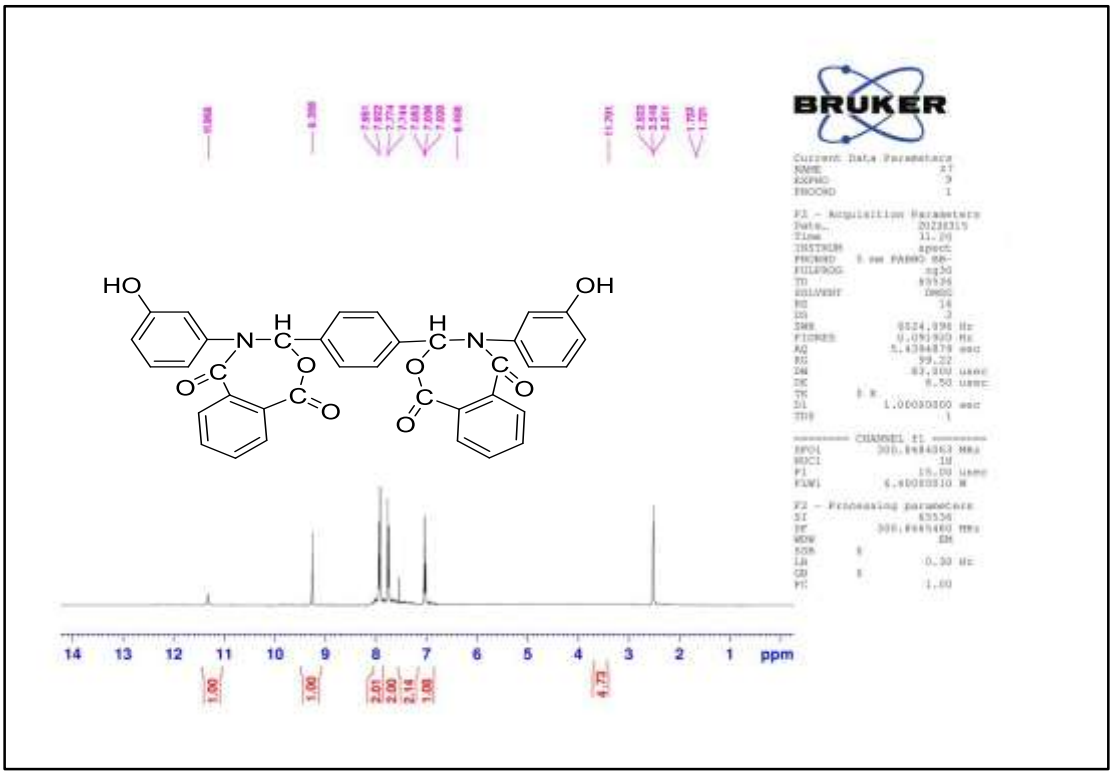


Figure (2):^{1}H-NMR spectrum for compound(Z7)

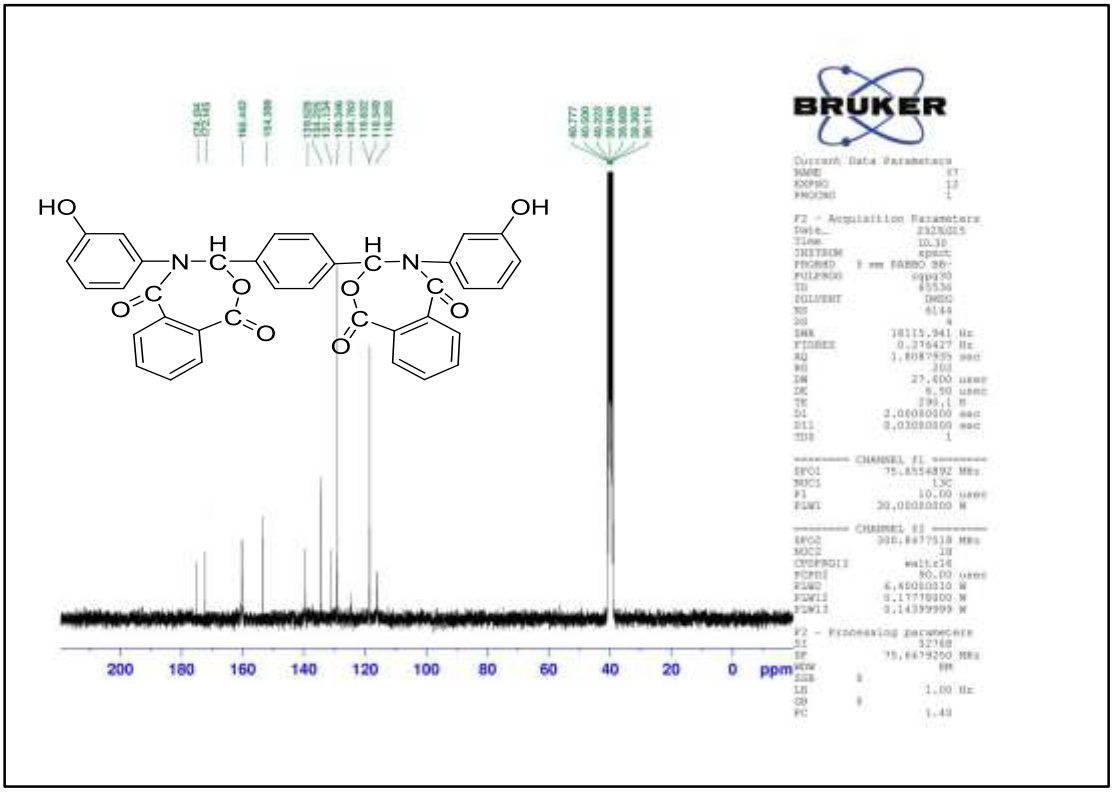


Figure (3):^{13}C-NMR spectrum for compound(Z7)

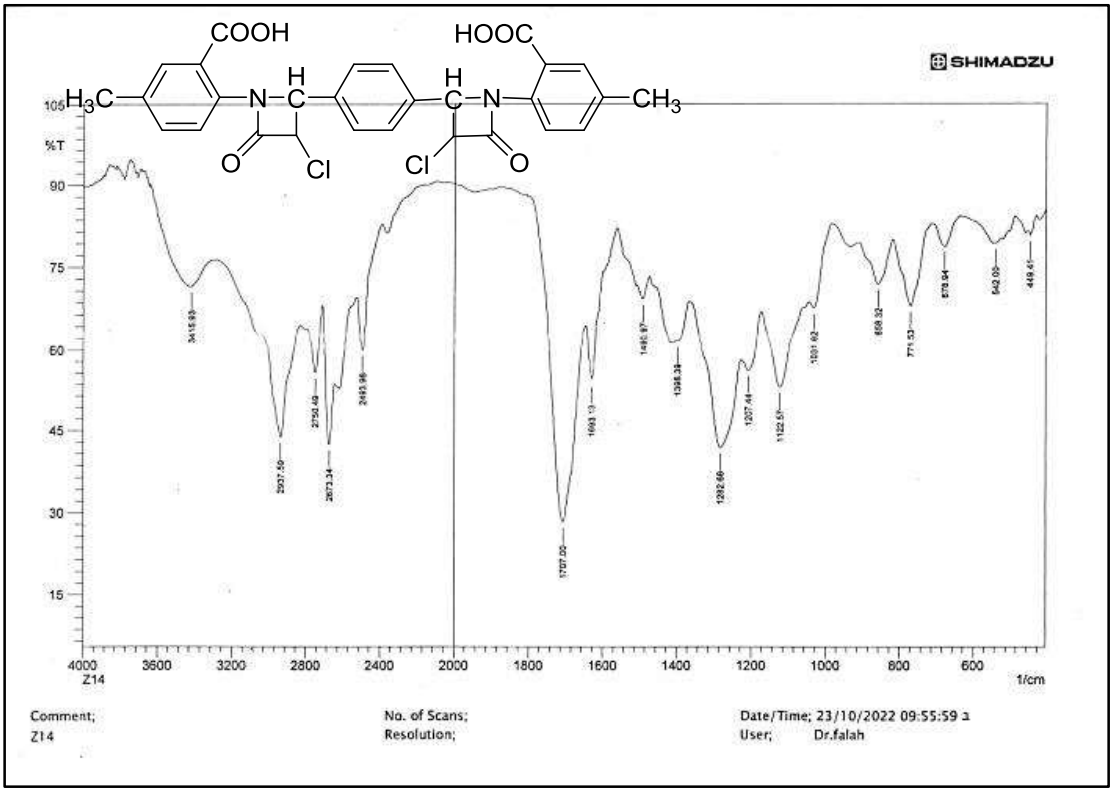


Figure (4):FT-IR spectrum for compound(Z14)

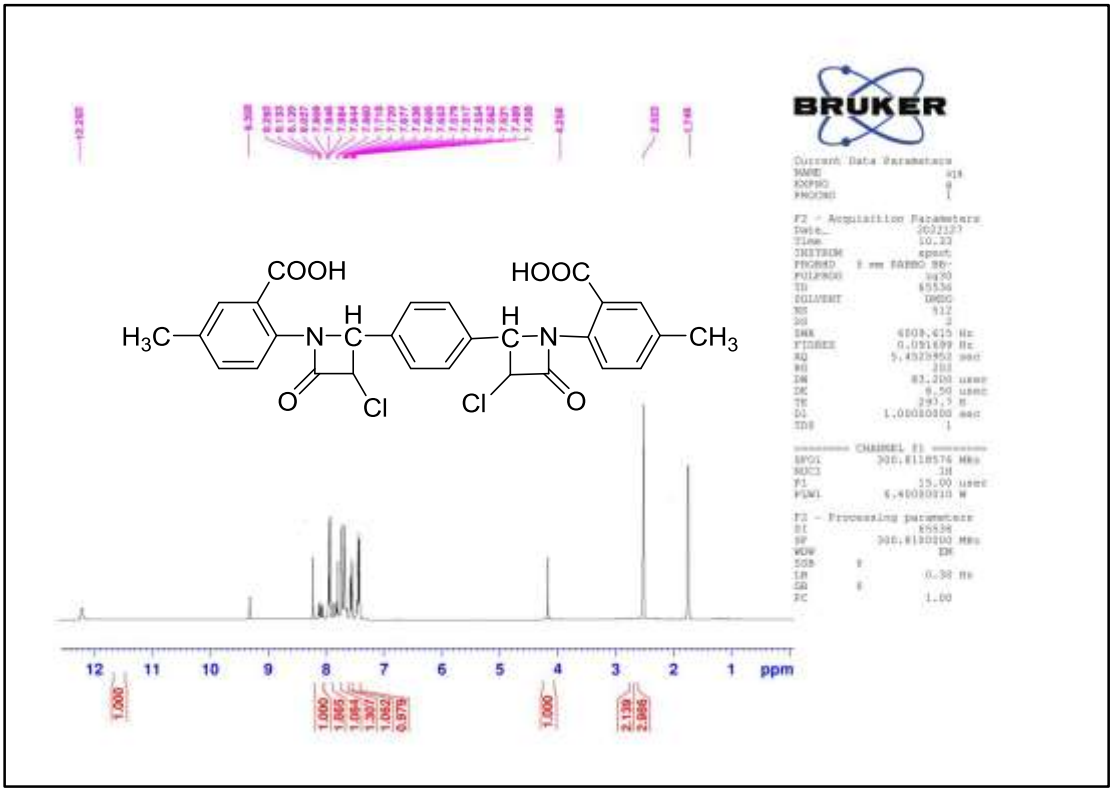


Figure (5): ^{1}H-NMR spectrum for compound(Z14)

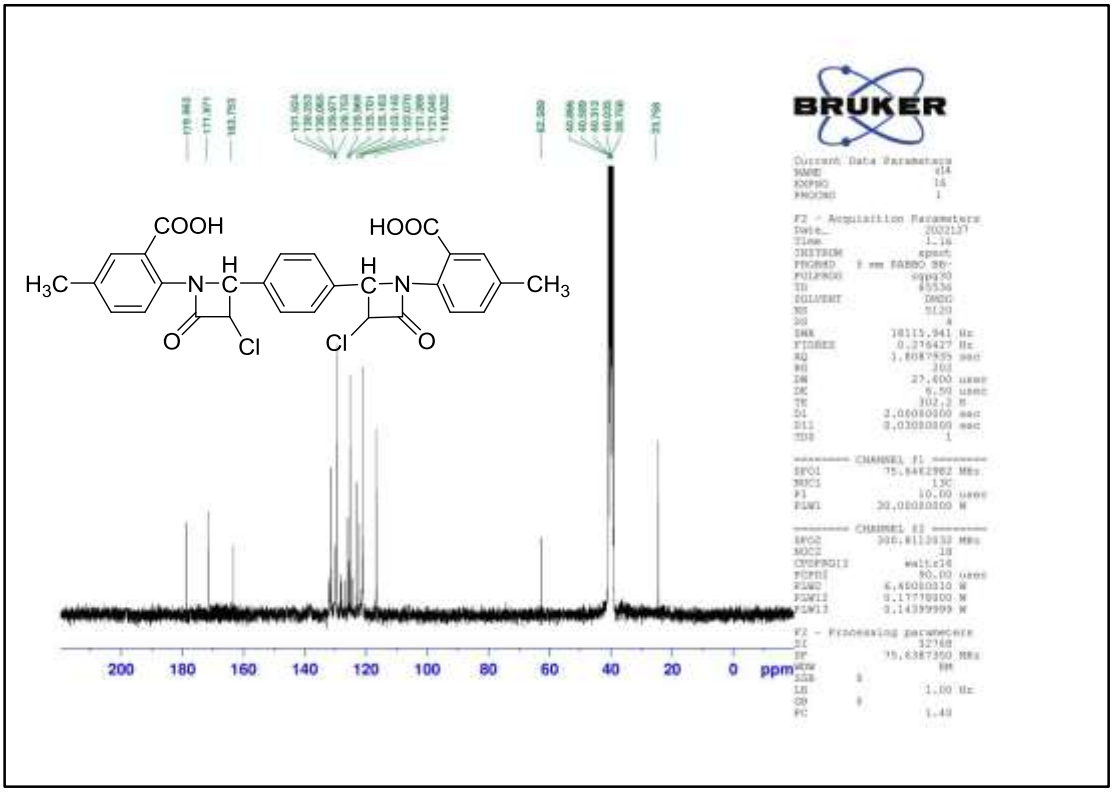


Figure (6): ^{13}C-NMR spectrum for compound(Z14)

Biological activity [22]

The study used two types of isolated pathogenic bacteria, Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia Coli), where solutions were prepared for some of the compounds and to be evaluated for their biological effectiveness against two types of bacteria by taking concentrations (0.1mg/ml, 1mg/ml) of each compound and dissolved it in (5ml) of the solvent (DMSO). The sensitivity of the compounds was investigated using the method of spreading bacteria on the surface of the dishes in the culture medium (Agar Mueller-Hinton) using (Loopful), and four holes were made in the dishes with a diameter of 9mm by corkscrew (Cork borer) sterilized with alcohol, with an appropriate distance between one hole and another to avoid the inhibition zones between them overlapping. Where the prepared solutions were applied to these wells in a volume of 0.1 ml using a (Micropipette) and incubated for 24 hours at 37°C. The compounds' inhibitory zones were then measured on a millimeter scale are shown in Table 2.

Table 2:The biological activity of the compounds

compounds	Anti-Bacterial Activity			
	Escherichia coil		Staphylococcus aureus	
	0.1mg/ml	1mg/ml	0.1mg/ml	1mg/ml
Z5	0	0	13	21
Z6	0	0	14	15
Z7	0	0	12	14
Z8	0	12	11	15
Z10	12	15	10	11
Z11	11	15	10	11
Z12	15	16	11	13
Z13	15	17	12	13
Z14	0	13	10	11

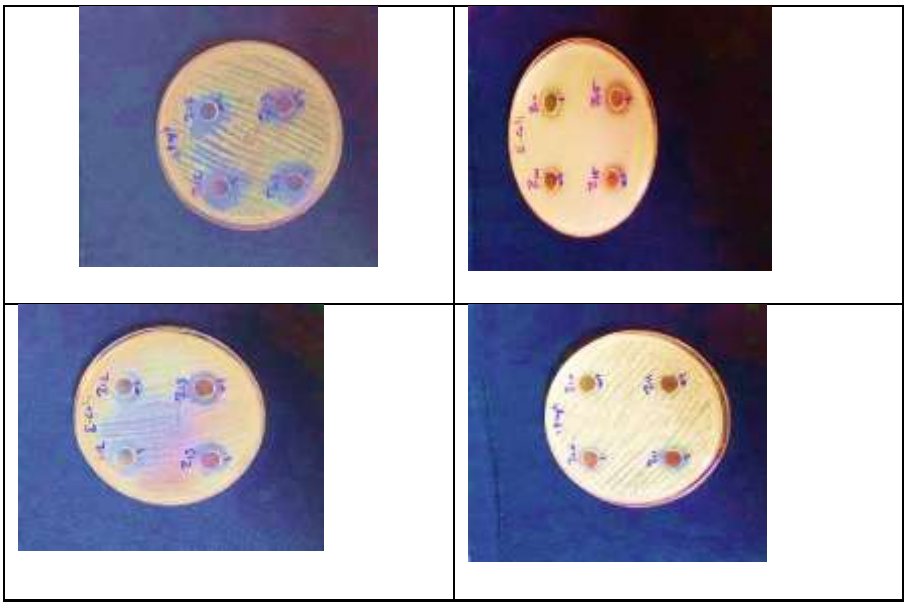


Figure (7): The Anti-Bacterial activity

3. CONCLUSIONS:

The synthesis of the designed chemicals has been completed successfully. Physical characteristics, FT-IR spectroscopy, 1H-NMR, and 13C-NMR spectra were used to corroborate the characterization and identification of the target compounds. Following that, the compounds were tested for antibacterial activity against gram-positive and gram-negative bacteria strains. The majority of the produced compounds exhibit extremely promising antibacterial action against some bacteria strains, whereas others exhibit no activity against specific bacterium strains.

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