Article

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Synthesis , Characterization and study the Antimicrobial Activity For Benzo pyran -2-imine and Tetrazole Ring Derivatives

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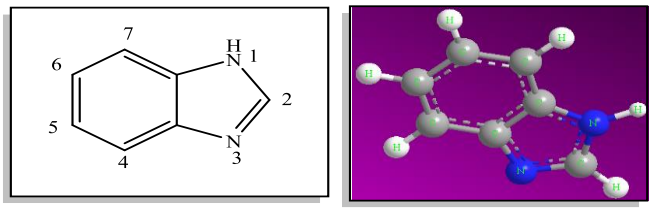
ABSTRACT:

The derivatives of [3-(1H-benzo[d]imidazol-2-yl)-2H-chromen-2-imine]L8 and [2-((1H-tetrazol-5-yl)methyl)-1H-benzo[d]imidazole] L7,L9 have been obtained using -(1H-benzo[d]imidazol-2-yl)acetonitrile as starting material .The latter compound was utilized as a key intermediate for the synthesis of new heterocyclic compounds L8,7,9 with good yields. Newly synthesized compounds have been screened for their antimicrobial activity and characterized by analytical and spectral data.

Keywords: Benzimidazole, Knoevenagel condensation, Benzo pyran, tetrazole.

Introduction

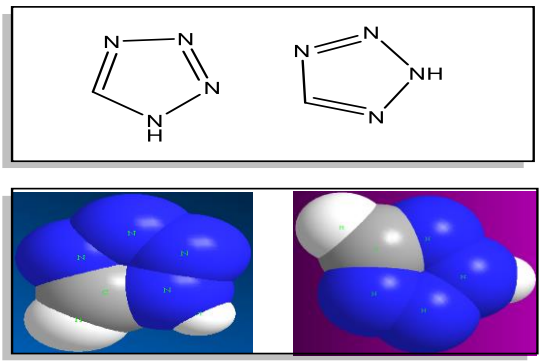
Benzimidazole is one of the imaidazole derivatives, which is position 2 in the imaidazole acetonitrile episode, which includes from two rings one of them is an imidazole heterocyclic ring and the other is a hemogeneous benzene ring[1,2].



Benzimidazoleis a desirable structure in pharmaceutical chemistry and an important pharmacophore. The most chronic benzimidazole compound in nature is (N-ribosyl-dimethyl benzimidazole), which serves as an axial ligand for cobalt in vitamin B12 [3].They have great importance and task because of acid and basic properties. The NH group found in benzimidazole is relatively strong and weak. Another property of benzimidazole is that they have the ability to form salts. . ScaFold benzimidazole is a

useful provisional amendment to the development of pharmaceutical or biological interest molecules. The benzimidazole derivatives were found alternately or the best wide and vintage therapeutic applications such as anti-golf, antennosis, antibiotics, anti-cruel and stopped pressure. Improvement of benzimidazole-based structures led to different drugs [4-6].

Tetrazoles It is heterocyclic rings and includes its composition on carbon atom and four netrogen atoms containing one of the netrueled atoms on hydrogen and is Show two isomer[7]



Itis an aromatic ring that completely obeys Hückel's law and has six delocalized electrons [8]. 1H-Tetrazole is a light yellow, odorless crystalline powder .shows solubility Temperature point at 155-157 Celsius Upon heating, tetrazoles decompose

and release toxic nitrogen Fumes . Tetrazole dissolves in water, acetonitrile, etc. In general , 1H-tetrazole diluted in acetonitrile is used for DNA synthesis in biochemistry [9, 10] .The use of amides , nitriles , thiamides , chlorides, imidoyl and heterogeneous accumulated compounds such as carbodiimides, isocyanates, isothiocyanates and others, which was one of the ways to prepare 1,5-disubstituted tetrazole derivatives (1,5-DSTs). The best method for preparing the tetrazole ring is the Huisgen method, which involves a dipolar cycloaddition reaction between nitriles and azides[11]. There are many prepared tetrazoles that have anti-hypertensive[12], anti-inflammatory [13], antifungal [14]and anti-diabetic[15] properties.

Chemistry

Materials and Methods :

All chemicals were of the highest purity , supplied by Fluka and Merck-company. Measurements of the melting points were recorded by using electro-thermal 9300," melting point engineering LTD,U.K".Thin Layer Chromatography (T.L.C) was performed on silica gel,and spots were visualized by Iodine vapors." FT-IR" spectra,Fourier transform infrared shimadzu (8400) using potassium bromide (KBr pellets) (where by the values are expressed in cm^{-1}),^{1}H-NMR&^{13}C-NMR-spectra in (ppm) unit were operating in *DMSO -d6* as solvent using (**Agilent Varian500 MHz**)-Tehran university/Iran , Microwave 280wt and 120 wt, MICROWAVE OVEN , MODEL NO: MWO-251-01 ,230-240V-50Hz 700W S/N 2004261012019 / MADE IN CHINA, Incubator Binder Italy, Autoclave Hirayama HVE-50.

Synthesis of 3-(1H-benzo[d]imidazol-2-yl)-2H-chromen-2-imine [L_8] Under Microwave irradiation [16] :

Mixture of (0.001mole , 0.157g) 2-(1H-benzo[d]imidazol-2-yl)acetonitrile with(0.001mole , 0.122g)2-hydroxybenzaldehyde in dry clean Crucible. Dissolve the mixture in (5ml) absolute ethanol with 3 drops of triethylamine .The contents were subjected to microwave irradiation at 120 W about 8 min. Progress of the reactions was monitored by TLC using a solvent,(Ethanol 2.5ml :Benzene 2.5ml).After the completion of the reactions cold distilled water were added to the component, Then filterate and take a precipitate with re-crystallization in hot absolute ethanol .

, Yield%:58 , m.p^0 C : 238-240 R_f: 0.78 , M.F : $C_{16}H_{11}N_3OL8$: Yellow , M.Wt: 261

FT-IR (KBr) (V_{max} , cm^{-1}): *3429 (NH benzoimidazole), 1647 (C=N benzoimidazole) (3163(C=NH),1598(C=NH).* ***^{1}H-NMR(DMSO-d6 , ppm):*** *10.58(s, H,NH benzoimidazole), 7.4(=CH pyran ring) , 7.53-7.81(m, H, phenyl rings), 8.42(S,H,C=NH).* ***^{13}C-NMR(DMSO-d6 , ppm):*** 158(C ,C-O), 154 (C,C=N in six ring), 129.9 (C ,C=N benzoimidazole), 116 -129.7 (C , C=C, phenyl rings).

Synthesis of tetrazoles two methods :-

First method:-

Synthesis of 2-((1H-tetrazol-5-yl)methyl)-1H-benzo[d]imidazole [L_7] Under Microwave irradiation [17] :

Grinding of (0.000064 mole , 0.01 g) 2-(1H-benzo[d]imidazol-2-yl)acetonitrile with(0.000064 mole, 0.004g) azide sodium respectively in dry clean Crucible. Dissolve The mixture in (1ml) G.A.A with 3 drops of DMF .

The contents were subjected to microwave irradiation at 120 W about 8 min. Progress of the reactions was monitored by TLC using a solvent,(Ethanol 1ml :Benzene 4ml). After the completion of the reactions distilled water were added to the component, Then filterate and take a precipitate with re-crystallization in absolute ethanol.

M.Wt : 200 , Yield% : 80% , m.p^{0}c:171-174 , M.F: $C_9H_8N_6$, L7 : Light brown

R_f : 0.94

FT-IR (KBr) (V_{max} , cm^{-1}): *3471 (NH tetrazole), 3414 (NH benzoimidazole), 1616 (C=N tetrazole), 1539 (C=C Arom) .*

Second method:-

Synthesis of 2-((1H-tetrazol-5-yl)methyl)-1H-benzo[d]imidazole [L_9] by refluxed method [18] :

(0.008mol, 0.048g) of urea dissolve in 2ml of distilled water with (1mL G.A.A) in baker after dissolve (0.008mol , 0.052g) azid sodium in 3ml of distilled water in other baker . (0.00064mol, 0.1g) 2-(1H-benzo[d]imidazol-2-yl)acetonitrile dissolved with 5ml of DMF then added the mixture in baker one with two into round bottom flask perform refluxed at (80) ^{0} C for (17hrs) The reaction was followed by TLC using a solvent (Ethanol 2.5ml:Benzene 2.5ml) after neutralized the solution at PH=7 by using HCl. leave it for 24hour After the completion of the reactions and distilled water was added to the component Then extracted it twice with chloroform , then removed the bottom organic layer and recrystallized it with absolute ethanol.

L9 : Light brown , M.Wt : 200 , Yield% : 40% , M.F: $C_9H_8N_6$ m.p^{0}C :171-174

R_f : 0.91

FT-IR (KBr) (V_{max} , cm^{-1}): 3408 (NH $_{tetrazole}$), overlapping (NH $_{benzoimidazole}$), 1622 (C=N $_{tetrazole}$), 1539 (C=C $_{Arom}$) . **^{1}H-NMR(DMSO-d6 , ppm):** 10.59(s, H,NH $_{benzoimidazole}$), 9.51(S,H, NH $_{tetrazole}$), 7.76 -7.98, 7.03-7.36 (m, H, phenyl rings), 3.79(S,2H ,-CH2). **^{13}C-NMR(DMSO-d6 , ppm)** 157(C,C=N $_{in\ ring\ benzimidazole}$), 161(C,C=N $_{in\ ring\ tetrazole}$), 112-130 (C,C=C phenyl rings), 24(C,CH2).

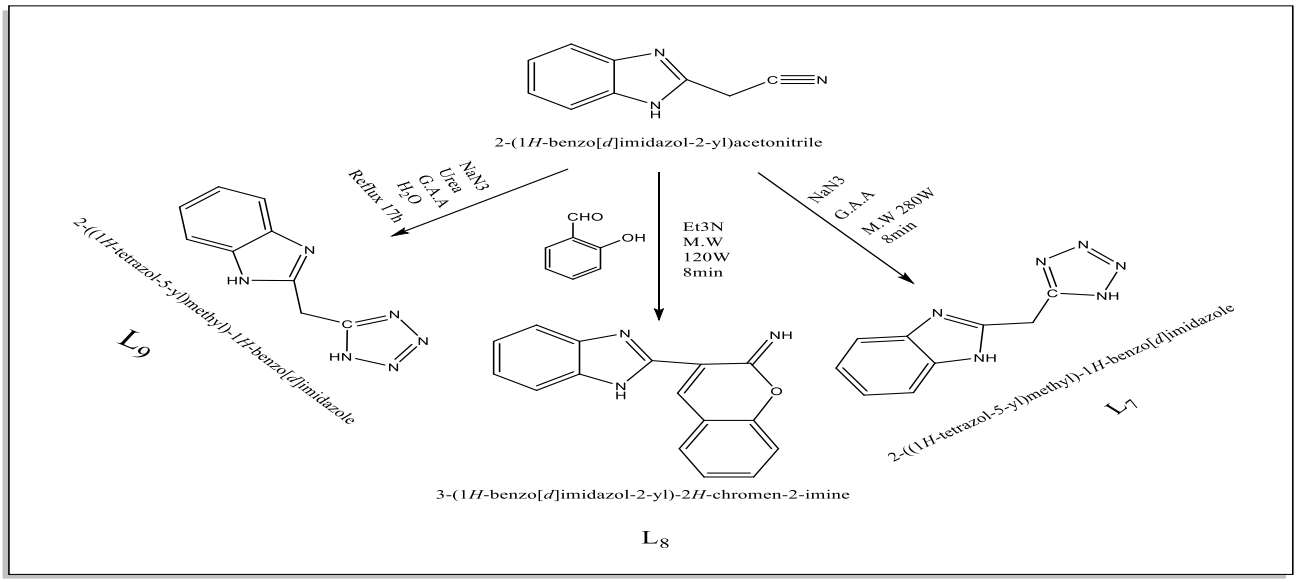
Biological activity Anti bacterial activity [19,20] :-

Two strains of The first type is [*Staphylococcus aureus*], while the second type is [*klebsilla*]. have been chosen. Bacterium is used for all experiments, and grown in[Muller Hinton agar]. All types of bacteria used for all experiments, and grown in [Muller Hinton agar] . All types of bacteria have been grown at (36h) and have been incubated at 37 °C . Different concentrations have been prepared as (0.01mg/ml, 1mg/ml, 0.1mg/ml) following , added separately to the wells on the plates that already have bacteria growth. The plates were incubated for (24 h) to test

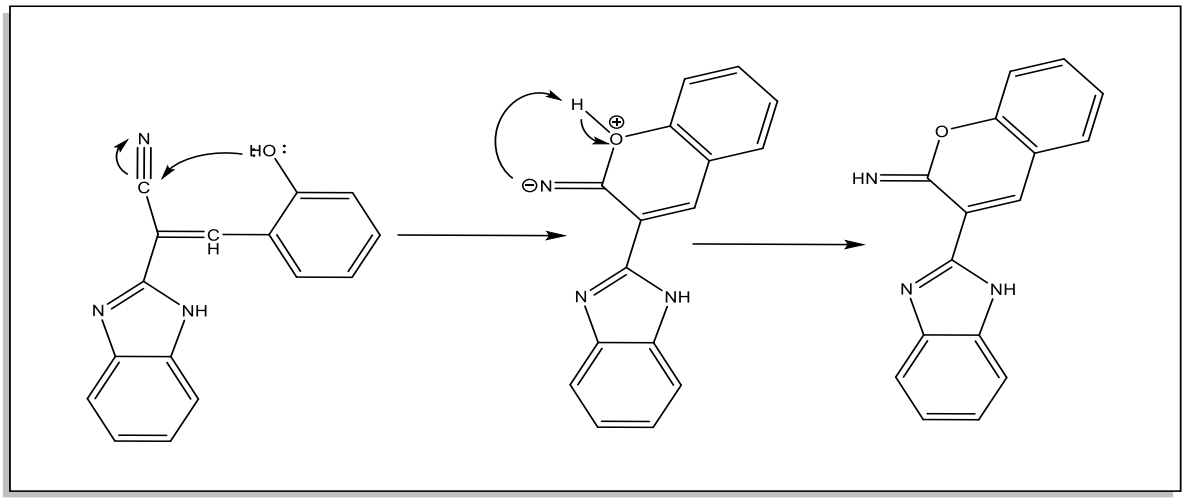
the anti-bacterial effects; a ruler is used to measure the inhibition zone to the nearest millimeter (mm).

Results and discussion:

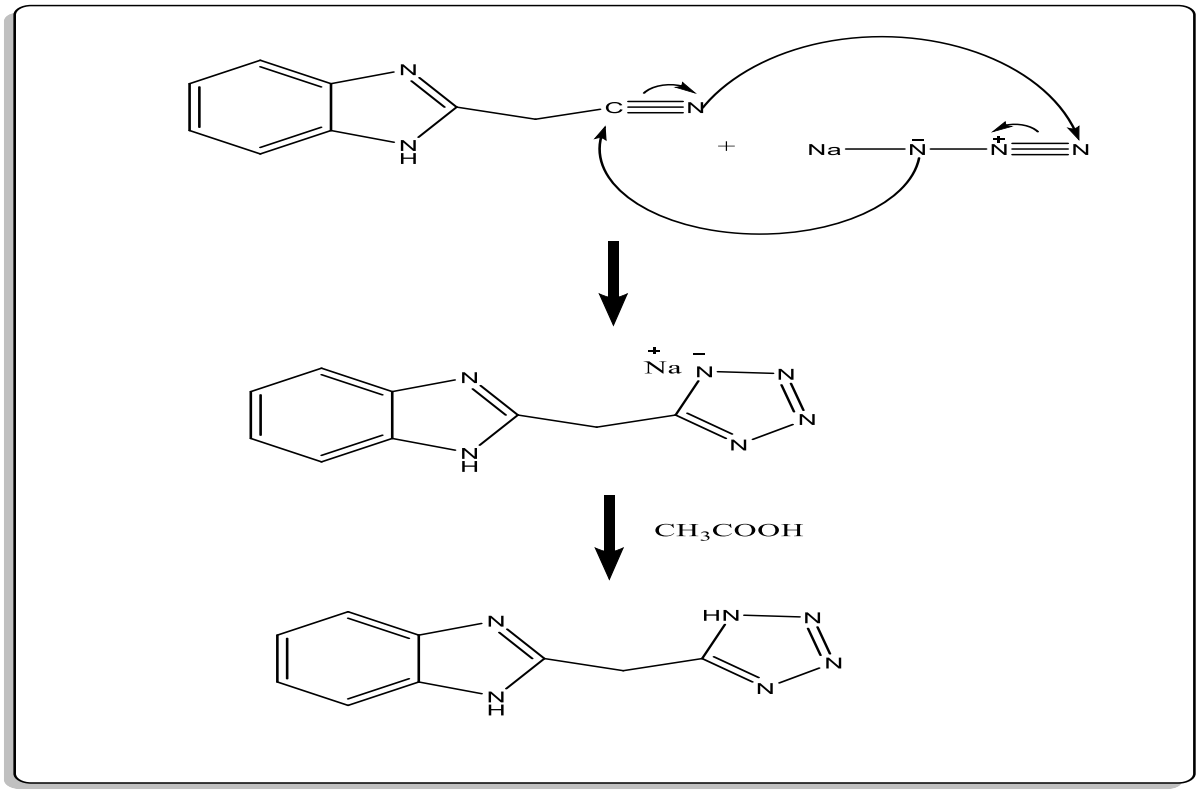
Chemistry :



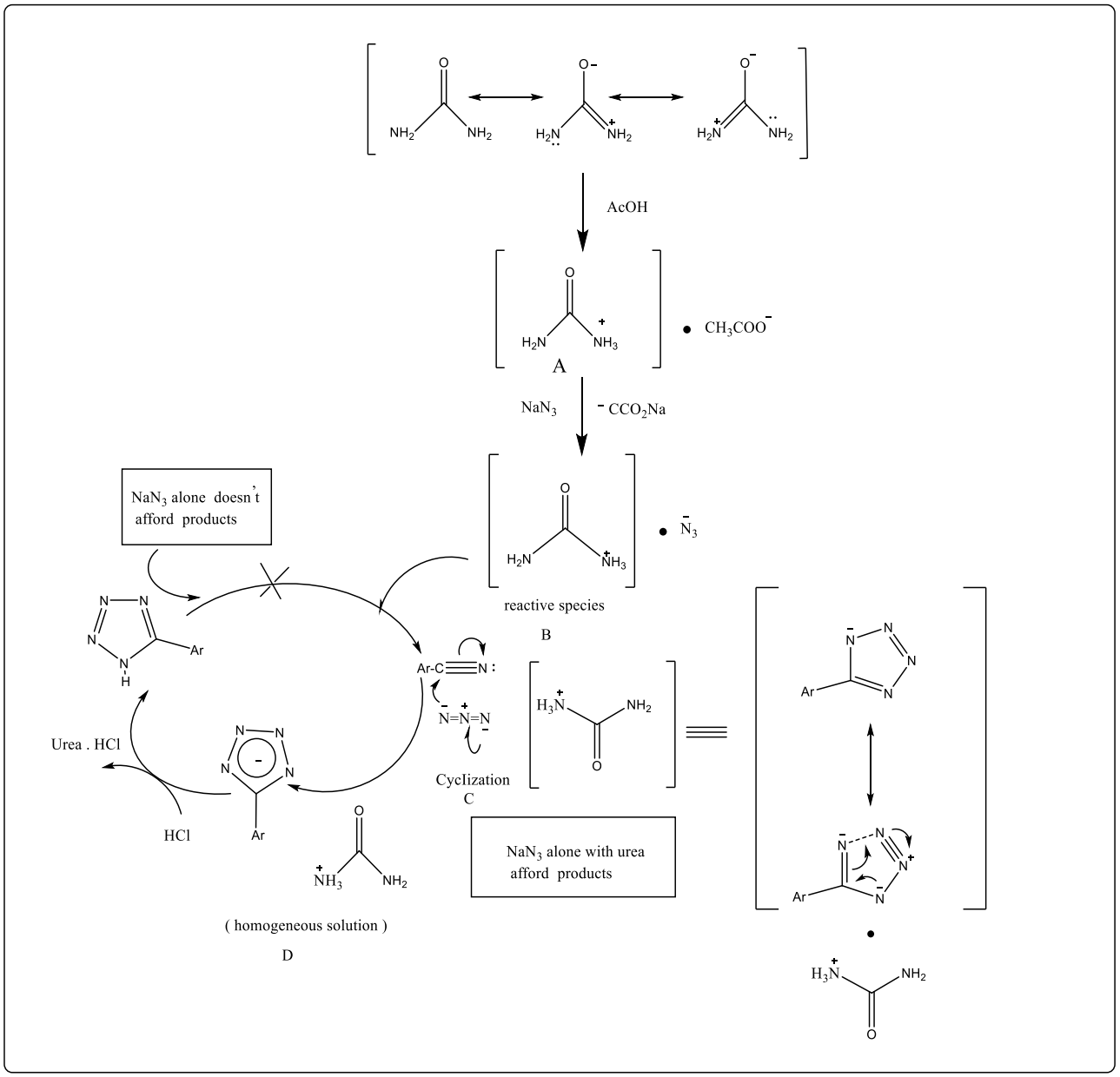
Scheme 1: Synthesis of rings tetrazoles L_7 , L_9 and ring derivatives chromene L_8



Scheme 2: The proposed mechanism for preparation of 2-imine-pyran ring



Scheme 3: The proposed mechanism preparation of ring tetrazoles derivatives L7



Scheme 4: mechanism preparation of ring tetrazoles derivatives L_9

All prepared Compounds were characterized by using the FT-IR,in addition to ^{1}HNMR, and ^{13}CNMR spectra. in FT-IR spectrum for L8 showed for L8 showed disappeare of Nitrile group this confirm with showed a band appeared at position 3429 cm^{-1} belonging to the NH in the benzoimidazole and the appearance of a new band at position 3163 cm^{-1} dating back to C=NH, while all of the prepared compounds were L7.9. the disappearance of the CN-nitrile group and A new band appeared at position 3417cm^{-1} for L7, and while it appeared overlapping with the NH band in the benzoimidazole for L9, a new band also appeared at position

1622.1620cm^{-1}, dating back to C=N for both compounds L7.9. As for the ^{1}HNMR spectrum of the compound L8 A single signal appeared at position 10.58 ppm, belonging to the NH group in the benzoimidazole, with a single signal appearing at position 8.42, belonging to the C,C=NH group. As for compound L9, a singlet signal appeared at position 10.59 ppm, belonging to the NH group in the benzoimidazole, and a single signal appeared at position 9.51. ppm refers to the NH of the tetrazole ring. In the ^{13}CNMR spectrum, a signal appeared at the 158ppm site belonging to C, C=NH, and a signal appeared at the 154ppm site belonging to C, C=N inside the benzoimidazole ring. As for compound L9, a signal appeared at the 161ppm site belonging to the C,C=Nin ring tetrazole group, and a signal appeared at the 157ppm site belonging to the C,C=N in ring benzimidazole group.

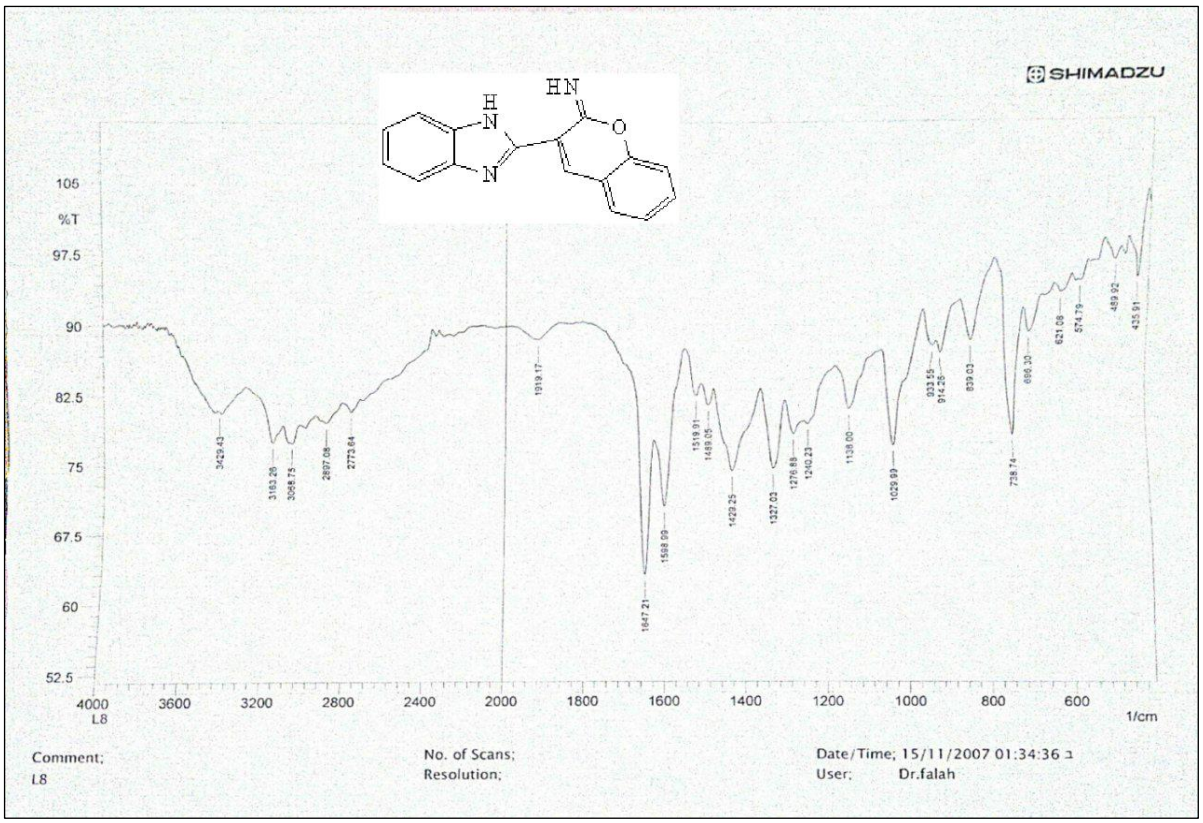


FIGURE 1 : FT-IR Spectrum for L8 chromene derivative

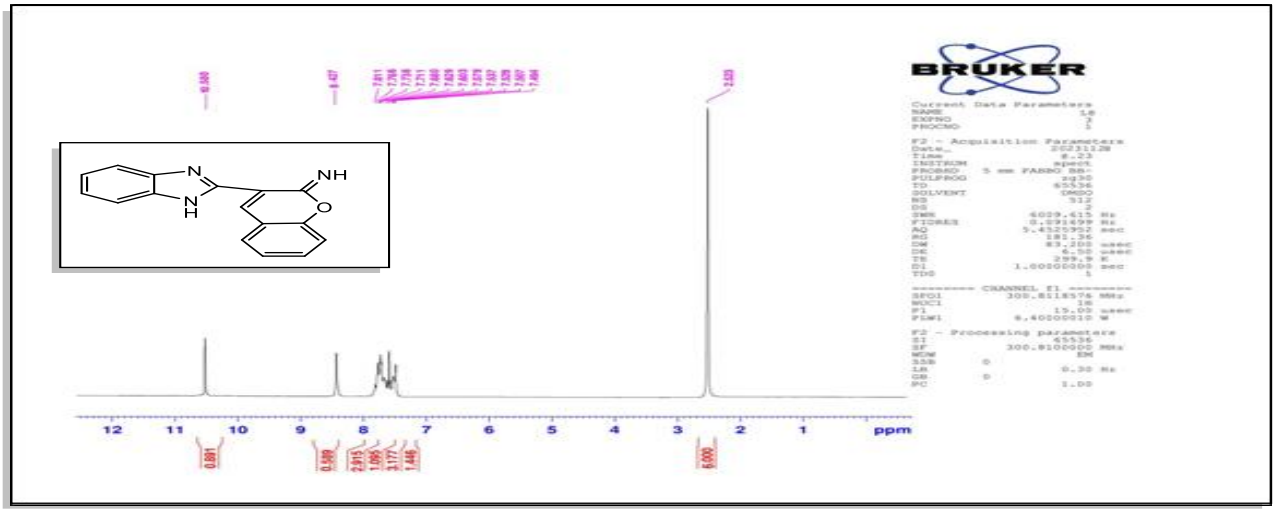


FIGURE 2 : 1H-NMR Spectrum for L8 chromene derivative

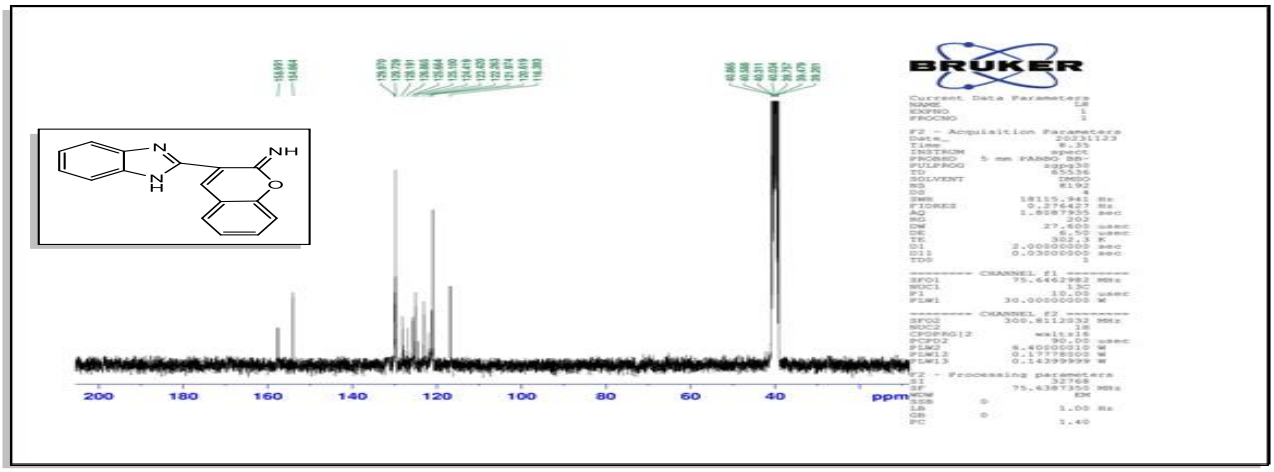


FIGURE 3 : 13C-NMR Spectrum for L8 chromene derivative

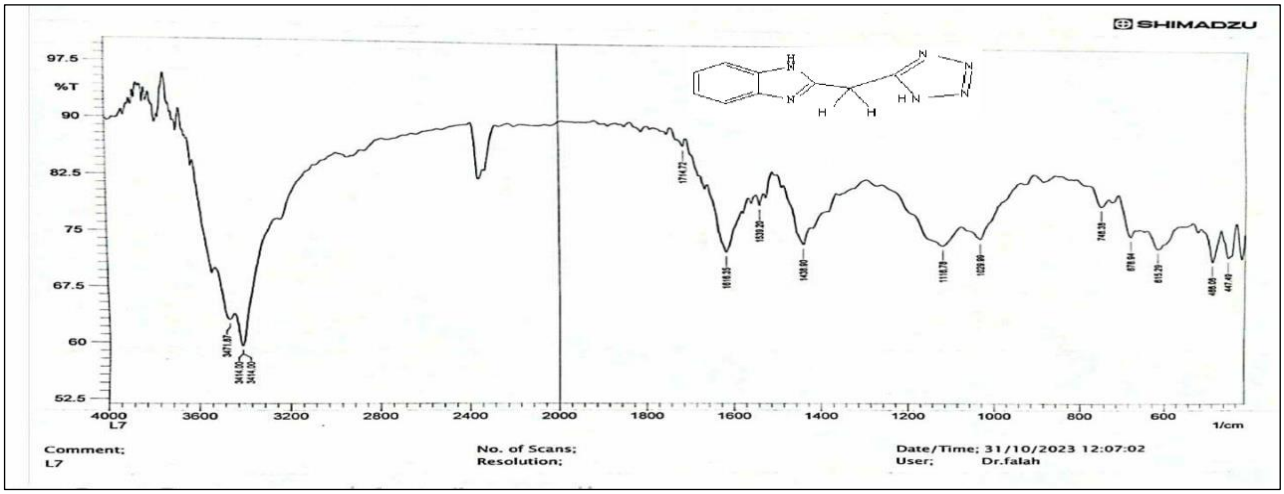


FIGURE 4 : FT-IR Spectrum for L7 tetrazoles derivative

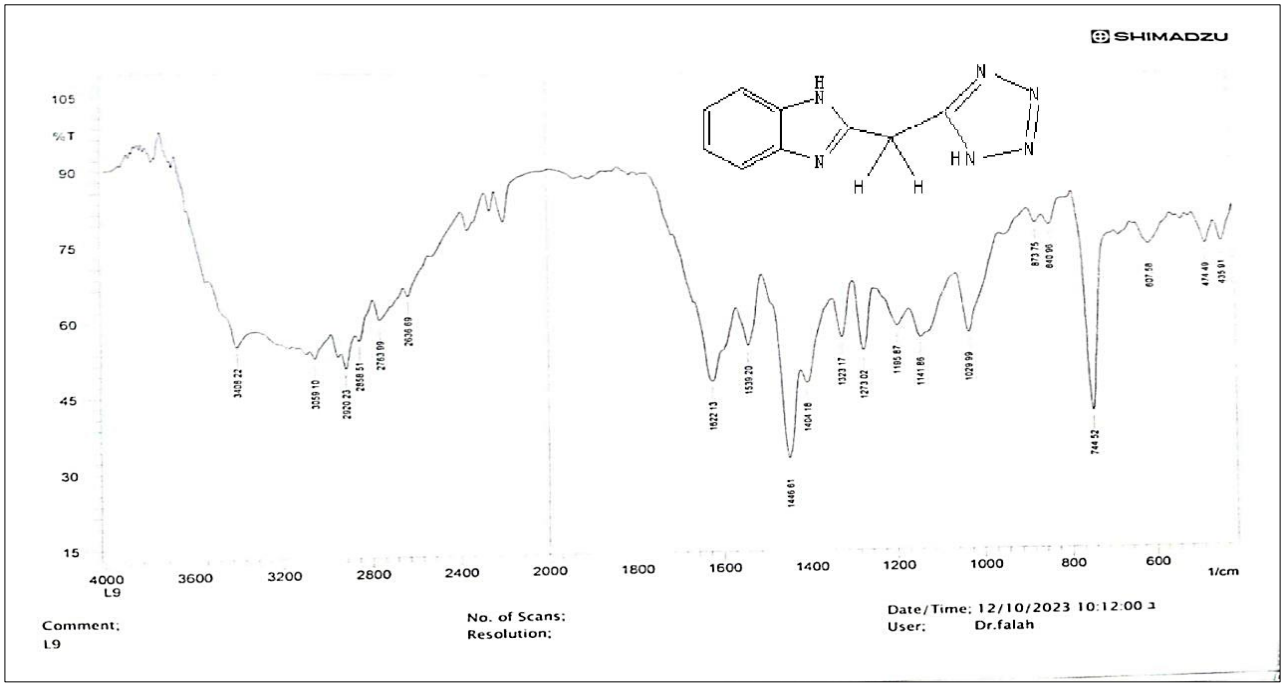


FIGURE 5 : FT-IR Spectrum for L9 tetrazoles derivative

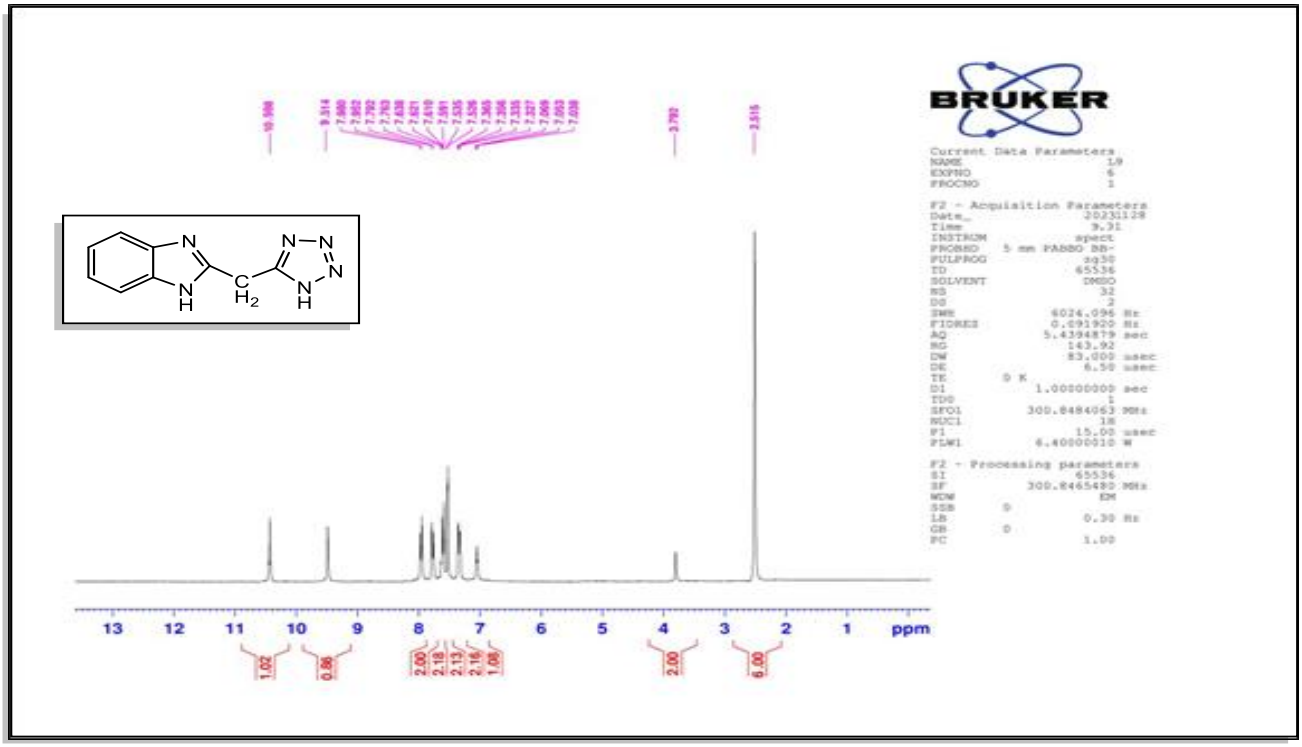


FIGURE 6 : 1H-NMR Spectrum for L9 tetrazoles derivative

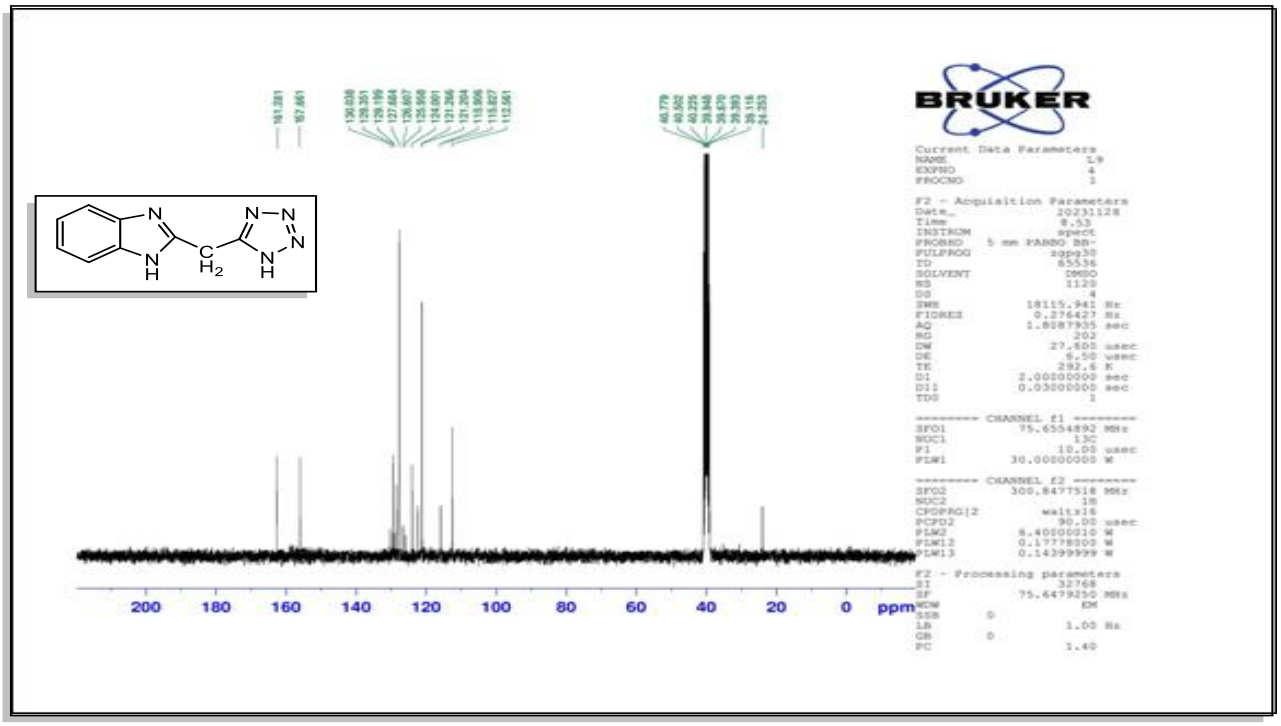


FIGURE 7 : 13C-NMR Spectrum for L9 tetrazoles derivative

TABLE 1 :The physical properties for the prepared derivatives L_7 , L_8 , L_9

Comp No	M.F	M.Wt	m.p°c	Yiled %	Color	R_f
L_7	**$C_9H_8N_6$**	**200**	171-174	80%	**Light brown**	(Ethanol :Benzene) (1:4) 0.94
L_8	**$C_{16}H_{11}N_3O$**	**261**	238-240	58%	**Yellow**	(Ethanol:Benzene) (2.5:2.5) 0.78
L_9	**$C_9H_8N_6$**	**200**	171-174	40%	**Dark Orange**	(Ethanol:Benzene) (2.5:2.5) 0.91

Molecular Docking Study :-

The molecular binding of the two compounds L7,8 to two types of bacteria, the first type ***Staphylococcus*** and the second type ***klebsilla***, with proteins (T2P1, 4OR7) was studied. The results were Lowest Binding Ee nergy for the type of ***Staphylococcus*** bacteria with the T2P1 protein L7 = -7.36, L8 = -7.61 and for the type ***klebsilla*** with the 4OR7 protein. It was L7=-6.22,L8 = -6.84.

Results *Staphylococcus* bacteria with the T2P1 protein for compound L7,8

Comp. NO.	Lowest Binding Energy	RUN
L7	-7.36	41
L8	-7.61	2

Compound L7:-

Hydrophobic Interactions

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	65A	ILE	3.20	2786	41
2	89A	PRO	3.22	2781	264

Hydrogen Bonds

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor ?	Side chain	Donor Atom	Acceptor Atom
1	65A	ILE	1.78	3.10	168.98	√	×	35 [Nam]	2796 [N2]
2	108A	GLU	2.04	3.05	160.21	×	×	2789 [Npl]	433 [O2]
3	109A	SER	2.65	3.50	137.97	×	√	2793 [Npl]	445 [O3]
4	110A	LEU	2.27	3.18	152.92	√	×	448 [Nam]	2794 [N2]

Compound L8 :-

Hydrophobic Interactions

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	65A	ILE	3.23	2782	39
2	89A	PRO	3.31	2799	264
3	110A	LEU	3.29	2788	455

Hydrogen Bonds

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	108A	GLU	1.93	2.75	132.66	×	×	2801 [Npl]	433 [O2]

Results type *klebsilla* with the 4OR7 protein for compound L7,8

Comp. NO.	Lowest Binding Energy	RUN
L7	-6.22	1
L8	-6.84	1

Compound L7:-

Hydrophobic Interactions

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	101A	GLU	3.83	1807	985
2	105A	PRO	3.73	1809	1037

Hydrogen Bonds

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	127A	ASP	2.21	3.01	130.81	×	×	1815 [Npl]	1264 [O2]
2	129A	ASP	2.07	2.96	148.29	√	×	1284 [Nam]	1819 [Npl]
3	129A	ASP	3.42	4.08	122.3	×	×	1819	1287

					2			[Npl]	[O2]
4	133A	TRP	2.13	3.16	167.32	√	√	1327 [Nar]	1822 [N2]
5	158A	ARG	3.01	4.01	164.75	√	√	1617 [Npl]	1823 [N2]
6	158A	ARG	2.83	3.74	148.05	√	√	1614 [N3]	1820 [N2]

Compound L8 :-

Hydrophobic Interactions

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	28A	LEU	3.06	1815	256

Hydrogen Bonds

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	20A	MET	2.27	3.05	148.89	√	×	176 [Nam]	1813 [Npl]
2	20A	MET	1.93	2.77	136.94	×	×	1813 [Npl]	179 [O2]

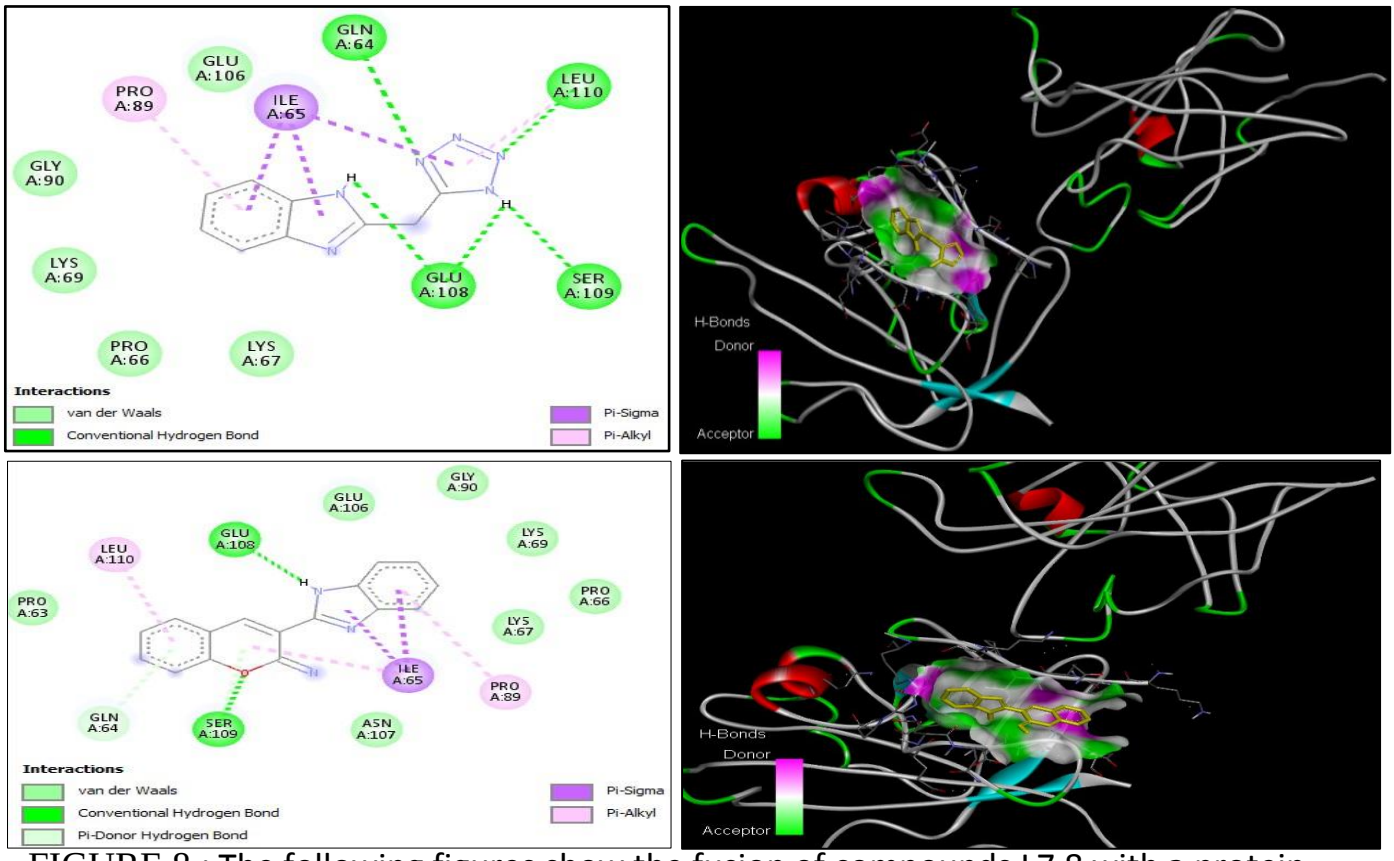


FIGURE 8 : The following figures show the fusion of compounds L7,8 with a protein T2P1

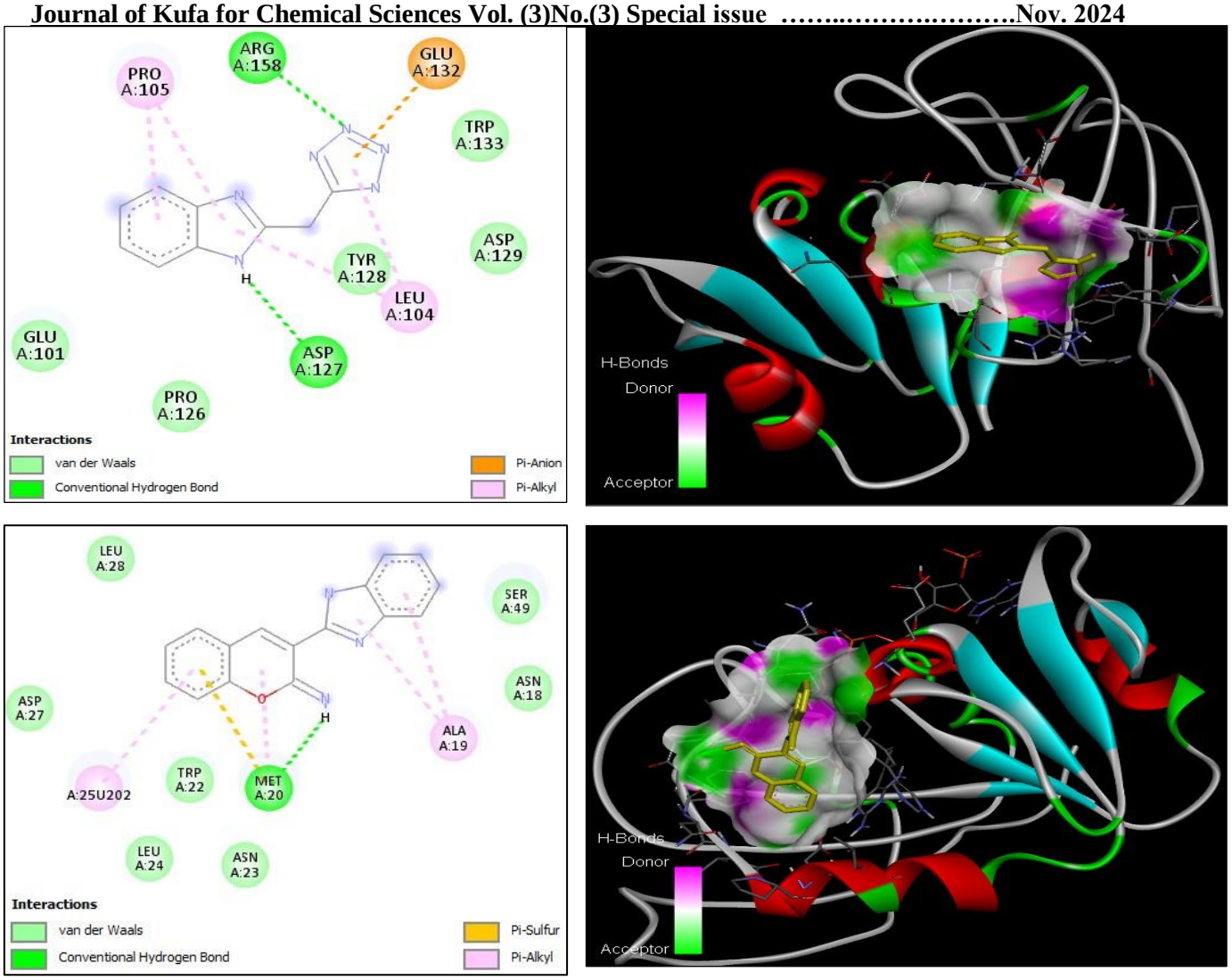


FIGURE 9 : The following figures show the fusion of compounds L7,8 with a protein 4OR7

Activity of anti-bacteria : -

The biological activity of some organic compounds (L7, L8) was studied to determine their antibacterial activity for two types of pathogenic bacteria after they were isolated and diagnosed from pathological cases and their properties were proven. The first type is Gram-positive bacteria [*Staphylococcus aureus*], while the second type is gram-negative bacteria [*klebsilla*]. it gave a high percentage of inhibition. Likewise, the organic compounds (L7, L8) were compared with two types of drugs amoxicillin and ciprofloxacin, as in table 2:-

TABLE 2 : the inhibition zones for (L7, L8) derivatives compared with two types of drugs amoxicillin and ciprofloxacin

No	Materail	Concentration	Inhibition Zone	
			klebsilla	Staph. Aureus
1	L7	1 mg/ml	22	22
		0.1 mg/ml	20	19
		0.01 mg/ml	17	16
2	L8	1 mg/ml	24	25
		0.1 mg/ml	20	22
		0.01 mg/ml	18	19
3	CIP	1 mg/ml	28	25
		0.1 mg/ml	26	21
		0.01 mg/ml	23	19
4	AMOX	1 mg/ml	21	24
		0.1 mg/ml	19	21
		0.01 mg/ml	17	19

TABLE 3 FT-IR Spectrum for compound

Comp.	NH tetrazole	NH benzoimidazole	C=N tetrazole	C=N benzoimidazole	C=C Arom	=NH	C=NH
L_7	3471	3414	1610	1616	1539	———	———
L_8	———	3429	———	1647	1519	3163	1598
L_9	Overlapping with the NH bond	3408	1619	1622	1539		—

TABLE 4 ^{1}H-NMR Spectrum for compound

Comp	S,H, NH $_{tetrazole}$	s, H,NH $_{benzoimidazole}$	m, H, phenyl rings	S, 2H ,-CH2	S,H,C=NH
L_8	--------	10.58	7.53-7.81	----------	8.42
L_9	9.51	10.59	7.76 -7.98 7.03-7.32	3.79	--------

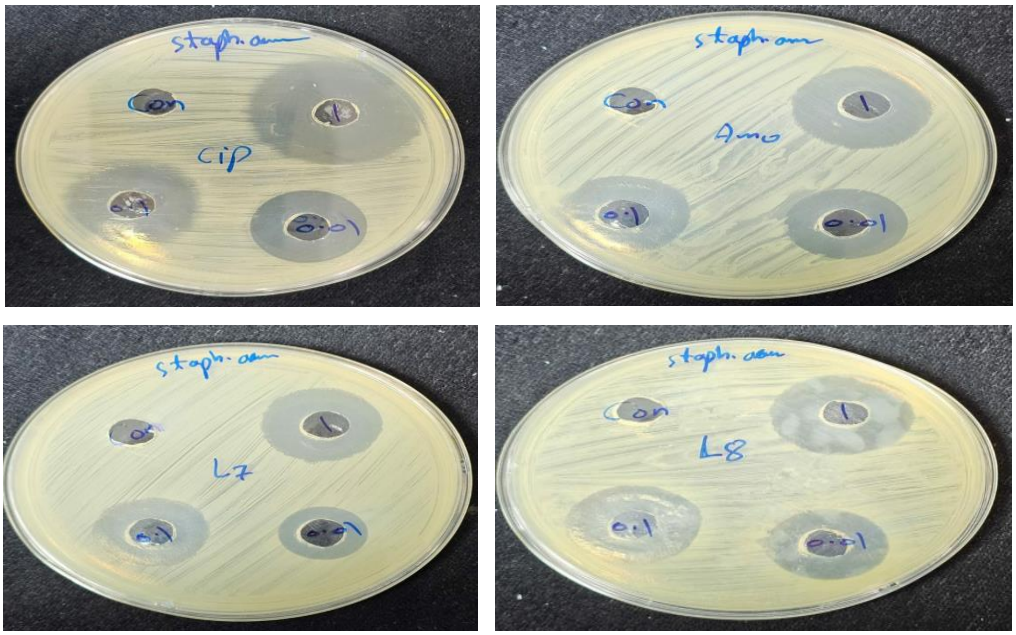


FIGURE 7: Shows the inhibition of the prepared compounds (L7, L8) of the bacteria Staphylococcus aureus and compared with CIP and AMOX..

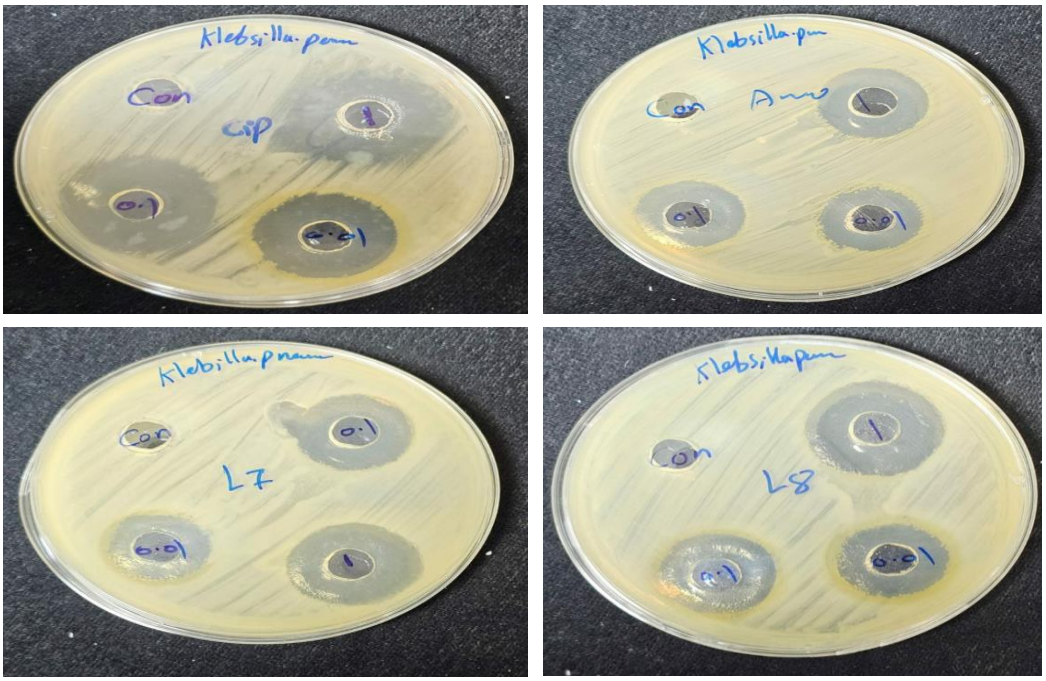


FIGURE 8: Shows the inhibition of the prepared compounds (L7, L8) of the bacteria klebsilla and compared with CIP and AMOX..

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