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Synthesis and identification of benzo[d][1,3]oxazin-4-one derivatives and testing of antibacterial activity of some of them

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***Abstract*---**Schiff bases (M1-M9) were prepared from a reaction of 4,4-methylenedianiline (MDA) 4,4-Methylenedianiline with aldehydes (2-bromobenzaldehyde, 2-chlorobenzaldehyde, benzaldehyde and 4-methoxy benzaldehyde) were prepared by microwave irradiation. Schiff bases (M10-M18) were reacted Prepared in the previous paragraph with salicylic acid to prepare 4,3-dihydro-1,3 -oxazine-4-one derivatives by microwave method. Nuclear magnetic resonance and infrared spectroscopy, as well as physical changes such as melting points and color change, were used to diagnose the produced compounds. Some of the prepared compounds were tested against two types of bacterial isolates, *Staphylococcus aureus* gram-positive and *Klebsiella pneumonia* gram-negative. The results showed the antibacterial activity of these compounds, at different concentrations (5,10,15) mg/ml against this bacterium compared with antibiotic ciprofloxacin. Some compounds, including the compound M5, showed good activity against *Staphylococcus aureus*, and some were moderately effective against *Klebsiella pneumonia*.

***Keywords*---**methylenedianiline, Schiff bases, microwave.

Introduction

Many 4,4-Methylenedianiline derivatives were prepared during the previous periods because of its many uses, as it is mainly used for the manufacture of 4,4-methylenediisocyanate diphenyl(1), isocyanates and polyisocyanates,

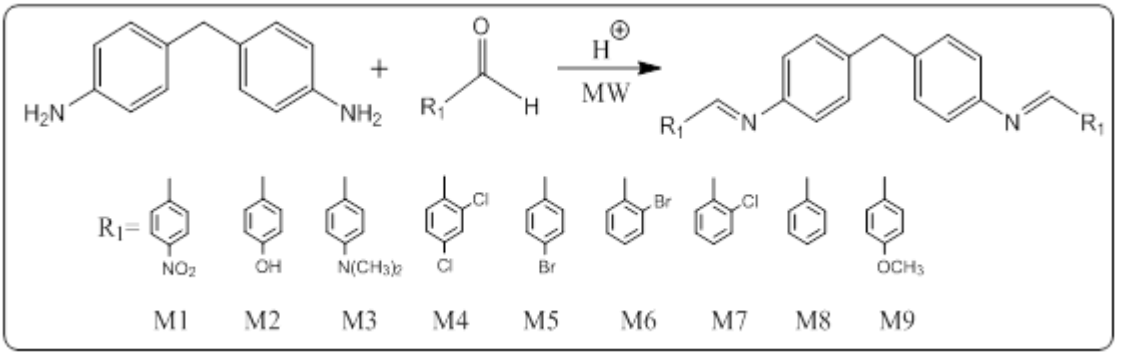
polyurethane, as well as its uses in the preparation of materials Buffer and organic dyes(3,2). Its derivatives are also used as antimicrobial materials (4), as N,N'-Bis(acetylacetone)4,4'-diaminodiphenylmethane was prepared from a condensation reaction between acyl acetone with a solution of 4,4-Diaminodiphenylmethane in the coloring (5), Also (E)-2-((4-(4-aminobenzyl)phenyl)imino)methyl)-6-ethoxyphenol was prepared from condensation of ethoxysalicylaldehyde with 4,4-Diaminodiphenylmethane (6). The importance of Schiff bases has increased as it has many applications, including medical applications such as antibacterial, anticancer, antifungal (7). The preparation of 4,3-dihydro-3,1-oxazine-4-un derivatives (or the so-called oxazinone) has attracted great interest among researchers, as dihydro-1-arylnaphtho[1,2-e] [1,3]oxazine was prepared -3-one/thione by condensation of 2-naphthol, benzaldehyde with urea (8). It also prepared tetrahydrooxazin-4-ones from the reaction of 2 2-aza-3-trimethylsilyloxy-1,3-diene with aldehydes, as some of its derivatives have biological and medicinal efficacy, as some of its derivatives act as antimicrobial, hypoglycemic, anti-obesogenic (9-11).

Practical part

Chemical materials and solvents manufactured by international companies were used without purification. The infrared spectrum was measured using a Fourier Transform Infrared Spectrophotometer (FTIR-8400S) device supplied by the Japanese company Shimadzo. The proton nuclear magnetic resonance spectrum was also measured in the laboratories of the University of Basra by using Bio spin device (AVIII-HD_800) with a power of 400MH supplied by German company Bruker, using tetramethylsilane as internal reference and dimethyl sulfoxide as sample solvent. Thin-layer chromatography was used to follow up the reaction process and to ensure the purity of the compounds that were prepared using several solvents. The stains were developed using ultraviolet rays, and a home microwave oven (MH2450, Universal-850 Watt) was used, of Chinese origin. Mass spectrometry measurements were carried out in the laboratories of the Ministry of Science and Technology / Department of Environment and Water Research using a device GCMS-QP2010 SE: Shimadzu Spectrometer.

Preparation of Schiff Bases using Microwave (M1-M9)

0.001mol from 4-4methylenedianiline was dissolved in 20 ml absolute ethanol in a round flask، and 0.002 mol of different aldehydes were added to it with stirring. Drops of glacial acetic acid were added to the reaction mixture as a catalyst, refluxed of the mixture in the microwave at 8-10 min (425 watts,). The reaction was followed up using TLC and after the reaction was finished, the mixture was left at room temperature. The reaction mixture was poured over cold water, and yellow-orange crystals were obtained, filtered, washed with cold water, and dried. Table (1) shows some physical properties of the prepared compounds.



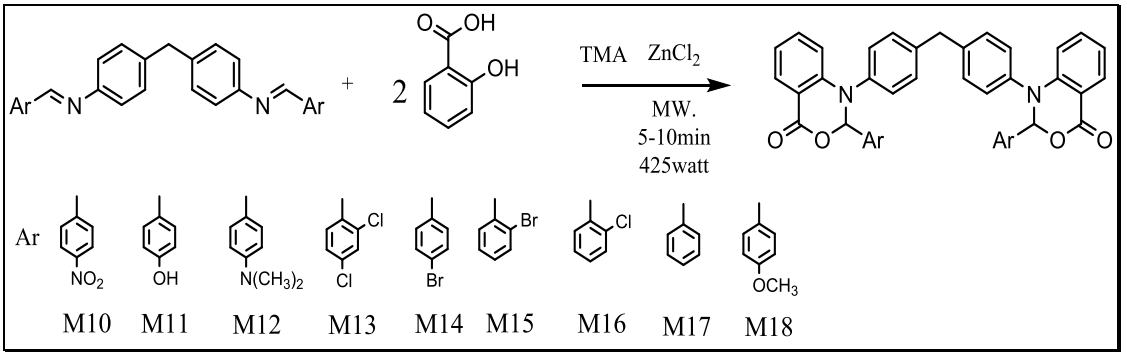
Equation of preparation of compounds M1-M9

Table (1) physical properties of the prepared compounds (M1-M9)

Comp. No.	Molecular Formula	Molecular Weight	Color	M.P ^{0}C	Yield %	RF
M1	$C_{27}H_{20}N_4O_4$	464.48	Dark yellow	188-191	84	0.88
M2	$C_{27}H_{22}N_2O_2$	406.49	Light yellow	229-232	88	0.87
M3	$C_{31}H_{32}N_4$	460.63	Orange	170-172	75	0.81
M4	$C_{27}H_{18}Cl_4N_2$	512.26	Light brown	126-130	77	0.89
M5	$C_{27}H_{20}Br_2N_2$	532.28	Light brown	188-191	85	0.82
M6	$C_{27}H_{20}Br_2N_2$	532.28	Light yellow	129-130	85	0.87
M7	$C_{27}H_{20}Cl_2N_2$	443.37	Brown	124-126	92	0.89
M8	$C_{27}H_{22}N_2$	374.49	Light yellow	110-112	89	0.91
M9	$C_{29}H_{26}N_2O_2$	434.54	Brown	153-154	76	0.85

Preparation of benzo[d][1,3]oxazin-4-one derivatives (M10 – M18)[12]

(0.001mol) of the prepared Schiff bases (M1-M9) were dissolved in 25ml of ethanol, after then (0.002 mol) of salicylic acid was added to it with a few drops of triethylamine and ZnCl2 as a catalyst. The reaction mixture was heated in a microwave for (5-10 minutes) (425watt) and followed up the reaction using TLC. After completion of the reaction, the solvent was evaporated, washed with cold water, and the solution was filtered. Table (2) shows some physical properties of the prepared compounds (M10-M18).



Equation of preparation of compounds (M10-M18)

Table (2) physical properties of the prepared compounds (M10-M18)

Comp. No.	Molecular Formula	Molecular Weight	Color	M.P ^{0}C	Yield %	Rf
M10	$C_{41}H_{28}N_4O_8$	704.70	Dark yellow	301-202	64	0.94
M11	$C_{41}H_{30}N_2O_6$	646.70	Yellow	364-365	68	0.92
M12	$C_{45}H_{40}N_4O_4$	700.84	Orange	100-103	70	0.95
M13	$C_{41}H_{26}Cl_4N_2O_4$	752.47	Dark yellow	148-149	72	0.91
M14	$C_{41}H_{28}Br_4N_2O_4$	772.49	Light yellow	295-297	73	0.92
M15	$C_{41}H_{26}Br_4N_2O_4$	772.49	Yellow	277-280	73	0.94
M16	$C_{41}H_{28}Cl_2N_2O_4$	683.59	Light brown	284-283	70	0.95
M17	$C_{41}H_{30}N_2O_4$	614.70	Yellow	257-260	66	0.90
M18	$C_{43}H_{34}N_2O_6$	674.75	Light yellow	289-291	96	0.95

Results and Discussion

The compounds (M1-M9) were diagnosed by spectroscopic methods. The prepared compounds showed in the infrared spectrum a peak at the range 3044-3064 cm-1 for the stretching of the aromatic C-H bond. The peak at the range 2902-2964 cm-1 for the stretching of the aliphatic C-H bond, and the absorption at the range -16511657 for the stretching of the bond C=N [13,14]. See table (3).

Table 3: FTIR data of Schiff bases derivatives M1-M9

Comp. No.	R	ν (C-H) Arom.	ν(C-H) Aliph. Asy,sy	(C=N) ν	Others
M1	4-NO_2	3058	2966 2875	1649	(-NO_2) 1504 and 1340
M2	4-OH	3089	2958 2889	1645	OH 3316
M3	4-$N(CH_3)_2$	3104	2953 2877	1655	-$N(CH_3)_2$ 1217
M4	2,4-Cl	3070	2956 2866	1640	C-Cl 706
M5	4-Br	3062	2902 2875	1652	C-Br 775

M6	2-Br	3050	2920 2850	1656	C-Br 776
M7	2-Cl	3044	2955 2884	1657	C-Cl 693
M8	H	3064	2935 2882	1653	----
M9	4-OCH_3	3054	2964 2878	1654	O-CH_3 1200

The spectrum of 1H-NMR for compound M7 in DMSO-d6 substituted with deuterium showed a signal at the 4.01 ppm position, which belongs to the C-H_2 protons. A multiple signals in the range 7. 27-8.14 ppm, which belongs to the protons of the benzene rings, and a signal at 8.86 ppm which belongs to a proton azomethine (N= C-H) [15-17]. See Figure 1.

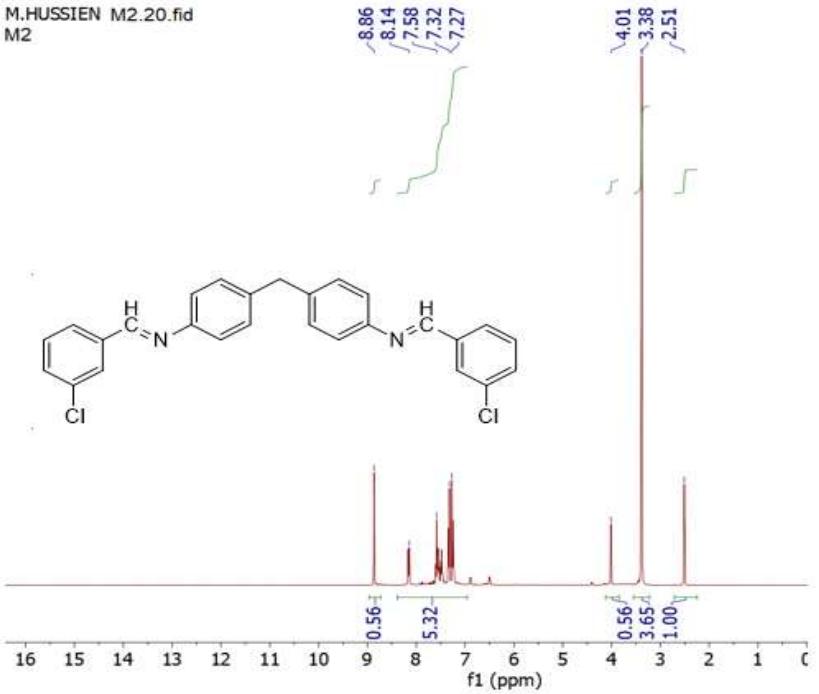


Figure (1): [1]H-NMR spectrum of compound M7

The spectrum of [1]H-NMR of compound M8 in DMSO-d6 substituted with deuterium showed the appearance of a singlet signal at the 3.99 ppm, which belongs to the C-H_2 protons and the spectrum showed a multiple signal in the range 7. 24-7.92 ppm, which belongs to the protons of the benzene rings. The position is 8.62 ppm which belongs to the C-H = proton [15-17] See Figure (2).

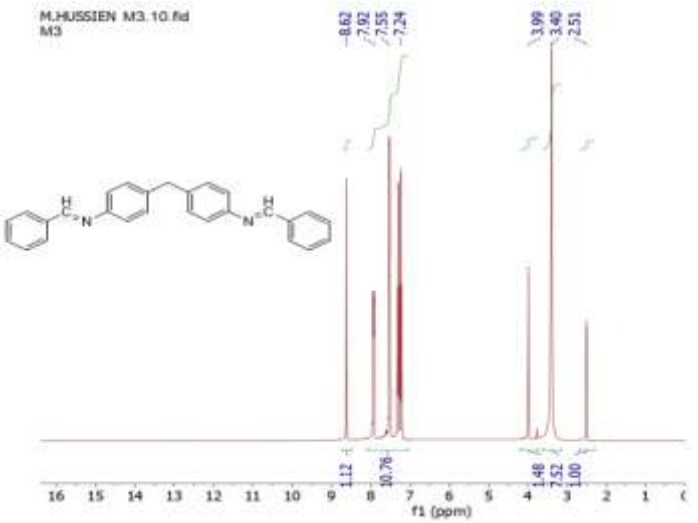


Figure (2): ^{1}H-NMR spectrum of compound M8

The compounds (M10-M18) were diagnosed by spectroscopic methods. The prepared compounds showed in the infrared spectrum a beam at the range 3026-3068 cm-1 for the stretching of the aromatic C-H bond, and absorption at the range 2877-2937 cm-1 for the stretching of the aliphatic C-H bond. And the spectrum showed absorption at the range 1220-1216 cm-1 for the stretching of the C-N bond[13,14]. See table (4).

Table (4): FTIR data of benzo[d][1,3]oxazin-4-one derivatives M10-M18

Comp. No.	R	(C-H)ν Arom.	ν(C-H) Aliph.asy.sy	(C-N)ν	ν (C=O) Oxazine	(C-O)ν	Others
M10	4-NO_2	3026	2977 2889	1216	1722	1212	NO_2 asy 1553 sy 1374
M11	4-OH	3028	2978 2881	1218	1725	1210	O-H 3463
M12	4-$N(CH_3)_2$	3033	2988 2877	1217	1721	1215	-$N(CH_3)_2$ 1220
M13	2,4-Cl	3044	2920 2876	1216	1726	1218	C-Cl 670
M14	4-Br	3067	2911 2872	1219	1722	1188	C-Br 666
M15	2Br	3033	2937 2866	1218	1720	1215	C-Br 777
M16	2-Cl	3030	2920 2887	1211	1722	1214	C-Cl 690
M17	H	3068	2914 2892	1216	1715	1220	----
M18	4-OCH_3	3062	2922 2883	1220	1719	1212	O-CH_3 Ether 1120

The spectrum of ^{1}H-NMR for compound M11 in DMSO-d6 substituted with deuterium showed the appearance of a signal at position 3.95 ppm that belongs to the C-H_2 protons, and the spectrum showed a signal at position 6.46 ppm that belongs to the C-H proton of Oxazin ring. The spectrum showed a multiple signal in the range 7. 74-6.79 ppm which belongs to the benzene ring protons, and the spectrum also showed a binary signal at the position 10.17 ppm which belongs to a proton and which belongs to the proton O-H[15-17] see Figure (3).

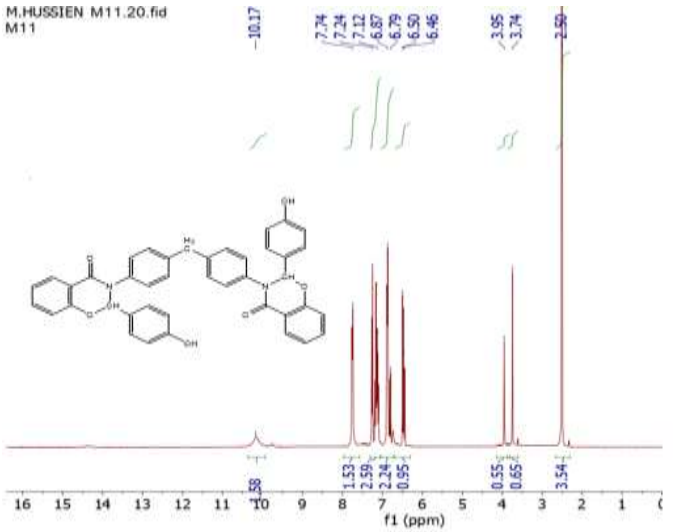


Figure (3): ^{1}H-NMR spectrum of compound M11

The ^{1}H-NMR spectrum of the compound M15 in the deuterium substituted DMSO-d6 solvent showed the appearance of a signal at the 4.05 ppm that belongs to the C-H_2 protons. Signal at the 7.30 ppm position that belongs to the C-H of Oxazin ring and the spectrum showed a multiple signal in the range 8.18-7.30 ppm that belongs to the protons of the benzene ring [15-17]. See Figure (4).

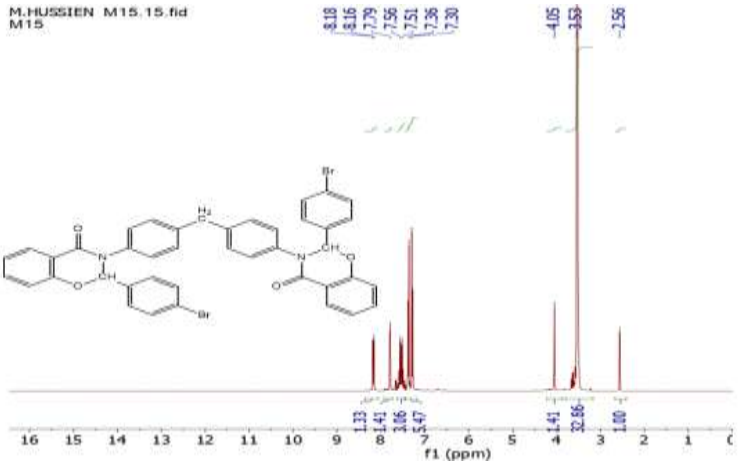


Figure (4): ^{1}H-NMR spectrum of compound M15

The H-NMR1 spectrum of compound M17 in DMSO-d6 substituted with deuterium showed the appearance of a signal at the 3.99 ppm position that belongs to the C-H_2 protons, the spectrum showed a signal at the 6.51 ppm position that belongs to the C-H of Oxazin ring and the spectrum showed a multiple signal in the range 7. 92-6.88 ppm that belongs to the benzene ring protons [15-17]. see figure (5)

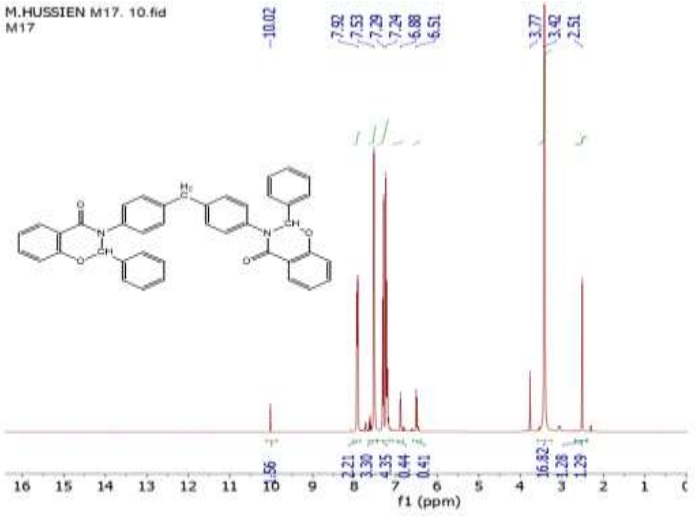


Figure (5): ^{1}H-NMR spectrum of compound M17

The mass spectrum of the compound (M30) was measured, where it showed a peak at (700 m/z) with a relative abundance (20%) due to the molecular ion of the compound, as well as a base peak at (147 m/z) and with a relative abundance (100%). And the mass spectrum of the compound (M36) was measured, where it showed a peak at (675 m/z) with a relative abundance (17%) due to the molecular ion of the compound, as well as a base peak at (81 m/z) and with a relative abundance (100%). See Figure (6 and 7).

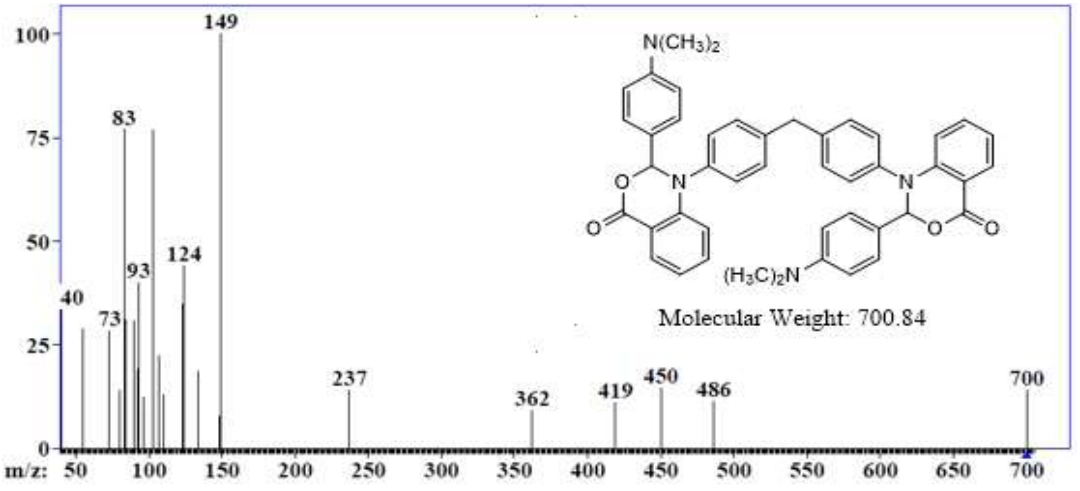


Figure (6): The mass spectrum of the compound M12

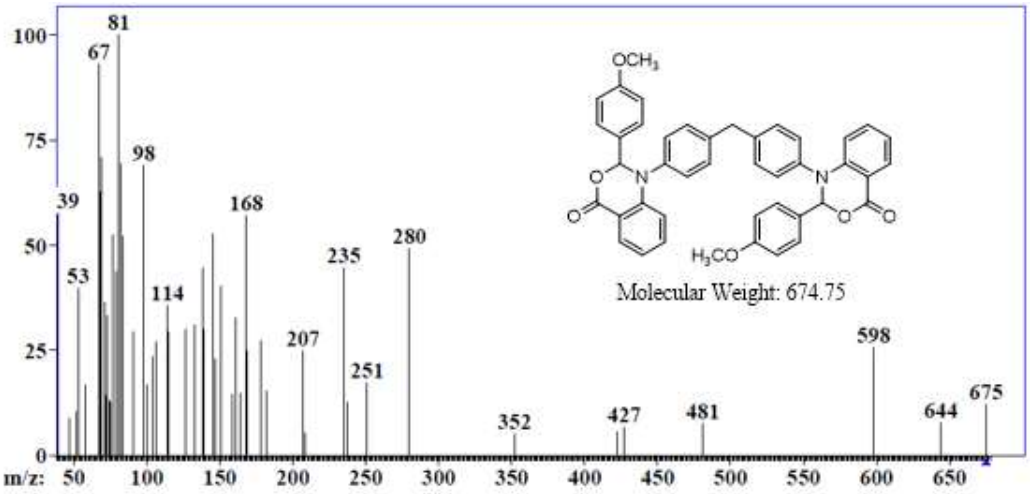


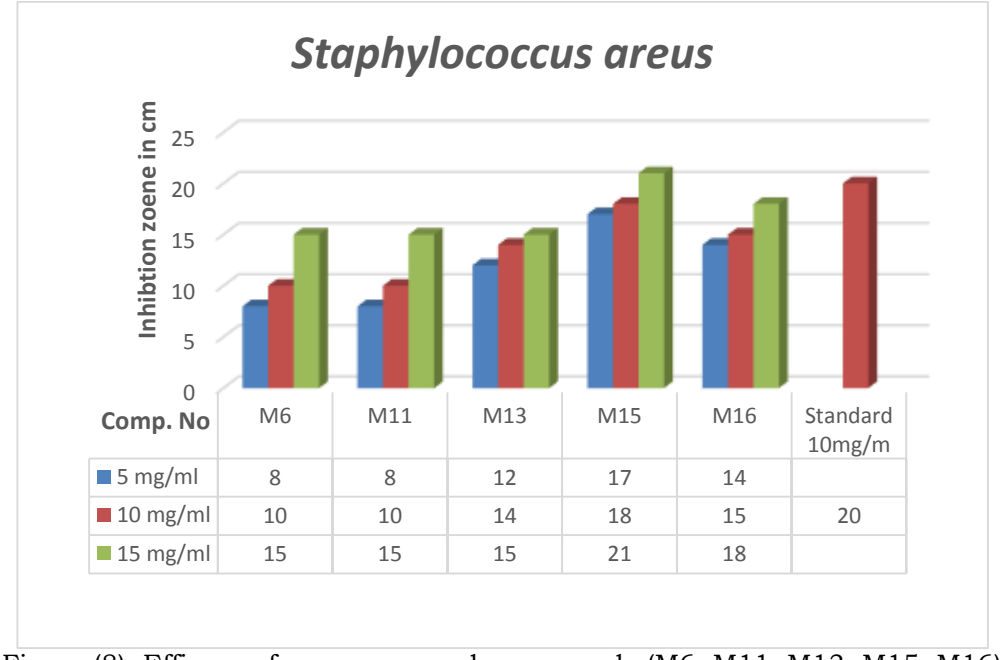
Figure (7): The mass spectrum of the compound M18

Antibacterial activity [18-20]

The antibacterial activity of some prepared compounds M6, M11, M13, M15, and M16 was measured on two types of bacterial isolates, *Staphylococcus aureus* gram-positive and *Klebsiella pneumonia* gram-negative. The results were compared with the antibiotic ciprofloxacin, the results showed that some of the prepared compounds possess inhibitory activity against bacteria at concentrations of the compounds (5,10,15) mg/ml, especially the compound M5, at the concentration of 15 mg/ml compared to the antibiotic ciprofloxacin. See Table (5) and Figures (8) and (9).

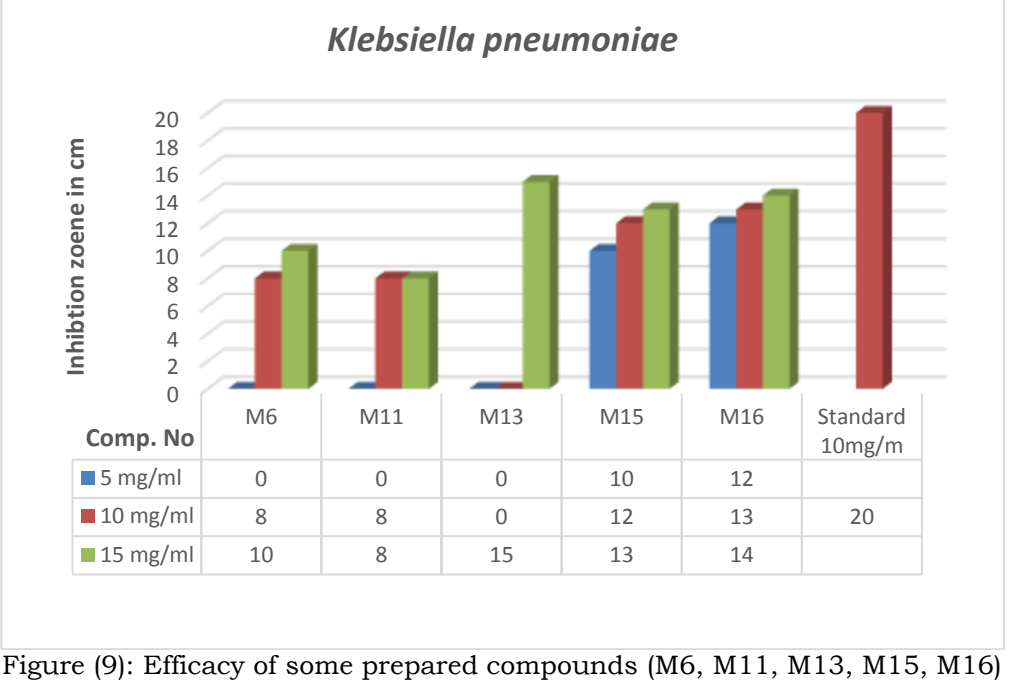
Table (5): Antibacterial activity data for some of the prepared compounds measured in millimeters

	Staphylococcus aureus			*Klebsiella pneumoniae*		
Comp. No.	5 mg/ml	10 mg/ml	15 mg/ml	5 mg/ml	10 mg/ml	15 mg/ml
M6	8	10	15	0	8	10
M11	8	10	15	0	8	8
M13	12	14	15	0	0	15
M15	17	18	21	10	12	13
M16	14	15	18	12	13	14
Standard 10mg/m		20			20	



Comp. No	M6	M11	M13	M15	M16	Standard 10mg/m
5 mg/ml	8	8	12	17	14	
10 mg/ml	10	10	14	18	15	20
15 mg/ml	15	15	15	21	18	

Figure (8): Efficacy of some prepared compounds (M6, M11, M13, M15, M16) against *Staphylococcus aureus*



Comp. No	M6	M11	M13	M15	M16	Standard 10mg/m
5 mg/ml	0	0	0	10	12	
10 mg/ml	8	8	0	12	13	20
15 mg/ml	10	8	15	13	14	

Figure (9): Efficacy of some prepared compounds (M6, M11, M13, M15, M16) against *Klebsiella pneumonia*

The following pictures show the areas of inhibition of the prepared compounds for the two types of bacteria used. See Figure 10 and 11

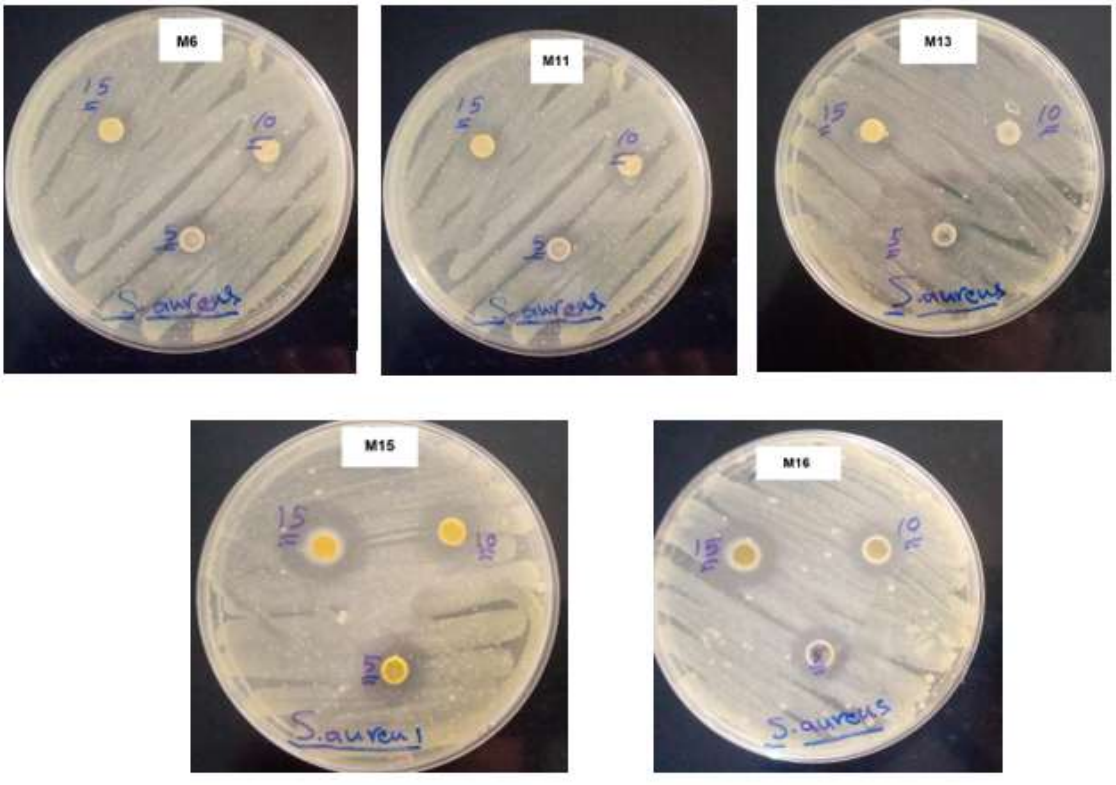


Figure 10: Inhibition zone diameter (mm) on *Staphylococcus aureus* + GIVe

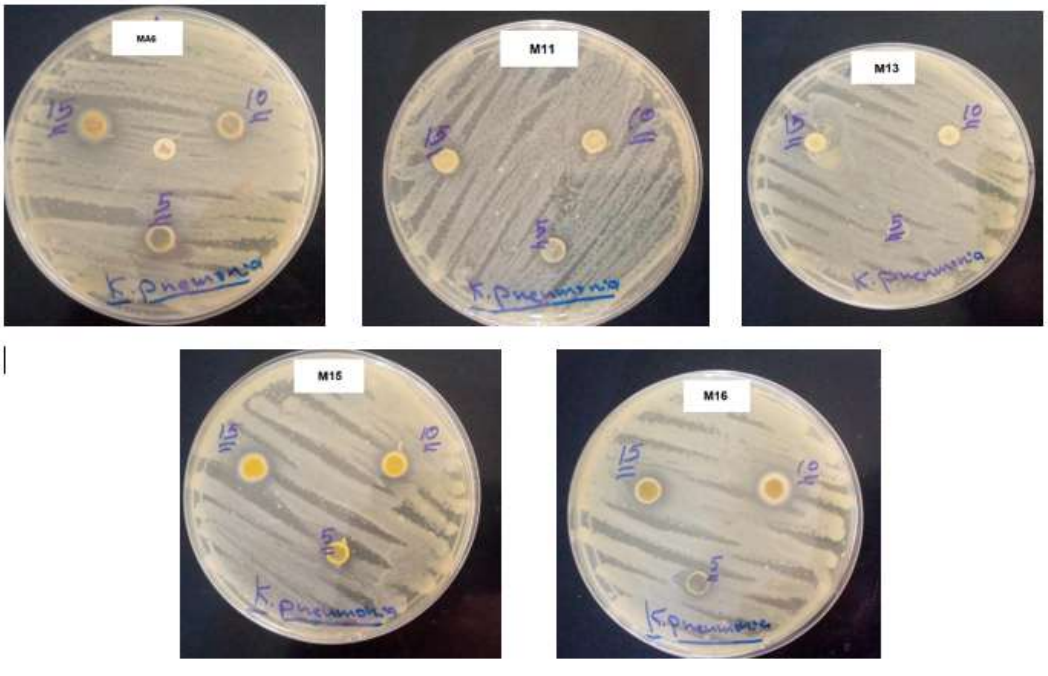


Figure 11: Inhibition zone diameter (mm) on *Klebsiella pneumoniae* – GIVe

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