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Synthesis and identification of morpholinium-5-amino-1,3,4-thiadiazole-2-thiolate, and study of the biological activity of some of their Schiff bases

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Abstract
A new series of morpholinium-5-amino-1,3,4-thiadiazole-2-thiolate salts (M1-M10) was produced by reacting different benzaldehyde derivatives with 5-amino-1,3,4-thiadiazole-2-thiol in the presence of morpholine. Infrared spectroscopy was used to evaluate our new Schiff bases of morpholinium-5-amino-1,3,4-thiadiazole-2-thiolate salts, and nuclear magnetic resonance spectroscopy and GC-MS were used to characterise some of them. The biological activity of the produced compounds was tested as an antibacterial against four species of bacteria, two of which are Gram-negative (*Pseudomonas aeruginosa* and *Klebcellacoccus*) and two of which are Gram-positive (*Staphylococcus aureus* and *Streptococcus*).
Keywords: 2-amino-1,3,4-thiadiazole, 5-thiol, Schiff bases, morpholinium salts, biological activity.

1. Introduction

Infections caused by bacteria are becoming more common as the population of immunocompromised people grows. Infection produced by these bacteria poses a significant challenge to the medical community, emphasising the need for the development of a novel, more effective, and selective non-traditional antimicrobial agent(Singh, et al., 2020).

Based on the foregoing, we chose to contribute to the creation of antibacterials. After examining various papers in this field, we observed that thiadiazole derivatives might be used as a template for the discovery and design of more potent medicinal agents through modification or derivatization(Serban et al., 2018). In addition, many of the previous studies demonstrated that thiadiazole compounds had antibacterial, antifungal, anti-inflammatory, antidepressant, antipsychotic, and antipyretic activity.

Because the majority of previous studies focused on the preparation of new Schiff bases derivatives from 2-amino-1,3,4-thiadiazole.The primary goal of the study was to design other types of Schiff bases for the 4-morpholinium-5-amino-1,3,4-thiadiazole-2-thiolate salts(Slaihim et al., 2019; Khairuddean et al., 2020), as a possible antibacterial in response to the reported biological activities of the aforementioned derivatives and as part of our ongoing research on new biologically active compounds.

2. Experimental

2.1. Chemicals

In this research of all the generated compounds, the following substances and reagents were used: 20 x 20 cm aluminum sheet, TLC silica gel 60 F254 (Merck, Germany); Carbon disulfide CS2, (ALPHA Chemika, India); Thiosemicarbazide, (ALPHA Chemika, India); Morpholine, (BDH, England); 100% Ethanol; p-Bromobenzaldehyde; p-Chloro benzaldehyde; o-Bromobenzaldehyde,(Fluka); p-Hydroxybenzaldehyde (Sigma, brand); 4-Hydroxy-3-methoxy benzaldehyde,(BDH, England);p-methyl benzaldehyde,(Fluka, brand); 2,4-dihydroxybenzaldehyde,(Sigma); p-Nitrobenzaldehyde; p-Dimethylamino benzaldehyde; Dimethyl sulfoxide-*d6* (DMSO-*d6*), (Sigma-Aldrich, USA).

2.2. Instruments

All devices or apparatus used to determine the structure of produced substances, with the exception of a Bruker Avance (400 MHz), are located at the University of Samarra's College of Applied Sciences. The proton spectra of nuclear magnetic resonance (1H-NMR) were obtained using a Bruker Avance (400 MHz) and DMSO-d6

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solvent at Basra University's College of Education. Using a Fourier Transform Infrared Spectrophotometer (FTIR-8400S) instrument provided by the Japanese company Shimadzu, infrared spectra were captured, and samples were manufactured as KBr discs. Mass spectra were obtained using a Shimadzu GC-MS-QP 2010 Ultra.

2.3 Biological assay

2.3.1 Compounds and cells

All test chemicals were dissolved in DMSO at a concentration of 0.032 mg / mL and serially diluted before being used in a growth medium. The following harmful microorganisms were used: *Pneumonia klebsiella* and *Pseudomonas aeruginous* are both gram-negative (Gr-ve). Furthermore, *Staphylococcus aureus* and *Streptococcus mutans* are both gram-positive (Gr+ve). The four bacterial species were tested in the Microbiology Laboratory/Pathological Analysis Department/College of Applied Sciences/ Samarra University.

2.3.2 Antibacterial assay

The organic solvent DMSO was used to make test solutions for the compounds M1–M10, and several concentrations were prepared from it, (0.032, 0.016, 0.008, 0.004, 0.002, 0.001, 0.0005) for each chemical, which were administered to the four aforementioned bacteria. The seven concentrations were spread on two plates using the agar well diffusion method(Baron & Finegold, 1990), and seven 5 mm-diameter holes were drilled in the center of the agar around the circumference of each plate. Then, 50–70 (µL) of varying concentrations of solutions were injected into each hole. At a temperature of 37 °C, a micropipette was used to inject each hole and record the results after 18–24 hours.

2.4. Synthesis method

2.4.1 For the production of compound PS, the method described by Yusuf et al. (2008) was used.

2.4.2 General procedure for the preparation of Schiff base compounds (M1-M10).

In a round bottom flask, an equimolar of compounds (0.001 mol) of one of the aromatic aldehyde derivatives, PS and morpholine, were mixed in (50 ml) of 100% ethanol to form a new series of Schiff bases (M1-M10). For 6 hours, the ingredients of the reaction were refluxed while stirring. The mixture is concentrated and gradually cooled. The resulting precipitate was filtered, dried, and purified using suitable techniques.

Table 1: Physical Properties of new Schiff Bases series (M1-M10).

Chemical Symbol	M .W	M.P C°	Color	Yeild%
M1	387.31	101-103	Orange	**64**
M2	342	133-135	Yellow	**83**
M3	351.49	209-211	dark Orange	**47**
M4	353.42	100-102	Purple	**93**
M5	387.31	239-241	Yellow	**85**
M6	354.44	gummy	Chocolate	**88**
M7	338.44	156-158	Yellow	**66**
M8	324.42	94-96	dark Orange	**53**
M9	322.45	218-220	Brown	**40**
M10	342.40	192-189	Orange	**73**

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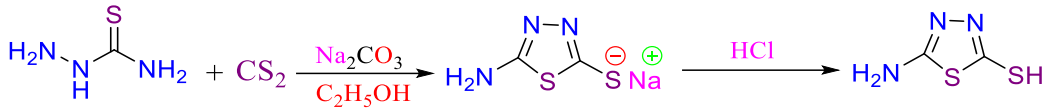
3. Results and Discussion

3.1. Spectra of Morpholinium Thiadiazole Salts of the New Schiff Base (M1-M10)

As (Scheme1), the starting material compound (PS) was synthesize via retrograde escalation of thiosemicarbazide with carbon disulfide in 100% ethanol (C_2H_5OH) in the presence of sodium carbonate for three hours(Yusuf et al., 2008). In the presence of sodium carbonate, Na_2CO_3, the intermediate state suffers from ring closure. The final produce 2-amino-4,3,1-thiadiazole-5-thiol (PS) is precipitated when the content is added to distilled water and acidified with drops of concentrated HCl.



thio simicabazide

5-amino-1,3,4-thiadiazole-2-thiol

Scheme 1: Preparation pathway of the starting material 2-amino-4,3,1-thiadiazole-5-thiol

The particular structure of the starting material compound was successfully confirmed using ^{1}H-NMR,^{13}C-NMR, IR, and GC-MS spectroscopy (PS). The spectra of the compound (PS) are depicted in the following four figures.

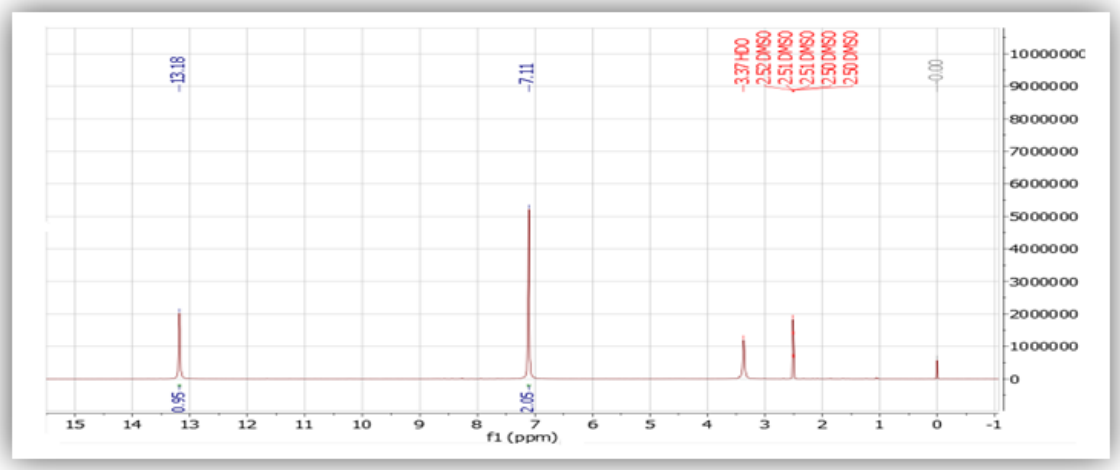


Figure 1: ^{1}H-NMR spectrum of the compound PS;(400 MHz, DMSO-*d6*)

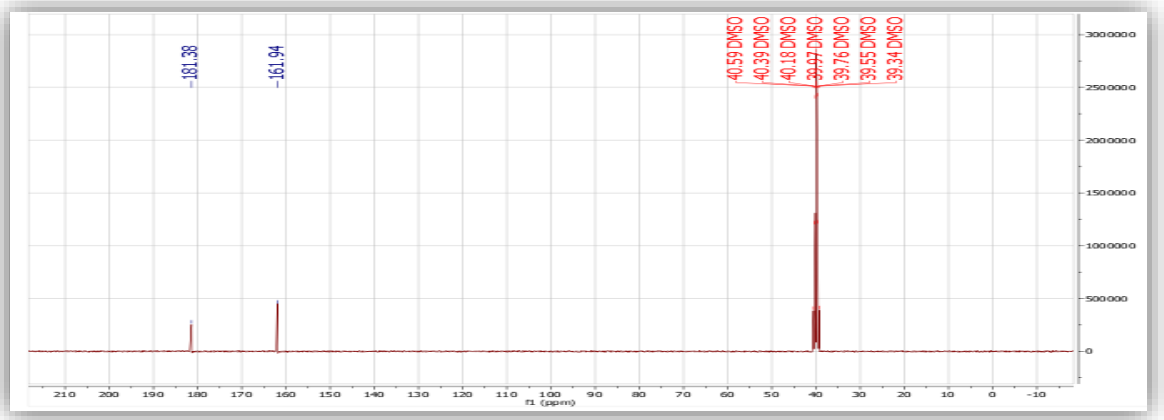


Figure 2: ^{13}C-NMR spectrum of the compound PS; (100 MHz, DMSO-*d6*)

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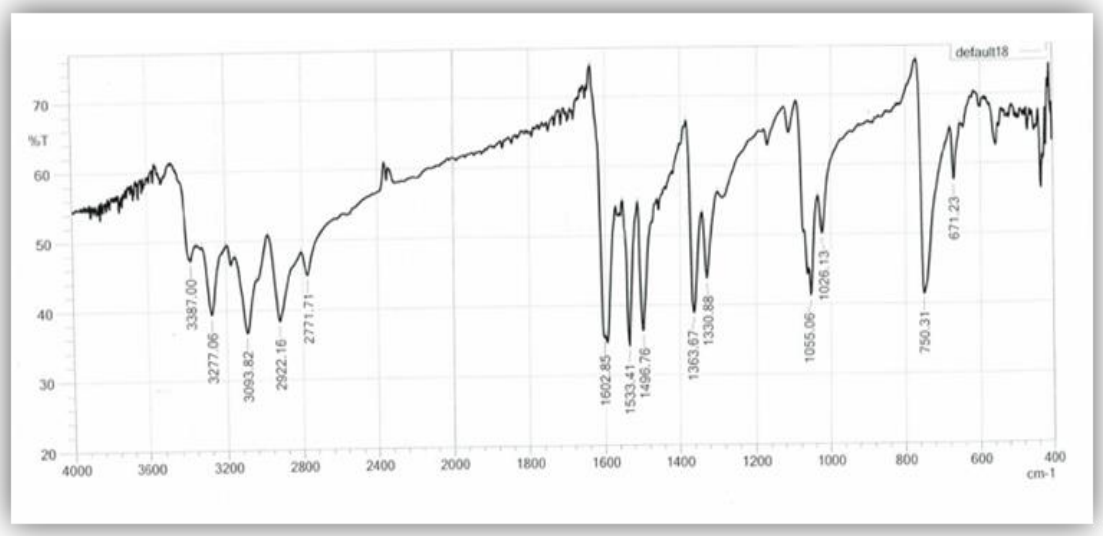

Figure 3: IR spectrum of 2-amino-4,3,1-thiadiazole-5-thiol(PS).

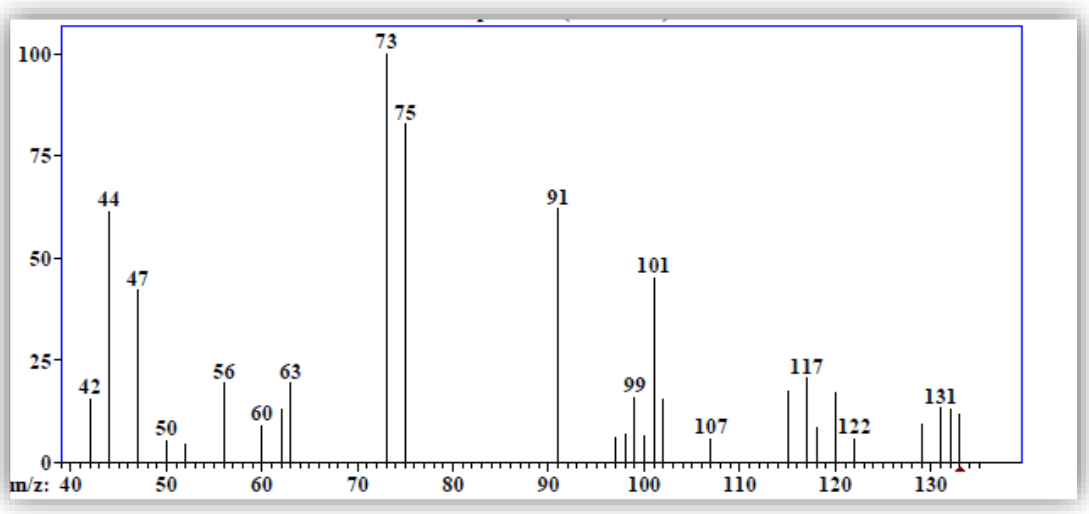

Figure 3: Mass spectrum of 2-amino-4,3,1-thiadiazole-5-thiol(PS).

In the present work, the new Schiff base series (M1-M10) was synthesised in accordance with the following scheme (2):

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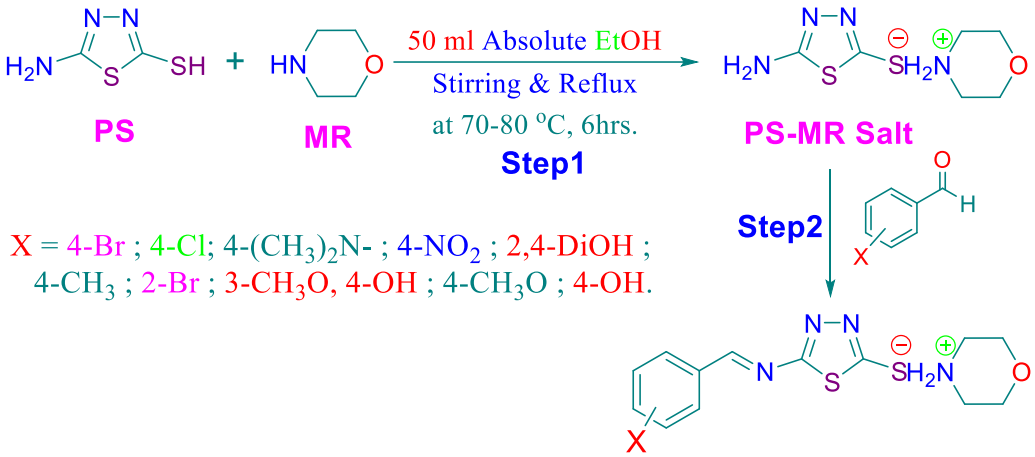

Scheme 2: Synthesis pathway of Schiff bases compounds (M1-M10).

Furthermore, the novel Schiff base series (M1–M10) was structurally characterised. Physical properties, 1H-NMR, IR, GC-MS, and MICs data from the new series (M1-M10) are shown in five tables, numbered 1, 2, 3, 4, and 5 respectively. The Schiff base scaffold is supported by the use of important organic identification methods such as IR, 1H-NMR, and GC-MAS spectroscopy.The -NH_2 or carbonyl groups were not present in the IR spectra, but the imine group's N=CH absorption band was visible at a wavenumber of 1650–1580 cm^{-1}, with the appearance of a stretching band at 1602–1506cm^{-1} and 1570–1473 cm^{-1}ranges in response to the stretching of the double aromatic (C=C) bond. as well as absorption bands caused by aromatic (C-H) bond stretching in the 3132–3010 cm^{-1} range. Aliphatic (C-H) stretch bands formed in the range 2970–2890 cm^{-1}, and the red spectra of all the produced compounds revealed the removal of bands associated with carbonyl group stretching frequencies that were present in the blue spectra (1660–1712 cm^{-1}).The Schiff base, or imine (-N=CH-), appears as a singlet in the ^{1}H-NMR spectrum between 8.76 and 8.19 ppm.All of the extra notable peaks and signals were found in the IR, 1H-NMR, and GC-MS spectra.

Table 2: ^{1}H-NMR characteristic data of compounds L1-L5

Structure	Chemical Shift (δ) ppm	Signal Features	No. of Protons	Type of Protons
L1/M4	8.51	s	H	(CH=N-)imine
	8.30	d, *J* = 8.5 Hz	2H	aromatic
	7.99	d, *J* = 8.4 Hz	2H	aromatic
	3.81	t, *J* = 4.0 Hz	4H	morpholinium
	3.09	t, J = 4.0 Hz	4H	morpholinium
L2/M5	8.19	s	H	(CH=N-)imine
	7.81	d, *J* = 8.7 Hz	1H	aromatic
	7.18	s	1H	aromatic
	6.91	d, *J* = 8.5 Hz	1H	aromatic
	3.77	t, *J* = 4.9Hz,	4H	morpholinium
	3.57	s	2H	-OH
	3.10	t, *J* = 4.9Hz	4H	morpholinium
L3/M7	8.76	s	H	(CH=N-)imine
	7.86	dd, *J* = 8.0 Hz	1H	aromatic
	7.81	d, *J* = 8.7 Hz	1H	aromatic
	7.71	t, *J* = 8.0 Hz	1H	aromatic

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	7.62	dd, *J* = 8.0 Hz	1H	aromatic
	3.79	t, *J* = 4.0 Hz	4H	morpholinium
	3.10	t, *J* = 4.0 Hz	4H	morpholinium
L4/M9	8.58	s	H	(CH=N-)imine
	7.95	d, *J* = 8.8 Hz	2H	aromatic
	7.88	d, *J* = 8.8 Hz	2H	aromatic
	3.87	s	3H	-OCH_3
	3.77	t, *J* = 4.0 Hz	4H	morpholinium
	3.11	t, *J* = 4.0 Hz	4H	morpholinium
L5/M10	8.40	s	H	(CH=N-)imine
	7.81	d, *J* = 8.7 Hz	2H	aromatic
	6.90	d, *J* = 8.6 Hz	2H	aromatic
	3.76	t, *J* = 4.0 Hz	4H	morpholinium
	3.08	t, *J* = 4.0 Hz	4H	morpholinium

Table 3:IR (v, cm^{-1}) characteristic bands of new Schiff Bases series (M1–M10) series.

No.	$(C-H)_{Ar}$	$(C-H)_{Al}$	$(C=N)_{im}$	$(C=C)_{Ar}$	$(-^{+}NH_2)_{MR}$	Others
M1	3095	2900	1622	1514,1480	3284	657C-Br
M2	3022	2970	1600	1564,1489	2244	C-Cl 748
M3	3089	2893	1616	1533,1512		C-CH_3 1369
M4	3040	2900	1606	1556,1519	3400	C-NO_2 1348
M5	3012	2968	1580	1506,1473	3261	OH with $(-^{+}NH_2)_{MR}$
M6	3010	2939	1640	1600,1514	3271	
M7	3064	2954	1600	1570,1523	3415	C-Br 673
M8	3030	2926	1602	1577,1508	3263	3126-3253O-H
M9	3132	2926	1610	1552,1477	3340	
M10	3022	2890	1650	1602,1570	3115	with $OH(-^{+}NH_2)_{MR}$

Table 4:Molecular weight ions with a base peak in the mass spectra

Produ N	Chemi form	Exact Mass	Mass spectrum m\z (relative intensity) of fragments

M2	$C_{13}H_{15}N_4OS$	307	308[M^+,23%	280[M^+,24%]	267[M^+,22%]	**88[M^+,100]**
M7	$C_{14}H_{18}N_4O_2S$	338	339[M^+,10%	5%]+294 [M^+,	224[M^+, 17%]	**117 [M^+,100%**
M8	$C_{13}H_{16}N_4O_2S$	324	325[M^+,10%	298[M^+,20%]	212[M^+,19%]	**141[M^+,100%**
M9	$C_{14}H_{18}N_4OS$	322	323[M^+,2%	285[M^+,5 %]	222[M^+,6%]	**102[M^+,100]**
M10	$C_{13}H_{16}N_4O_3S$	340	341[M^+,10%	250[M^+,80%]	194[M^+,48%]	**160 [M^+,100%**
PS	$S_2H_3N_3S_2$	131	132 [M^+,20%	101[M^+,4 6%	73[M^+,100%]	----------

3.2. Evaluation of the biological activities of the new Schiff bases series (T1-T10)

The Schiff base derivatives were tested against four different gram-positive and gram-negative bacterial species to determine their biological effects. This study looked at four bacteria: *Streptococcus mutans*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus.*

The majority of the chemicals tested inhibited at least one of the four bacterial species studied. The **MIC** values for this activity were discovered to range from weak to strong, as follows:At **MICs of 0.0005 mg/mL**, compound **(M2)**demonstrated the biggest inhibition zone **(15, 20, 16, and 16 mm)**against *Staphylococcus aureus*, *Streptococcus mutans*, *Pneumonia klebsiella*, and *Pseudomonas aeruginosa*. Similarly, at **MICs of 0.0005 mg/mL**, compounds **(5), (M7),** and**(M9)**displayed greater inhibition zones of **13, 18, and 15 mm**against *Streptococcus mutans bacteria*. Furthermore, at **MICs** of **0.0005 mg/mL**, the compounds**(M7)** and**(M9)**inhibited *Pseudomonas aeruginosa* with inhibition zones of **12** and**14 mm**, respectively.

Table 5: MICs of some new Schiff bases of series M1-M10 against gram-negative and gram-positive bacterial strains.

4. Conclusion

In the present study, a series of novel Schiff bases derivatives containing 1,3,4-thiadiazole scaffolds modified with morpholinium salt was efficiently designed and synthesized. It also involved the preparation, characterization, and investigation of the biological activity of all new Schiff bases (M1–M10). The structures of the new compounds were elucidated using IR, ^{1}H-NMR, ^{13}C-NMR, and GC-MS spectroscopy.

Among the synthesized4-morpholinium-5-amino-1,3,4-thiadiazole-2-thiolate salts of Schiff bases, compound

OMPOUND Cons.: (M3)	***aphylococcus aureus***	***reptococcus mutans***	***neumonia klebsiella***	***Pseudomonas aeruginosa***
0.032	17	20	17	21
0.016	16	20	14	20
0.008	16	19	0	19
0.004	16	17	0	19
0.002	0	0	0	0
0.001	0	0	0	0
0.0005	0	0	0	0
OMPOUNDCons.:	***aphylococcus aureus***	***reptococcus mutans***	***neumonia klebsiella***	***Pseudomonas***

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(M5)				*aeruginosa*
0.032	24	24	16	19
0.016	24	27	16	18
0.008	23	23	10	16
0.004	16	20	0	0
0.002	15	14	0	14
0.001	15	14	0	12
0.0005	15	13	0	0
OMPOUND Cons.: (M6)	***ıphylococcus aureus***	***reptococcus mutans***	***ıeumonia klebsiella***	***Pseudomonas aeruginosa***
0.032	15	20	0	19
0.016	15	20	0	16
0.008	13	18	0	14
0.004	13	15	0	13
0.002	0	15	0	12
0.001	0	14	0	11
0.0005	0	0	0	11
OMPOUNDCons.: (M7)	***ıphylococcus aureus***	***reptococcus mutans***	***ıeumonia klebsiella***	***Pseudomonas aeruginosa***
0.032	26	25	16	21
0.016	26	24	10	19
0.008	14	20	14	15
0.004	16	20	12	12
0.002	0	20	0	14
0.001	0	19	0	11
0.0005	0	18	0	12
OMPOUNDCons.: (M8)	***ıphylococcus aureus***	***reptococcus mutans***	***ıeumonia klebsiella***	***Pseudomonas aeruginosa***
0.032	16	29	15	14
0.016	13	26	12	12
0.008	14	17	11	10
0.004	11	17	0	12
0.002	11	0	0	11
0.001	0	0	0	0
0.0005	0	0	0	0
OMPOUNDCons.: (M9)	***ıphylococcus aureus***	***reptococcus mutans***	***ıeumonia klebsiella***	***Pseudomonas aeruginosa***
0.032	19	30	22	26
0.016	19	30	23	26
0.008	17	26	20	29
0.004	15	20	16	18
0.002	0	20	15	17
0.001	0	19	0	12
0.0005	0	15	0	14
OMPOUNDCons.: (M10)	***ıphylococcus aureus***	***reptococcus mutans***	***ıeumonia klebsiella***	***Pseudomonas aeruginosa***

0.032	25	29	15	20
0.016	21	20	0	18
0.008	16	17	0	16
0.004	14	15	0	0
0.002	14	0	0	12
0.001	14	0	0	12
0.0005	13	0	0	0

(M2) showed the largest inhibition zone (15, 20, 16, and 16 mm) at MICs of 0.0005 mg/mL against *Staphylococcus aureus, Streptococcus mutans, Pneumonia klebsiella,* and *Pseudomonas aeruginosa*. Similarly, compounds (5), (M7), and (M9) had stronger inhibition zones of 13, 18, and 15 mm against *Streptococcus mutans bacteria* at MICs of 0.0005 mg/mL. Furthermore, compounds (M7) and (M9) inhibited *Pseudomonas aeruginosa* with inhibition zones of 12 and 14 mm, respectively, at MICs of 0.0005 mg/mL.

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