Microwave-assisted synthesis, structural characterization of amino pyridines, pyrrolidine, piperidine, morpholine, acetamides, and assessment of their antibacterial activity

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Abstract: A sequence of new acetamide derivatives **9-15** of primary, secondary amine, and *para*-toluene sulphinate sodium salt have been synthesized under microwave irradiation and assessed in vitro for their antibacterial activity against one Gram-positive and two Gram-negative bacterial species such as *S. pyogenes*, *E. coli*, and *P.mirabilis* using the Mueller-Hinton Agar diffusion (well diffusion) method. The synthesized compounds with significant differences in inhibition diameters and MICs were compared with those of amoxicillin, ampicillin, cephalothin, azithromycin and doxycycline. All of the evaluated acetamide derivatives were used with varying inhibition concentrations of 6.25, 12.5, 37.5, 62.5, 87.5, 112.5 and 125 µg/ml. The results show that the most important antibacterial properties exercised by the synthetic compounds **9** and **11** bearing *para*-chlorophenyl moiety incorporated into the 2-position moiety of acetamide 2. The molecular structures of the new compounds were determined using FT-IR, ^{1}H-NMR techniques.

Keywords: acetamide, pyridine, pyrrolidine, piperidine, antibacterial activity, heterocycles

1. Introduction

Many processes have reported the use of acetamide chlorides **3-8** as a useful building block for the synthesis of complex heterocyclic compounds. The derivatives of moiety **2** (figure 1) have shown potential for biological action, notably as antimalarial [1], anticancer [2], anti-diabetic [3], anti-tubercular [4], and anti-inflammatory [5] as well as industrial applications such as synthesis for stabilizers [6], plastics release agents

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[7], films [8], surfactants and soldering fluxes [9], organic fibers [10], and colorants [11].

-NHCO-CH_2-

2

Figure 1. Structure of acetylamide moeity 2

The compounds atorvastatin [12] **16**, lidocaine [13] **17**, paracetamol [14] **18**, amoxicillin [15] **19**, levobupivacaine [16] **20** are among the acetamide derivatives of **2** the most widely used drugs in medicines (figure 2) . Many derivatives of **2** containing secondary cyclic amines linked to 1 or 2-positions that were reported to have become an anticonvulsant. For instance, anticonvulsant compounds such as **21** and **22** used to treat epilepsy [17], whereas the combination of compounds **23** and **24** used to inhibit kinase enzyme and antihistamines [18].

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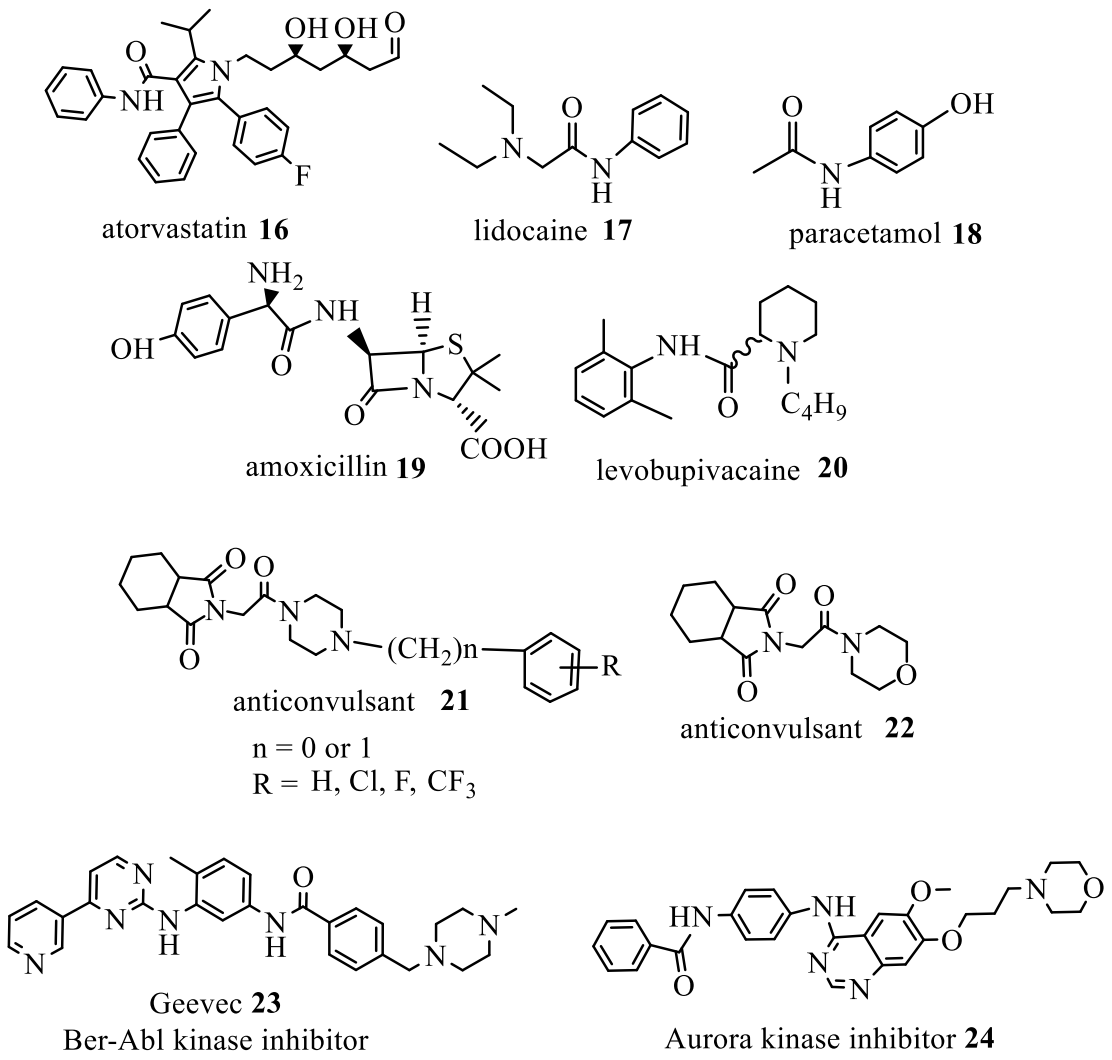

Figure 2. The most widely used drugs in medicines

Heterocycles of pyridine are present in certain biologically active molecules (figure 3). One of pyridine-containing medications is lunesta with active ingredient **25** used to treat insomnia, and pioglitazone **26** is effective in diabetes care. In addition, previous studies conducted by Nakamoto et al. [19] and Tang et al. [20] evaluated the compounds **27** and **28** as an antifungal and antituberculous agents respectively.

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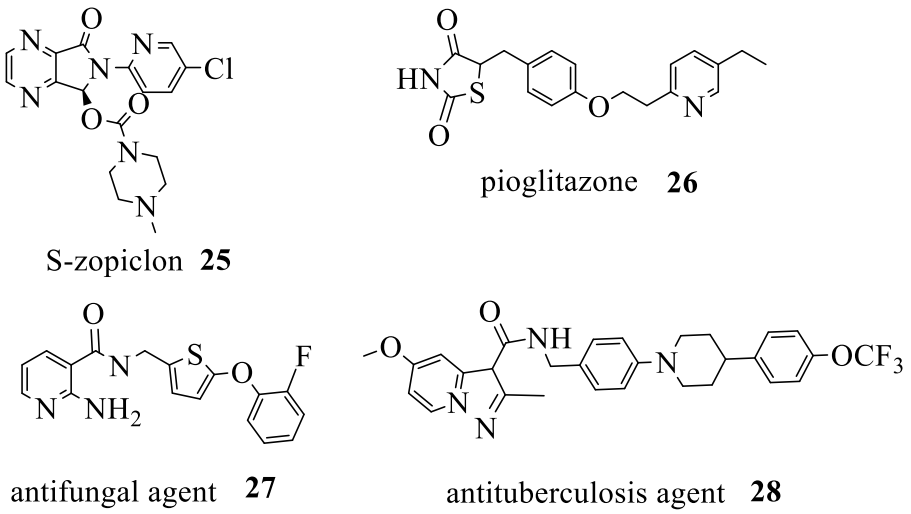

Figure 3. Some heterocycles of biologically active pyridines

Synthesis of broad spectrum compounds with antibacterial activity is required because of the ex-tense use of antibiotics in medicine and prevailing resistance in microorganism. Conventional organic preparation methods are sluggish to meet the rapid need for the synthesis of these compounds. Nowadays microwave plays an appealing role in organic synthesis. Herein, we report the use of microwave in organic synthesis of seven newly acetamides derivatives **9-15** form reaction of chloroacetyl chloride **1**, with primary, secondary amines and *para* toluene sulphinate sodium salts. Also, the aim of this study is also to assess the susceptibility profile of bacterial isolates species to synthesized compounds **9-15**, reference antibiotics, and to determine minimum concentrations of inhibition (MIC's). Compounds **9-15** were structurally determined by ^{1}H-NMR, and FT-IR and elemental analysis. Scheme 1 shows a central aspect of that approach.

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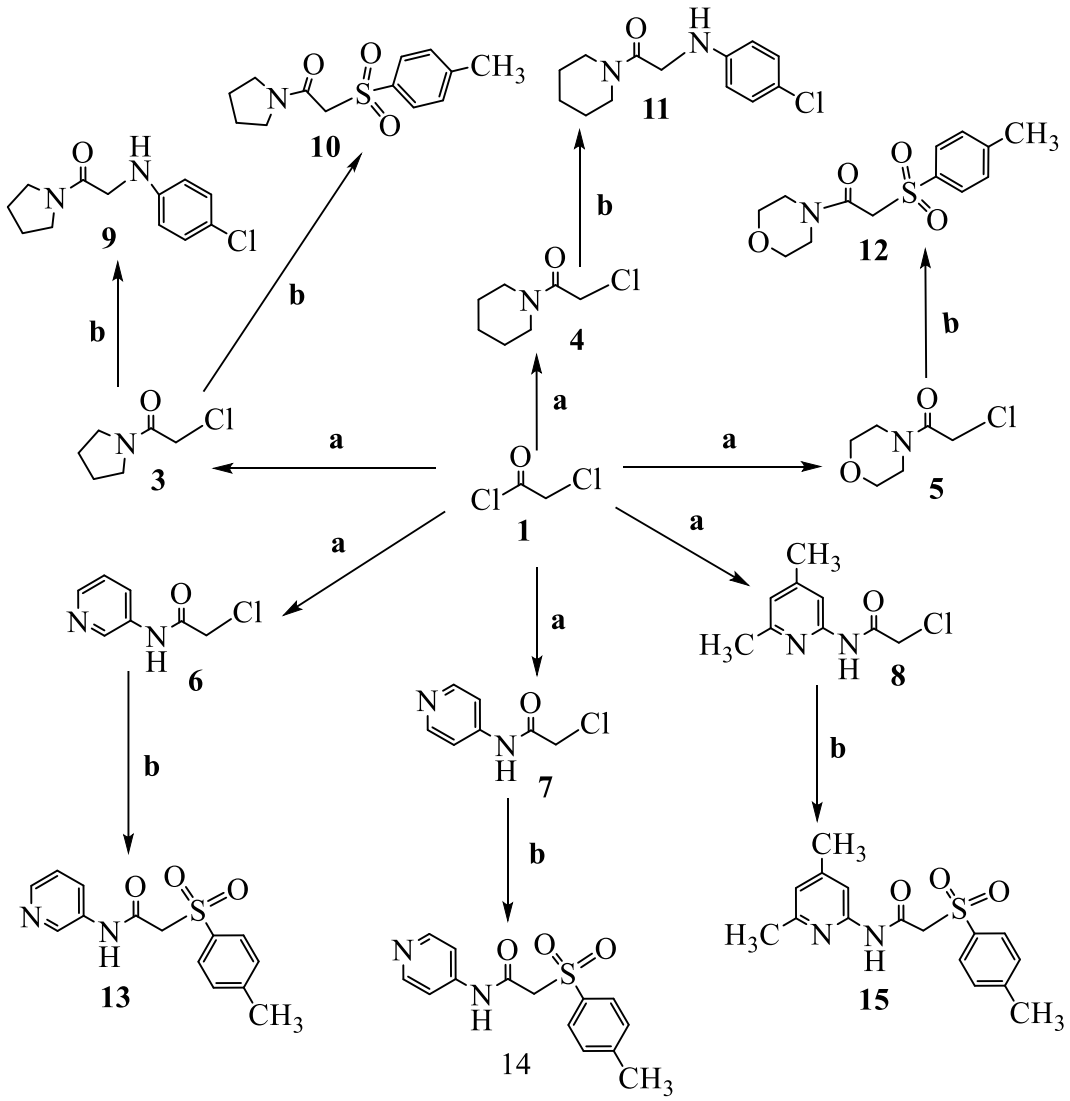

Scheme 1. Syntheic pathway to compounds **9-15**

2. Results and Discussion

2.1. Chemistry

We are engaged in intensive efforts to synthesize nitrogen-heterocyclic compounds with high yields. Following a previously described study [21], the acetamide intermediates **3-8** and the new targeted derivatives **9-15** synthesized in this work are depicted in Scheme (1). Over the past thirty years, microwave irradiation was used to enhance reaction rates [22, 23]. We have thus exploited the advance of microwave to accelerate the creation of C-N bonds. For the synthesis of the precursors **3-8**, the process was performed under mild conditions involving the addition of chloroacetyl

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chloride **1** to a mixture of amines and *para*-toluene sulphinate sodium salt in dry CH_2Cl_2 at 0 ºC and the isolated yields of these compounds range from 60 to 90% see Table 1. The synthesis of targeted compounds **9-15** was achieved through two routes: conventional heating and microwave irradiation methods. The heating process involved precursor **3-8**, amine, and sodium p-toluene sulphinate reactions in a CH_3CN solution containing Et_3N as a catalyst giving the compounds **9-15** in moderate yields reached of 60% at 70 ºC see Scheme 1 and Table 1, On the other hand, treatment of the precursors **3-8** with amines as well as sodium p-toluene sulphinate in a dry CH_3CN provided the desired compounds **9-15** at 65-70 ºC in good yields under microwave irradiation. Reactions took 5-10 min to complete and one of the advantages of this technique is allowed us to isolate products with high purity and without side products, see Tables 1, 2, and Table 3 for ^{1}H-NMR details.

Table 1. Melting points, colours, solvents for recrystallization, and yields of the compounds **3-8**

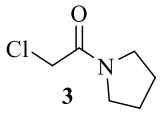
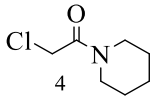
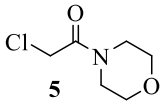
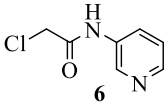
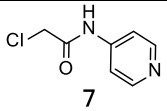
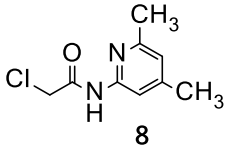
Compd. No.	Recryst. Solvent	Color	M.P. ºC	Yield %
 **3**	n-Hexane	Gray	143 - 145	78
 **4**	Ethanol	White	202 - 203	85
 **5**	Ethanol	Brown	276 - 278	60
 **6**	Hexane: methanol 1:3	Brown	196 - 198	83
 **7**	Ethylacetate:acetone 1:3	White	251 - 253	80
 **8**	n-Hexane	Brown	198 - 200	90

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Table 2. Melting points, colours, solvents for recrystallization, and yields of compounds **9-15**

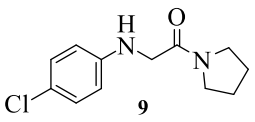
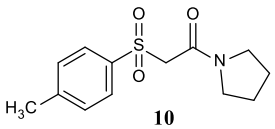
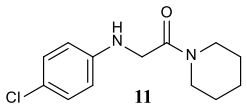
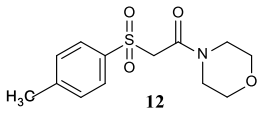
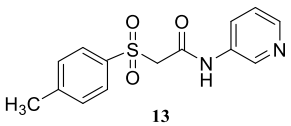
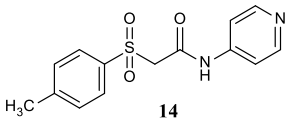
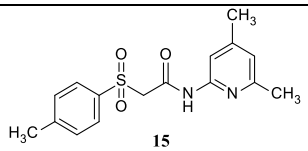
Compd. No.	Recryst. Solvent	Color	M.P. ^{o} C	Yield % of	Yields % of Convention
 **9**	n-Hexane	Gray	183-185	50	24
 **10**	Ethanol	Yellow	188-189	85	55
 **11**	n-Hexane: ethanol	White	197-198	78	55
 **12**	Ethylacetate: Ethanol	White	170-172	90	30
 **13**	Methanol	White	204-207	78	53
 **14**	Hexane: Methanol	White	251-253	76	60
 **15**	Ethanol	Brown	212-213	62	50

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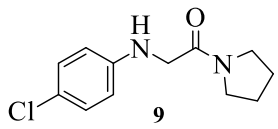
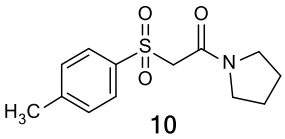
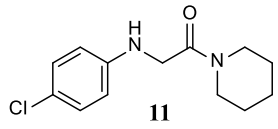
Table 3. ^{1}H-NMR data of compounds **9-15**

Structure.	Chemical shift (δ) ppm	Feature of signal	No. of protons	Type of protons
 **9**	7.20 - 6.75 6.32 3.36 and 1.75 3.30	dd, $J_{2,3}$ 5.25 Hz, $J_{2,6}$ 1.30 Hz s 2m s	4H 1H 8H 2H	aromatic NH pyrrolidinyl proton $-CH_2CO-$
 **10**	7.75 - 7.42 4..20 2.85 and 1.90 2.35	dd, $J_{2,3}$ 5.35, $J_{2,6}$ 1.30 Hz s 2m s	4H 2H 8H 3H	tolyl $-CH_2CO-$ pyrrolidinyl proton CH_3
 **11**	7.20 - 6.70 6.35 3.20 2.90 and 1.45	dd, $J_{2,3}$ 5. 0 Hz, $J_{2,6}$ 1.45 Hz s s 2m	4H 1H 2H 10H	aromatic NH $-CH_2CO-$ piperidinyl proton

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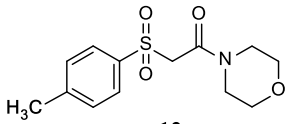
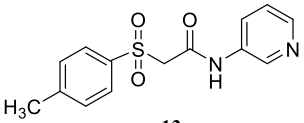
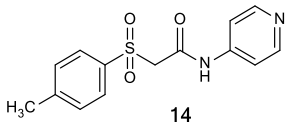
 **12**	7.75-7.42 4.15 3.70 and 2.50 2.40	dd, $J_{2,3}$ 5.26 Hz, $J_{2,6}$ 1.4 Hz s 2t s	4H 2H 8H 3H	tolyl protons $-CH_2CO$ morpholinyl protons CH_3
 **13**	10.65 9.0 8.41 - 7.10 7.70 - 7.35 4.20 2.35	s s dd, $J_{4,5}$ 5.31 Hz, $J_{5,6}$ 5.25 Hz dd, $J_{2,3}$ 5.25 Hz, $J_{2,6}$ 1.5 Hz s s	1H 1H 3H 4H 2H 3H	NH pridinyl protons tolyl protons CH_2 CH_3
 **14**	9.90 8.41 - 7.91 7.65 - 6.40 4.25 2.32	s dd, $J_{2,3}$ 5,31 Hz., $J_{2,6}$ 1.45 Hz dd, $J_{2,3}$ 5.20, $J_{2,6}$ 1.5 Hz s s	1H 4H 4H 2H 3H	NH pridinyl protons tolyl protons CH_2 CH_3

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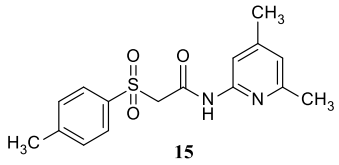 15	10.40 8.30 - 7.40 7.60-7.45 4.25 2.45 - 2.35	s 2s dd, $J_{2,3}$ 5.26 Hz, $J_{2,6}$ 1.55 Hz s 3s	1H 2H 4H 2H 9H	NH pridinyl proton tolyl protons CH_2 $3CH_3$

2.2. In vitro Antibacterial activity testing

Instructive bacterial infections such as tuberculosis, urinary tract infection, pneumonia, brain abscess, phyaryngitis, and tonsillitis are a series of life-threatening diseases commonly recognized in immunocompromised patients. Such infectious diseases are becoming more and more resistant to antibiotics in human patients. Resistance to microbial drugs is an inevitable consequence of the use of antibacterial drugs. Antimicrobial resistance to antibiotics has arisen due to overuse of these antibacterial compounds. Susceptibility to antibiotics by pathogens has now become a crucial factor in the effectiveness of antibacterial choice. Amoxicillin, ampicillin, doxycycline, azithromycin and cephalothin, the five reference antibiotics used in this research, are commonly used for the treatment of infections. Researchers continue to discover new antimicrobial agents as a high-resistance drug organism. This research was carried out to determine the susceptibility of seven newly synthesized compounds to certain bacterial organisms. Synthesized compounds **9-15** have been evaluated for antibacterial activity in comparison with the mentioned reference antibiotics.

Acetamide derivatives are well known to have a broad range of antibacterial action [24]. With regard to our recent research [21] on the synthesis of acetamide derivatives and the assessment of their biological role, in this in vitro study, three species of microorganisms were included, all isolates of the three species were susceptible to synthesized compounds **9-15** at MICs greater than 6.25 µg/ml.

Tests for the gram-negative species, *E. coli* and *P. mirabilis* cause urinary tract infections (UTI) in patients exhibited inhibition zones for compounds **9** and **10** ranging from 6.0 to 8.4 mm at MIC of 12.5 µg/ml. At MIC of 37.5 µg/ml, isolates of these bacterial species were not susceptible to compounds **13** and **15**, while inhibiting

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zones of other compounds ranging from 6–14 mm Table 4. The results for these two gram-negative species exhibited intermediate inhibition zone diameters are in agreement with other authors who reported 11-12 mm using amoxicillin (20 µg) and ampicillin (20 µg) [25, 26, 27] antimicrobial disks Tables 4 and 5.

Gram-positive species, *S. pyogenes* are one of the main causes of urinary tract infection and pose a significant health concern and cause a number of human diseases [28, 29]. Also, *S. pyogenes* considered to be the most common and significant cause of bacterial tonsillitis in children and adults, and isolates this bacterial species were obtained from Tikrit hospital, Tikrit, Iraq. All isolates at MICs more than 6.25 µg/ml were susceptible to synthesized compounds **9-15**, and compounds **14** and **15** did not display any inhibition zones in this test, even at MIC of 37.5 µg/ml Table 6.

For the sake of comparison, we compared the antibacterial susceptibility of all the isolates to five antibacterial agents amoxicillin (20 µg), ampicillin (25 µg), cephalothin (30 µg), azithromycin (15 µg) and doxycycline (30 µg) by the process of disc diffusion. Isolates of these three species are susceptible to five antibiotics and displaced inhibition zones ranging from 5.8 -19 mm. *S. pyogenes* isolates showed intermediate zones ranging from 5.8 -7.6 mm compared to other Gram-negative species isolates see Table 6.

However, all species included in this study were more susceptible to synthesized compounds **9-15** with a range of 37.5-125 µg/ml higher MICs relative to the related antibiotics, but this low activity could be caused by resistance-acquiring bacteria as a result of patients arbitrary use of antibiotics.

The aim of this analysis was also to observe any relation between the structure of the synthesized compounds **9-15** and the susceptibility of the bacterial isolates to these compounds. Thus, as described earlier, the antibacterial susceptibility of compounds **9-15** and antibiotics given as MIC values in µg/ml is used to achieve this goal. For compounds **9-15**, the figures 4, 5**,** and 6, clearly indicated that *E. coli* is more susceptible to compounds **9**, **10**, and **12**, as *P. mirabilis* which is more susceptible to compounds **9** and **11** so does *S. pyogenes* see figures 7, 8, and 9. The presence of *para* -chlorophenyl moiety at 2-position 2 of these compounds appears to be due to this effect.. Presence of chlorine atom presumably makes such compounds more active than other compounds against isolates of the three bacterial species. In case of antibiotics, *E. coli* tends to be more susceptible to these five antibiotics, and there

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were few variations among isolates towards antibiotics as it can be seen see figures 7, 8, 9.

Table 4. Zone diameter and minimum inhibitory concentration (MIC) for *E. coli* towards synthesized compounds **9-15** and referenced antibiotics

<table>
<tr><td colspan="9">Bacterial species – E. coli</td></tr>
<tr><td colspan="3">Synthesized compds.</td><td colspan="3">Synthesized compds.</td><td colspan="3">Synthesized compds.</td></tr>
<tr><td>Comp dno.</td><td>Conc. (µg /ml)</td><td>Zone Diameter Mean values ± SD (mm)</td><td>Comp dno</td><td>Conc. (µg /ml)</td><td>Zone Diameter Mean values ± SD (mm)</td><td>Comp dno.</td><td>Conc. (µg /ml)</td><td>Zone Diameter Mean values ± SD (mm)</td></tr>
<tr><td rowspan="7">9</td><td>6.25</td><td>0</td><td rowspan="7">12</td><td>6.25</td><td>0</td><td rowspan="7">15</td><td>6.25</td><td>0</td></tr>
<tr><td>12.5</td><td>*3.4 ± 1.8</td><td>12.5</td><td>*8.1 ± 3.5</td><td>12.5</td><td>*6..0 ± 2.6</td></tr>
<tr><td>37.5</td><td>*7.7 ± 3.7</td><td>37.5</td><td>n.s 12,6 ± 4.6</td><td>37.5</td><td>*7.5 ± 3.4</td></tr>
<tr><td>62.5</td><td>*10.0 ± 5.3</td><td>62.5</td><td>n.s 15.6 ± 4.8</td><td>62.5</td><td>n.s 10.5 ± 4.5</td></tr>
<tr><td>87.5</td><td>n.s 13.2 ± 5.6</td><td>87.5</td><td>n.s 19.4 ± 5.0</td><td>87.5</td><td>n.s 11.6 ± 5.0</td></tr>
<tr><td>112.5</td><td>n.s 14.3 ± 8.5</td><td>112.5</td><td>*21.5 ± 5.4</td><td>112.5</td><td>n.s 13.0 ± 6.0</td></tr>
<tr><td>125</td><td>n.s 16.6 ± 7.2</td><td>125</td><td>*24.9 ± 6.0</td><td>125</td><td>n.s 15.5 ± 7.2</td></tr>
<tr><td rowspan="4">10</td><td>6.25</td><td>0</td><td rowspan="4">13</td><td>6.25</td><td>0</td><td colspan="3">Antimicrobial agents</td></tr>
<tr><td>12.5</td><td>7.5 ± 4.5</td><td>12.5</td><td>*5.6 ± 2.6</td><td>Code</td><td>Disc content</td><td>Zone Diameter Mean values ± SD (mm)</td></tr>
<tr><td>37.5</td><td>n.s 12.0 ± 3.2</td><td>37.5</td><td>*8.2 ± 4.1</td><td>amx</td><td>20</td><td>12.3 ± 4.0</td></tr>
<tr><td>62.5</td><td>n.s 16.0 ± 2.5</td><td>62.5</td><td>n.s 10.3 ± 4.3</td><td>azi</td><td>15</td><td>17.0 ± 1.4</td></tr>
</table>

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	87.5	*21.7 ± 2.8		87.5	n.s 10.7 ± 2.5	doxy	30	19.0 ± 5.0
	112.5	*22.2 ± 3.1		112.5	n.s 12.6 ± 6.5	cep	30	13.5 ± 4.4
	125	*26.2 ± 4.0		125	*10.9 ± 5.8	am	25	16.0 ± 3.0
11	6.25	0	**14**	6.5		Previous studies		
	12.5	0		12.5	*2.9 ± 1.8	Code	Disc content	Zone DiameterMean values ± SD (mm)	Ref
	37.5	*5.8 ± 2.6		37.5	*2.9 ± 1.9	amx	20	11-12	[25]
	62.5	*6.5 ± 3.1		62.5	*4.4 ± 2.2	azi	15	15-21	[26]
	87.2	*7.2 ± 3.2		87.5	*4.8 ± 1.9	doxy	30	11-12	[26]
	112.5	*8.5 ± 3.8		112.5	*5.3 ± 2.5	cep	30	±	[26]
	125	*10.2 ± 4.2		125	*8.2 ± 3.9	am	25	11-12	[25]

* means there are significant differences, n.s. means there are no significant differences

Table 5. Zone diameter and minimum inhibitory concentration (MIC) for *P. mirabilis* towards synthesized compounds **9-15** and referenced antibiotics

Bacterial species – *P. mirabilis*								
Synthesized compds.			Synthesized compds.			Synthesized compds.		
Comp dno.	Conc. (µg /ml)	Zone Diameter Mean values ± SD (mm)	Comp dno	Conc. (µg /ml)	Zone Diameter Mean values ± SD (mm)	Comp dno.	Conc. (µg /ml)	Zone Diameter Mean values ± SD (mm)
9	6.25	0		6.25	0		6.25	0

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	12.5	*8.4 ±1.3	**12**	12.5	*8.0 ± 4.3	**15**	12.5	0
	37.5	n.s 14.2 ± 1.4		37.5	*9.0 ± 5.0		37.5	0
	62.5	n.s 17.1 ± 1.1		62.5	n.s 10.2 ± 6.0		62.5	*5.0 ± 4.0
	87.5	*20.9 ± 2.0		87.5	n.s 11.0 ± 2.0		87.5	*6.3 ± 1.4
	112.5	*24.1 ± 2.9		112.5	n.s 12.0 ± 3.0		112.5	*5.0 ± 3.3
	125	*29.3 ± 2.2		125	n.s 13.1 ± 5.2		125	*9.0 ± 6.0

10	6.25	0	**13**	6,25	0	Antimicrobial agents		
	12.5	0		12.5	0	Code	Disc content	Zone Diameter Mean values ± SD (mm)
	37.5	*7.0 ± 2.0		37.5	0	amx	20	12.8 ± 2.9
	62.5	*9.0 ± 2.5		62.5	n.s 12.0 ± 7.3	azi	15	11.3 ± 3.9
	87.5	n.s 10.0 ± 6.3		87.5	n.s 10.0 ± 7.1	doxy	30	13.0 ± 3.2
	112.5	n.s 11.0 ± 2.0		112.5	*6.0 ± 7.0	cep	30	11.0 ± 3.0
	125	n.s 12.2 ± 4.4		125	n.s 15.0 ± 13.0	am	25	7.5 ± 2.0

11	6.25	0	**14**	6,5	0	Previous studies			
	12.5	*7.4 ± 2.0		12,5	0	Code	Disc content	Zone Diameter Mean values ± SD (mm)	Ref
	37.5	n.s 12.9 ± 3.0		37.5	*6.0 ± 2.3	amx	20	11-12	[25]
	62.5	n.s 19.4 ± 7.2		62.5	*8.2 ± 4.4	azi	15	16-21	[26]
	87.2	*21.0 ± 7.0		87.5	*9.0 ± 2.0	doxy	30	18-24	[26]

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	112.5	*24.0 ± 8.2		112.5	*9.0 ± 7.0	cep	30	17-21	[25]
	125	*27.0 ± 8.1		125	n.s 16.0 ± 2.0	am	25	11-12	[25]

Table 6. Zone diameter and minimum inhibitory concentration (MIC) for *S. pyogenes* towards synthesized compounds **9-15** and referenced antibiotics

Bacterial species – *S. pyogenes*								
Synthesized compds.			Synthesized compds.			Synthesized compds.		
Comp dno.	Conc. (µg /ml)	Zone Diameter Mean values ± SD (mm)	Comp dno	Conc. (µg /ml)	Zone Diameter Mean values ± SD (mm)	Comp dno.	Conc. (µg /ml)	Zone Diameter Mean values ± SD (mm)
9	6.25	0	**12**	6.25	0	**15**	6.25	0
	12.5	0		12.5	*1.0 ± 1.0		12.5	0
	37.5	n.s 15.0 ± 5.0		37.5	*3.0 ± 3.0		37.5	0
	62.5	n.s 19.0 ± 5.0		62.5	*5.0 ± 3.2		62.5	*8.0 ± 4.3
	87.5	*23. 0 ± 4.2		87.5	*6.0 ± 2.0		87.5	*9.0 ± 2.4
	112.5	*30.0 ± 3.9		112.5	*7.0 ± 2.0		112.5	n.s 13.0 ± 4.0
	125	31.0 ± 4.0		125	*8.0 ± 2.0		125	n.s 15.0 ± 2.0
10	6.25	0	**13**	6,25	0	Antimicrobial agents		
	12.5	0		12.5	0	Code	Disc content	Zone Diameter Mean values ± SD (mm)
	37.5	*4.0 ± 5.0		37.5	0	amx	20	5.8 ± 6.3
	62.5	*4.0 ± 3.0		62.5	*3.0 ± 4.0	azi	15	6.5 ± 8.4
	87.5	*6.2 ± 2.8		87.5	*4.0 ± 6.0	doxy	30	7.3 ± 4.2
	112.5	*5.1 ± 3.4		112.5	*5.0 ± 8.4	cep	30	7.6 ± 4.3
	125	*7.0 ± 5.0		125	*9.2 ± 6.0	am	25	6.4 ± 5.5

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11	6.25	0	**14**	6,5	0	Previous studies			
	12.5	0		12,5	0	Code	Disc content	Zone Diameter Mean values ± SD (mm)	Ref
	37.5	n.s 13.0 ± 2.4		37.5	0	amx	20	30.93 ± 2.9	[32]
	62.5	n.s 15.0 ± 2.2		62.5	*4.0 ± 2.2	azit	15	14-17	[26]
	87.2	n.s 19.0 ± 2.1		87.5	*5.0 ± 2.0	doxy	30	13-15	[26]
	112.5	*22.0 ± 3.0		112.5	*7.0 ± 2.0	cep	30	±	N
	125	*23.0 ± 3.3		125	*8.0 ± 3.0	am	10	15-17	[26]

N: in vitro susceptibility of this antibiotic *S. pyogenes* is included in the CSLI 2017 guideline

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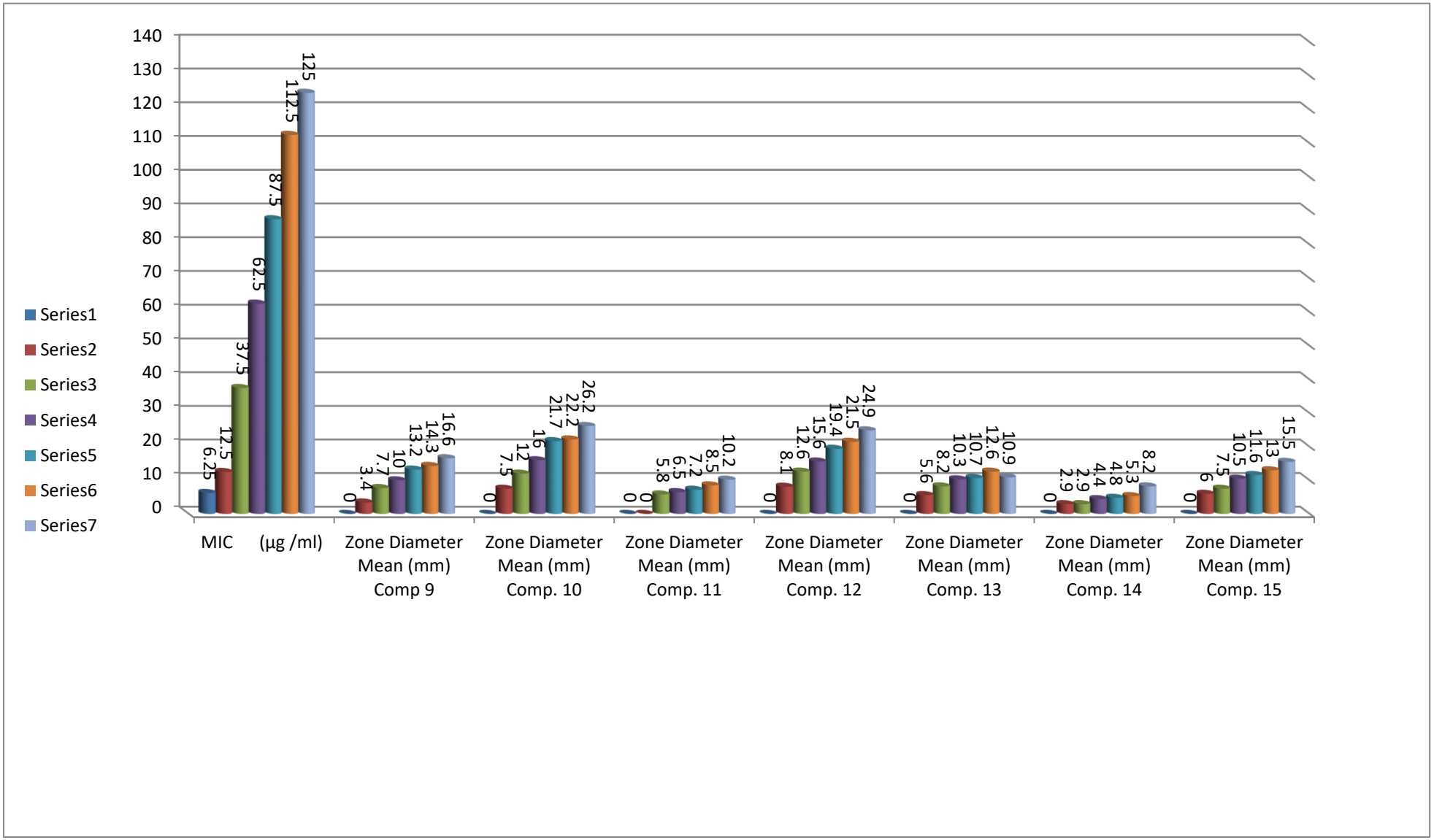

Figure 4. Antibacterial susceptibility of species *E . coli* given as MICs values in µg/ml for compounds **9-15**

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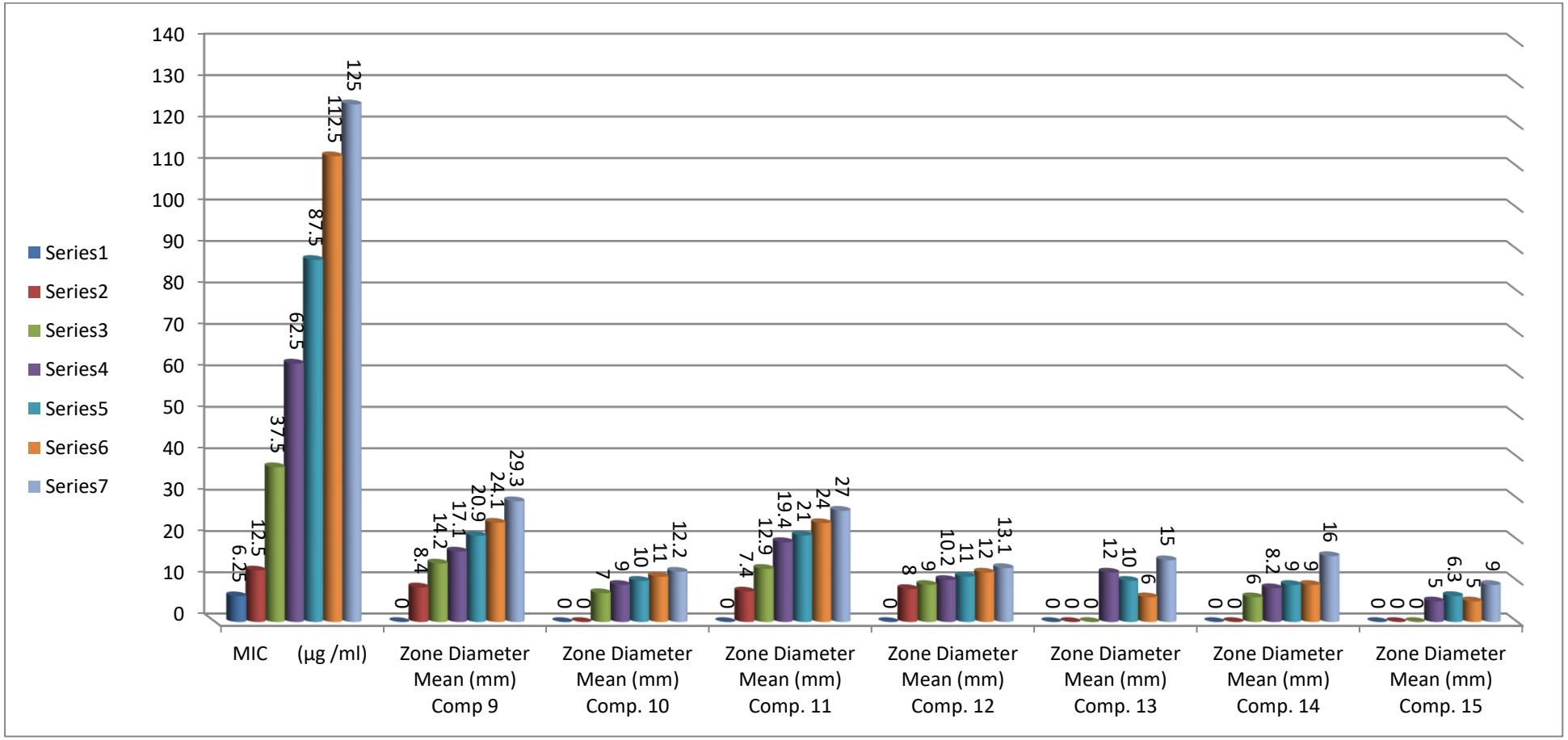

Figure 5. Antibacterial susceptibility of species *P. mirabilis* given as MICs values in µg/ml for compounds **9-15**

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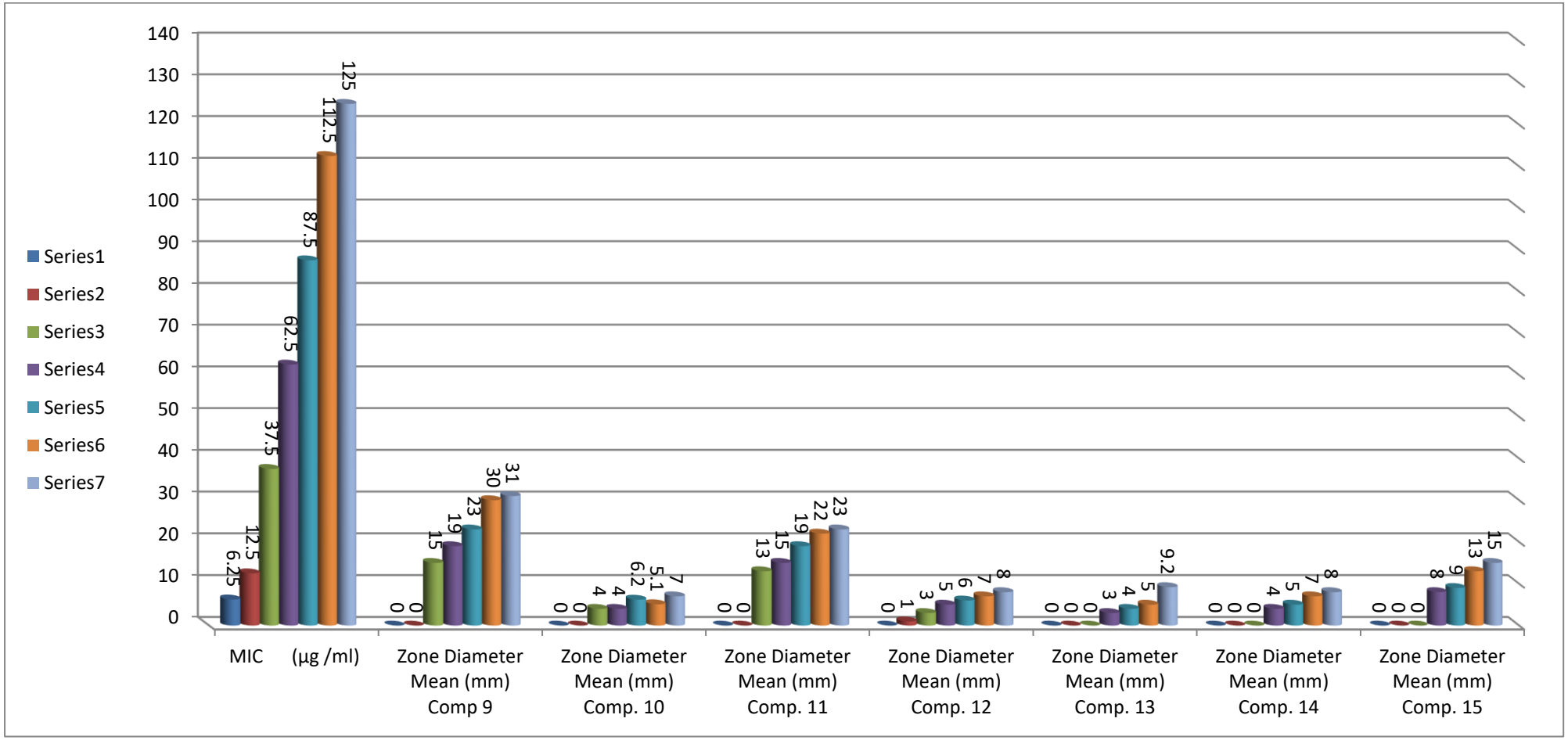

Figure 6. Antibacterial susceptibility of species *S. pyogenes* given as MICs values in µg/ml for compounds **9-15**

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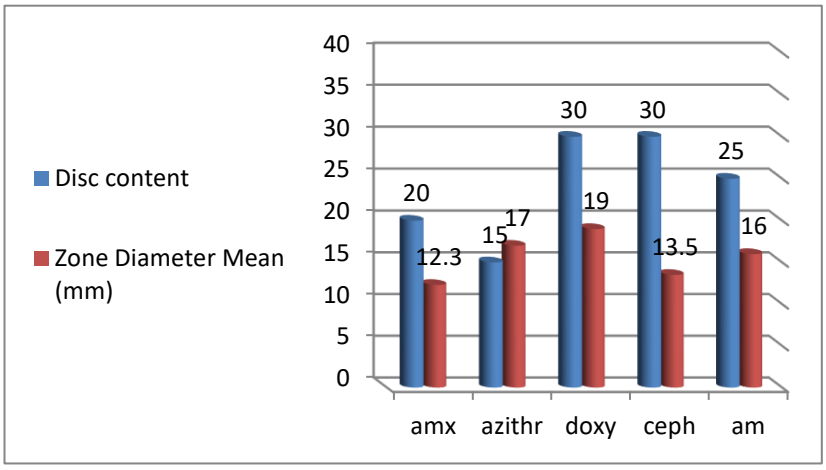

Figure 7. Antibacterial susceptibility of species *E. coli* given as MICs values in µg/ml for referenced antibiotics

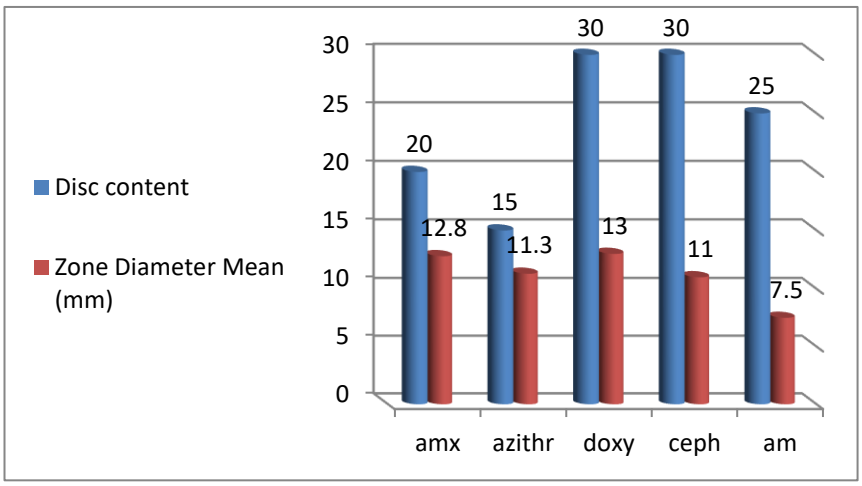

Figure 8. Antibacterial susceptibility of species *P. mirabilis* given as MICs values in µg/ml for referenced antibiotics

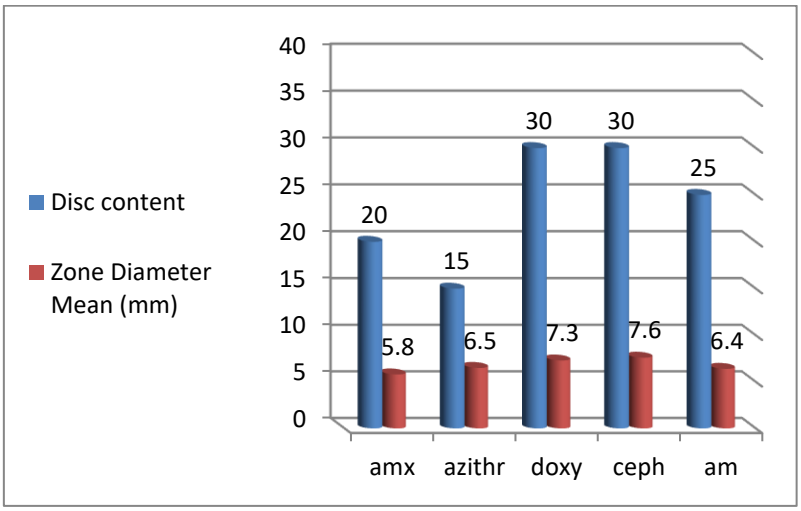

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Figure 9. Antibacterial susceptibility of species *S. pyogenes* given as MICs values in µg/ml for referenced antibiotics

3. Materials and Methods

3.1. Chemistry

On Buchi melting point apparatus, melting points were measured using an open capillary loop, and uncorrected. All the required chemicals used were purchased from Aldrich.. Thin layer chromatography (TLC) was carried out on already made 5 × 5 completes coated with silica 0.25 cm N-HR/UV_{254} obtained from Merck. IR spectra over a frequency spectrum of 4000–400 cm^{-1} were recorded on the Shimatzu FT-IR 8400S spectrophotometer. ^{1}H-NMR spectra were recorded on BRUKER spectrometer (300 MHz) by different solvents with TMS as internal reference Chemical shifts are expressed as internal standard at δ scale in ppm relative to TMS. The elemental analysis (CHN) was administered on the elemental (Eur.Vector EA 3000A), Germany. Microwave experiments were conducted using Microwave Synthesis WorkStation (MAS-II), Microwave chemistry technology.

General procedure for the preparation of derivatives **3-8**; Following a previous report [21], equivalent quantity of 2-chloroacetylchloride (2 g, 0.0176 mol) was added to the stirred and cooled solution of secondary amines (0.0176 mol), pyrrolidine, morpholine, piperidine, 2,4-dimethyl aminopyridine, 2-amino pyridine, 3-aminopyridine and 4-aminopyridine in dry dichloromethane (12 ml), containing equivalent quantities of triethylamine (0.0178 mol) as a base. At room temperature, the reactions were then stirred for 1-2 h. Reaction progress was tracked via TLC. Once the reaction had been completed, aqueous sodium carbonate was added with shaking until the medium became neutral. The organic layers were washed with water separately and dried over anhydrous magnesium sulfate. The solvents extracted in vacuum and the residual materials treated with ether to give crystalline products of 2-chloro (pyrrolidinyl, morpholinyl, piperazinyl, pyperidinyl, pyridin-2-yl, pyridin-3-yl, pyridin-4-yl, 4,6-dimethyl pyridine-2-yl) acetamides **3-8**, and recrystallization from different solvents gave pure derivatives of **3-8**.

*2-chloro-1-(pyrrolidin-1-yl)ethan-1-one **3**.*

Grey crystals, m.p., 143-145 ºC ; (Yield: 2.8 g, 78%); υ_{max} [KBr]: 2986 cm^{-1} (C-H aliphatic) ; 1635 cm^{-1} (C=O amide) ; 1452 cm^{-1} (C-N) ; 707 cm^{-1} (C-Cl).

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2-chloro-1-(piperidin-1-yl)ethan-1-one ***4***.

White crystals, m.p., 202-203 ºC ; (Yield: 2.5g, 85%); υ_{max} [KBr]: 2945 cm^{-1} (C-H aliphatic) ; 1708 cm^{-1} (C=O amide) ; 1506 cm^{-1} (C-N) ; 746 cm^{-1} (C-Cl).

2-chloro-1-morpholinoethan-1-one ***5***.

Brown crystals, m.p., 198-200 ºC ; (Yield: 2.7 g, 60%); υ_{max} [KBr]: 2975 cm^{-1} (C-H aliphatic) ; 1650 cm^{-1} (C=O amide) ; 1437 cm^{-1} (C-N) ; 732 cm^{-1} (C-Cl).

2-chloro-N-(pyridin-3-yl)acetamide ***6***.

Brown crystals, m.p., 196-198 ºC ; (Yield:4.1g, 83%); υ_{max} [KBr]: 3500 cm^{-1} (N-H amide) ; 3040 cm^{-1} (C-H aromatic) ; 2981 cm^{-1} (C-H aliphatic) ;1654 cm^{-1} (C=O amide) ; 1575 cm^{-1} (C=C) ; 1477 cm^{-1} (C-N) ; 740 cm^{-1} (C-Cl).

2-chloro-N-(pyridin-4-yl)acetamide ***7***.

White crystals, m.p., 251-253 ºC ; (Yield:3.9 g, 80%); υ_{max} [KBr]: 3346 cm^{-1} (N-H amide) ; 3051 cm^{-1} (C-H aromatic) ; 2941 cm^{-1} (C-H aliphatic) ;1677 cm^{-1} (C=O amide) ; 1550 cm^{-1} (C=C) ; 1460 cm^{-1} (C-N) ; 578 cm^{-1} (C-Cl).

2-chloro-N-(4,6-dimethylpyridin-2-yl)acetamide ***8***.

Brown crystals, m.p., 267-278 ºC ; (Yield: 2.6 g, 90%); υ_{max} [KBr]: 3307 cm^{-1} (N-H amide) ; 3074cm^{-1} (C-H aromatic) ; 2947 cm^{-1} (C-H aliphatic) ;1656 cm^{-1} (C=O amide) ; 1575 cm^{-1} (C=C) ; 1545 cm^{-1} (C-N) ; 743cm^{-1} (C-Cl)).

General procedure for the preparation of derivatives **9-15**

a- Conventional method

The compounds **9-15** synthesized as previously reported [21] by combining equivalent amounts of the acetamide **3** (0.2 g, 0.0013 mol) with substituted *para*-chloro aniline (0.17 g, 0.0013 mol) in dry acetonitrile (7 ml) containing equivalent amounts of triethylamine as a catalyst. The mixture is heated for 2-3 h at 70 ºC, and TLC tracked the progress of reactions until starting materials disappeared. Upon completion, the reaction mixture was cooled to room temperature, the solvent was drained by vacuum and the residue was taken in CH_2Cl_2 (10 ml), treated with aqueous K_2CO_3 until neutralization, the organic layer washed with water, isolated and dried

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over anhydrous magnesium sulphate. In vacuo, the solvent was extracted and the gummy materials treated with ether, resulting in crystalline derivative of **9** see table 2. 10-15 were synthesized similarly by using equivalent amounts of starting materials

2-((4-Chlorophenyl)amino)-1-(pyrrolidin-1-yl)ethan-1-one ***9***.

Grey crystals, m.p., 183-185 ^{o}C ; (Yield: 0.17 g, 50%); calculated. C, 60.31; H, 6.28; N, 11.76. $C_{12}H_{15}N_2OCl$ Founded. C, 60.01; H, 6.02; N, 11.45; υ_{max} [KBr]: 3132 cm^{-1} (N-H) ; 3078 cm^{-1} (C-H aromatic) ; 2989 cm^{-1} (C-H aliphatic) ; 1679cm^{-1} (C=O amide) ; 1583 cm^{-1} (C=C) ; 1541 cm^{-1} (C-N) ; 690 cm^{-1} (C-Cl). ^{1}H-NMR (DMSO-d_6): δ 7.20-6.75 (4H, dd, $J_{2,3}$ 5.25, $J_{2,6}$ 1.30 Hz, aromatic); 6.32 (1H, s, N-H); 3.36 and 1.60 (8H, 2m, pyrrolidinyl protons); and ppm 3.30 (2H,s, -CH_2CO-)

1-(Pyrrolidin-1-yl)-2-tosylethan-1-one ***10***.

Yellow crystals, m.p., 188-189 ^{o}C ; (Yield: 0.29g, 85%); calculated. C, 58.42; H, 6.23; N, 5.24. $C_{13}H_{17}NO_3S$ Founded. C, 58.10; H, 6.02; N, 5.11; υ_{max} [KBr]: 3068 cm^{-1} (C-H aromatic) ; 2931 cm^{-1} (C-H aliphatic) ; 1676 cm^{-1} (C=O amide) ; 1562 cm^{-1} (C=C) ; 1515 cm^{-1} (C-N) ; 1321 cm^{-1} ; 1153 cm^{-1} ; 611 cm^{-1} (S=O)) ; ^{1}H-NMR (DMSO-d_6): δ 7.75-7.42 (4H, dd, $J_{2,3}$ 5.35, $J_{2,6}$ 1.30 Hz, aromatic, tolyl protons); 3.20 (2H,s, -CH_2CO-); 2.90 and 1.45 ppm (8H, 2m, pyrrolidinyl protons).

1-(Piperidin-1-yl)-2-tosylethan-1-one ***11***.

White crystals, m.p., 204-207 ^{o}C ; (Yield: 0.27g, 78%); calculated. C, 61.17; H, 6.73; N, 11.08. $C_{13}H_{17}N_2OCl$ Founded. C, 60.85; H, 6.90; N, 10.30; υ_{max} [KBr]: 3298 cm^{-1} (N-H) ; 3031 cm^{-1} (C-H aromatic) ; 2977 cm^{-1} (C-H aliphatic) ; 1647 cm^{-1} (C=O amide) ; 1515 cm^{-1} (C=C) ; 1460 cm^{-1} (C-N) ; 1357 cm^{-1} ; 1174 cm^{-1} ; 580 cm^{-1} (S=O) ; ^{1}H-NMR (DMSO-d_6): δ 7.20-6.70 (4H, dd, $J_{2,3}$ 5.30, $J_{2,6}$ 1.45 Hz, aromatic); 6.35 (1H, s, N-H); 3.20 (2H, s, -CH_2CO-); 2.90 and 1.45 ppm (10H. 2m, piperidinyl protons)

1-Morpholino-2-tosylethan-1-one ***12***.

White crystals, m.p., 170-172 ^{o}C ; (Yield: 0.31g, 90%); calculated. C, 55.12; H, 6.0; N, 4.94. $C_{13}H_{17}NO_4S$ Founded. C, 54.90; H, 6.10; N, 5.08; υ_{max} [KBr]: 3099 cm^{-1} (C-H aromatic) ; 2974 cm^{-1} (C-H aliphatic) ; 1679 cm^{-1} (C=O amide) ; 1581 cm^{-1} (C=C) ; 1539 cm^{-1} (C-N) ; 1247cm^{-1} ; 1172 cm^{-1} ; 584 cm^{-1} (S=O);) ; ^{1}H-NMR (DMSO-d_6): δ

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7.75-7.42 (4H, dd, $J_{2,3}$ 5.25 Hz, $J_{2,6}$ 1.4 Hz, tolyl protons), 4.15 (2H, 2, -CH_2CO-); 3.70 and 2.50 ppm (8H, mm, morpholinyl protons)

N-(Pyridin-3-yl)-2-tosylacetamide ***13***.

White crystals, m.p., 204-207 ^oC ; (Yield: 0.27g, 78%); calculated. C, 57.93; H, 4.82; N, 9.65. $C_{14}H_{14}N_2O_3S$ Founded. C, 58.20; H, 5.02; N, 9.50; υ_{max} [KBr]: 3298 cm^{-1} (N-H) ; 3031 cm^{-1} (C-H aromatic) ; 2977 cm^{-1} (C-H aliphatic) ; 1647 cm^{-1} (C=O amide) ; 1515 cm^{-1} (C=C) ; 1460 cm^{-1} (C-N) ; 1357 cm^{-1} ; 1174 cm^{-1} ; 580 cm^{-1} (S=O).) ; ^{1}H-NMR (DMSO-d_6): δ 10.61 ppm (N-H, s), ; δ 10.65 (1H, s, N-H); 9.0 (1H, s, pridinyl proton); 8.41-7.10 (3H, dd, $J_{4,5}$ 5.31, $J_{5,6}$ 5.25 Hz, $J_{2,6}$ 1.5 Hz, pridinyl proton); 7.70-7.35 (4H, dd, $J_{2,3}$ 5.25 Hz, tolyl protons); 4.20 (2H, s, -CH_2CO-); and 2.35 ppm (3H, s, CH_3).

N-(Pyridin-4-yl)-2-tosylacetamide ***14***.

White crystals, m.p., 251-253 ^oC ; (Yield: 0.26 g, 76%); calculated. C, 57.93; H, 4.82; N, 9.65. $C_{14}H_{14}N_2O_3S$ Founded. C, 57.60; H, 5.0; N, 9.61; υ_{max} [KBr]: 3344 cm^{-1} (N-H) ; 3080 cm^{-1} (C-H aromatic) ; 2977 cm^{-1} (C-H aliphatic) ; 1639 cm^{-1} (C=O amide) ; 1531 cm^{-1} (C=C) ; 1461 cm^{-1} (C-N) ; 1365 cm^{-1} ; 1103 cm^{-1} ; 557 cm^{-1} (S=O); ; ^{1}H-NMR (DMSO-d_6): δ 9.90 (1H, s, N-H); δ 8.41-7.91(4H, dd, $J_{2,3}$ 5.31 Hz, $J_{2,6}$ 1.5 Hz, pridinyl protons); δ 7.65-6.40 (4H, dd, $J_{2,3}$ 5.20 Hz, $J_{2,6}$ 1.5 Hz, tolyl protons); δ 4.25 (2H, s, -CH_2CO-); and 2.32 ppm (3H, s, CH_3).

N-(4,6-Dimethylpyridin-2-yl)-2-tosylacetamide ***15***.

Brown crystals, m.p., 212-213 ^oC ; (Yield: 0.21 g, 62%); calculated. C, 60.37; H, 5.66; N, 8.80. $C_{16}H_{18}N_2O_3S$ Founded. C, 60. 57; H, 5.42; N, 8.91; υ_{max} [KBr]: 3463 cm^{-1} (N-H) ; 3085 cm^{-1} (C-H aromatic) ; 2945 cm^{-1} (C-H aliphatic) ; 1677 cm^{-1} (C=O amide) ; 1596 cm^{-1} (C=C) ; 1544 cm^{-1} (C-N) ; 1336 cm^{-1} ; 1150 cm^{-1} ; 524 cm^{-1} (S=O) ; ^{1}H-NMR (DMSO-d_6): δ 10.40 (1H, s, N-H); 8.30-7.40 (2H, 2s, pridinyl protons); 7.60-7.45 (4H, dd, $J_{2,3}$ 5.25 Hz, $J_{2,6}$ 1.55 Hz, tolyl protons); 4.25 (2H, s, -CH_2CO-); and 2.45-2.35 ppm (9H, s, 3CH_3).

b- Microwave method

The compounds **9-15** synthesized as previously stated [21] by combining equivalent quantities mentioned in above experiments, substituted aniline and *para*-toluene sulfonate sodium salt with acetamides **3-8** in dry acetonitrile (7 ml) containing

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equivalent quantities of triethylamine as a catalyst. The mixture displayed at 65-70 °C with a capacity of 400 watts for 5-10 min under microwave irradiation. The reaction progress monitored with TLC until starting materials had disappeared. After the reaction was completed, the mixture cooled to room temperature, the solvent washed away by vacuum, and the residue was taken in CH_2Cl_2 (10 ml) and treated with aqueous K_2CO_3 until neutralization, then the organic layer washed with water, separated and dried over anhydrous magnesium sulfate. The solvent was removed in vacuum, and the resulting gummy materials of **9-15** were treated with ether to give crystalline **9-15** products. See Table 2.

3.2. Antibiotics

The following antibiotics, amoxicillin (20 mcg), ampicillin (25 mcg), cephalothin (30 mcg), azithromycin (15 mcg) and doxycycline (30 mcg) were obtained from Samarra drug industries, Samarra, Iraq and were used in the disk diffusion method.

3.3. Bacterial species

Isolates of the three different bacterial species, mamely, two Gram-negative bacteria *E. coli* plus *P. mirabilis* and one Gram-positive *S. pyogenes* bacteria were collected from Tikrit teaching hospital, Tikrit, Iraq. All the isolates of *E. coli* and *P. mirabilis* were collected from urine samples of urinary tract infection (UTI) patients, and the isolates of *S. pyogenes* were collected from tonsillitis patients.

3.4. Antibacterial susceptibility Testing

Antibacterial resistance to seven new synthesized compounds **9-15** of the acetamide class and five antibiotics, including amoxicillin, ampicillin, cephalothin, azithromycin and doxycycline, was evaluated using the standard disk diffusion method [33] and the diffusion method (well diffusion) for synthesized compounds **9-15**. Both studied bacterial species were pre-cultivated on 24 h incubated nutrient agar plates and bacterial suspensions of each pure isolate were prepared for in vitro antibacterial treatment in 0.6 McFarland turbidity nutrient broth tubes. Mueller Hinton agar plates (Oxoid Ltd, Hampshire, UK), 12 cm in diameter, were prepared as directed by the manufacturer and incubated at 37 ^{o}C for 24 h. So, a sterile borer was used to make equidistant wells with a diameter of 6 mm. 50 mg of each of the synthetic compounds **9-15** was dissolved in DMSO (1 ml), followed by dilution of 2.5, 5, 15, 25, 35, 45 and

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50 µL of each to 1ml DMSO to obtain concentrations of 0.125, 0.250, 0.750, 1.25, 1.75, 2.25 and 2.5 mg/ml which equivalent to 6.25, 12.5, 37.5, 62.5, 87.5, 112.5 and 125 µg/50 respectively. 50 µL of each of the later concentrations were used to assess antibacterial susceptibility and minimum inhibitory concentrations (MICs) by filling the growth media wells with it accompanied by 24-hour incubation at 37 ^{o}C and growth inhibition monitor in.

3.5. Analysis of results

SPSS software (version 20) was used to evaluate the effects of the antimicrobial susceptibility study. Mean values and standard deviations for the inhibition zone diameters were determined. The findings were presented as average values ± SD. Statistically standard deviation, variations are calculated relative to normal antibiotics and the amount of dispersion between them.

4. Conclusions

2-Chloroacetamides are versatile intermediates in organic synthesis The synthesis method is based primarily on chloroacelation by chloroacetyl chloride of amines. The simple replacement of chlorine atom allowed us to prepare many of the acetamide derivatives through the reaction with amines and *para*-tosyl sodium salt. A series of new acetamide derivatives were successfully synthesized by adding primary and secondary amines to chloroacetyl chloride **1**. With the help of microwave irradiation in hand, we have been able to synthesize seven compounds in an attempt to increase yields and reduce the reaction time. Moderate to good yields and reduction of the reaction time from 2-3 h to few minutes were achieved. The application of the compounds **9-15** against Gram-positive and Gram-negative bacterial species demonstrated encouraging antibacterial potency relatively good in comparison with used reference antibiotics. Isolates of two of the tested species, namely, *E. coli and P. mirabilis* displaced higher susceptibility than *S. pyogenes*. The results show that among synthetic compounds **9-15** compounds **9** and **11** exert the most important antibacterial properties bearing *para*-chlorophenyl moiety in the acetamide 2-position of moiety **2**. We successfully developed a synthetic method has proven to be a fast, environmentally friendly technique with moderate to good performance in microwave irradiation, and high acceleration of reaction rates has been achieved in the presence of Et_3N as a base.

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