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

Abdulmajeed S. H. Alsamarrai 1*, Saba S. Abdulghani 2

1 Department of chemistry, College of Applied Sciences, University of Samarra
13/1333, Salahaldin, Iraq; abdulmajeedsalihamad@yahoo.com

2 Department of chemistry, College of Applied Sciences, University of Samarra
13/1333, Salahaldin, Iraq; sabasabdulghani@gmail.com

* Correspondence: abdulmajeedsalihamad@yahoo.com; Tel.:+9647707805130

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, 1H-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
[7], films [8], surfactants and soldering fluxes [9], organic fibers [10], and colorants [11].

$$\text{-NHCO-CH}_2\text{-}$$

2

**Figure 1.** Structure of acetylamide moiety 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].
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.

**Figure 2.** The most widely used drugs in medicines
Synthesis of broad spectrum compounds with antibacterial activity is required because of the excessive use of antibiotics in medicine and prevailing resistance in microorganisms. 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, from 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.

**Figure 3.** Some heterocycles of biologically active pyridines
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...
chloride 1 to a mixture of amines and para-toluene sulphinate sodium salt in dry CH₂Cl₂ 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 CH₃CN solution containing Et₃N 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 CH₃CN 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 ¹H-NMR details.

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

| Compd. No. | Recryst. Solvent | Color     | M.P. °C | Yield % |
|------------|------------------|-----------|---------|--------|
| 3          | n-Hexane         | Gray      | 143 - 145 | 78     |
| 4          | Ethanol          | White     | 202 - 203 | 85     |
| 5          | Ethanol          | Brown     | 276 - 278 | 60     |
| 6          | Hexane: methanol | Brown     | 196 - 198 | 83     |
| 7          | Ethylacetate:acetone | White | 251 - 253 | 80     |
| 8          | n-Hexane         | Brown     | 198 - 200 | 90     |
Table 2. Melting points, colours, solvents for recrystallization, and yields of compounds 9-15

| Compd. No. | Recryst. Solvent | Color       | M.P. °C     | Yield % of | Yields % of | Convention Method |
|------------|------------------|-------------|-------------|------------|-------------|-------------------|
| 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          |                   |
Table 3. $^1$H-NMR data of compounds 9-15

| Structure | Chemical shift ($\delta$) ppm | Feature of signal | No. of protons | Type of protons |
|-----------|-------------------------------|-------------------|----------------|----------------|
| ![Structure 9](image) | 7.20 - 6.75 | dd, J$_{2,3}$ 5.25 Hz, J$_{2,6}$ 1.30 Hz s | 4H | aromatic |
|          | 6.32                         |                   | 1H | NH |
|          | 3.36 and 1.75                |                   | 8H | pyrrolidinyl proton |
|          | 3.30                         | s                 | 2H | -CH$_2$CO- |
| ![Structure 10](image) | 7.75 - 7.42 | dd, J$_{2,3}$ 5.35, J$_{2,6}$ 1.30 Hz s | 4H | tolyl |
|          | 4.20                         |                   | 2H | -CH$_2$CO- |
|          | 2.85 and 1.90                |                   | 8H | pyrrolidinyl proton |
|          | 2.35                         | s                 | 3H | CH$_3$ |
| ![Structure 11](image) | 7.20 - 6.70 | dd, J$_{2,3}$ 5.0 Hz, J$_{2,6}$ 1.45 Hz s | 4H | aromatic |
|          | 6.35                         |                   | 1H | NH |
|          | 3.20                         |                   | 2H | -CH$_2$CO- |
|          | 2.90 and 1.45                |                   | 10H | piperidinyl proton |
| Chemical Structure | Chemical Shifts | Multiplicity, Coupling Constants, Integration | Number of Hydrogens | Assignments |
|--------------------|-----------------|---------------------------------------------|---------------------|-------------|
| ![Chemical Structure 12](image12.png) | 7.75-7.42 | dd, J\(_{2,3}\) 5.26 Hz, J\(_{2,6}\) 1.4 Hz | 4H | tolyl protons |
| | 4.15 | s | 2H | -CH\(_2\)CO |
| | 3.70 and 2.50 | 2t | 8H | morpholinyl protons |
| | 2.40 | s | 3H | CH\(_3\) |
| ![Chemical Structure 13](image13.png) | 10.65 | s | 1H | |
| | 9.0 | s | 1H | NH |
| | 8.41 - 7.10 | dd, J\(_{4,5}\) 5.31 Hz, J\(_{5,6}\) 5.25 Hz | 3H | pridinyl protons |
| | 7.70 - 7.35 | dd, J\(_{2,3}\) 5.25 Hz, J\(_{2,6}\) 1.5 Hz | 4H | tolyl protons |
| | 4.20 | s | 2H | CH\(_2\) |
| | 2.35 | s | 3H | CH\(_3\) |
| ![Chemical Structure 14](image14.png) | 9.90 | s | 1H | NH |
| | 8.41 - 7.91 | dd, J\(_{2,3}\) 5.31 Hz, J\(_{2,6}\) 1.45 Hz | 4H | pridinyl protons |
| | 7.65 - 6.40 | dd, J\(_{2,3}\) 5.20, J\(_{2,6}\) 1.5 Hz | 4H | tolyl protons |
| | 4.25 | s | 2H | CH\(_2\) |
| | 2.32 | s | 3H | CH\(_3\) |
2.2. In vitro Antibacterial activity testing

Instructive bacterial infections such as tuberculosis, urinary tract infection, pneumonia, brain abscess, pharyngitis, 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...
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
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

| Bacterial species – *E. coli* | Synthesized compds. | Synthesized compds. | Synthesized compds. |
|-------------------------------|---------------------|---------------------|---------------------|
| Comp dno. | Conc. (µ g /ml) | Zone Diameter Mean values ± SD (mm) | Comp dno. | Conc. (µ g /ml) | Zone Diameter Mean values ± SD (mm) | Comp dno. | Conc. (µ g /ml) | Zone Diameter Mean values ± SD (mm) |
| 9 | 6.25 | 0 | 12.5 | *3.4 ± 1.8 | 12.5 | *8.1 ± 3.5 | 6.25 | 0 | 12.5 | *6.0 ± 2.6 |
| 6.25 | 12.5 | *3.4 ± 1.8 | 12.5 | *8.1 ± 3.5 | 6.25 | 0 | 12.5 | *6.0 ± 2.6 |
| 12 | 37.5 | *7.7 ± 3.7 | 37.5 | n.s 12.6 ± 4.6 | 6.25 | 0 | 12.5 | *6.0 ± 2.6 |
| 62.5 | *10.0 ± 5.3 | 62.5 | n.s 15.6 ± 4.8 | 6.25 | 0 | 12.5 | *6.0 ± 2.6 |
| 15 | 87.5 | n.s 13.2 ± 5.6 | 87.5 | n.s 19.4 ± 5.0 | 62.5 | n.s 10.5 ± 4.5 | 62.5 | n.s 10.5 ± 4.5 |
| 112.5 | n.s 14.3 ± 8.5 | 112.5 | *21.5 ± 5.4 | 87.5 | n.s 11.6 ± 5.0 | 112.5 | n.s 13.0 ± 6.0 |
| 125 | n.s 16.6 ± 7.2 | 125 | *24.9 ± 6.0 | 87.5 | n.s 11.6 ± 5.0 | 112.5 | n.s 13.0 ± 6.0 |
| 10 | 6.25 | 0 | 12.5 | 7.5 ± 4.5 | 12.5 | *5.6 ± 2.6 | Cod e | Disc conte nt | Zone Diameter Mean values ± SD (mm) |
| 6.25 | 12.5 | 7.5 ± 4.5 | 12.5 | *5.6 ± 2.6 | 6.25 | 0 | 12.5 | *6.0 ± 2.6 |
| 13 | 37.5 | n.s 12.0 ± 3.2 | 37.5 | *8.2 ± 4.1 | amx | 20 | 12.3 ± 4.0 |
| 62.5 | n.s 16.0 ± 2.5 | 62.5 | n.s 10.3 ± 4.3 | azi | 15 | 17.0 ± 1.4 | 62.5 | n.s 16.0 ± 2.5 | 62.5 | n.s 10.3 ± 4.3 | 62.5 | n.s 10.3 ± 4.3 |
* 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. |
|-----------------------------------|---------------------|---------------------|---------------------|
| Compdno.                          | Conc. (µg/ml)       | Zone Diameter Mean values ± SD (mm) | Compdno. | Conc. (µg/ml) | Zone Diameter Mean values ± SD (mm) | Compdno. | Conc. (µg/ml) | Zone Diameter Mean values ± SD (mm) |
| 9                                 | 6.25                | 0                   | 6.25                | 0                   | 6.25 | 0 |
| Code | Disc content | Zone Diameter Mean values ± SD (mm) | Ref |
|------|--------------|------------------------------------|-----|
| 12   | 12.5 0       | 12.5 0                             |     |
| 13   | 10 6.25 0    | 10 6.25 0                          |     |
| 14   | 11 6.5 0     | 11 6.5 0                           |     |

**Antimicrobial agents**

| Code | Disc content | Zone Diameter Mean values ± SD (mm) | Ref |
|------|--------------|------------------------------------|-----|
| 12   | 12.5 0       | 12.5 0                             |     |
| 13   | 10 6.25 0    | 10 6.25 0                          |     |
| 14   | 11 6.5 0     | 11 6.5 0                           |     |

**Previous studies**

| Code | Disc content | Zone Diameter Mean values ± SD (mm) | Ref |
|------|--------------|------------------------------------|-----|
| 12   | 12.5 0       | 12.5 0                             |     |
| 13   | 10 6.25 0    | 10 6.25 0                          |     |
| 14   | 11 6.5 0     | 11 6.5 0                           |     |

**Antimicrobial agents**

| Code | Disc content | Zone Diameter Mean values ± SD (mm) | Ref |
|------|--------------|------------------------------------|-----|
| 12   | 12.5 0       | 12.5 0                             |     |
| 13   | 10 6.25 0    | 10 6.25 0                          |     |
| 14   | 11 6.5 0     | 11 6.5 0                           |     |

**Previous studies**

| Code | Disc content | Zone Diameter Mean values ± SD (mm) | Ref |
|------|--------------|------------------------------------|-----|
| 12   | 12.5 0       | 12.5 0                             |     |
| 13   | 10 6.25 0    | 10 6.25 0                          |     |
| 14   | 11 6.5 0     | 11 6.5 0                           |     |
| 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) |
|----------|--------------|----------------------------------|----------|--------------|----------------------------------|----------|--------------|----------------------------------|
| 6.25     | 0            |                                  | 6.25     | 0            |                                  | 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 |                    |
| 6.25     | 0            |                                  | 6.25     | 0            |                                  | 6.25     | 0            | Antimicrobial agents |
| 12.5     | 0            |                                  | 12.5     | 0            |                                  | 12.5     | 0            | Cod e              |
| 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    |                    |

**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) |
|                     | 12                 | 15                  |
| 6.25                | 0                  | 6.25                | 0                  |
| 12.5                | 0                  | 12.5                | 0                  |
| 37.5                | n.s 15.0 ± 5.0     | 37.5                | *3.0 ± 3.0         |
| 62.5                | n.s 19.0 ± 5.0     | 62.5                | *5.0 ± 3.2         |
| 87.5                | *23.0 ± 4.2        | 87.5                | *6.0 ± 2.0         |
| 112.5               | *30.0 ± 3.9        | 112.5               | *7.0 ± 2.0         |
| 125                 | *31.0 ± 4.0        | 125                 | *8.0 ± 2.0         |
| 6.25                | 0                  | 6.25                | 0                  |
| 12.5                | 0                  | 12.5                | 0                  |
| 37.5                | *4.0 ± 5.0         | 37.5                | 0                  |
| 62.5                | *4.0 ± 3.0         | 62.5                | *3.0 ± 4.0         |
| 87.5                | *6.2 ± 2.8         | 87.5                | *4.0 ± 6.0         |
| 112.5               | *5.1 ± 3.4         | 112.5               | *5.0 ± 8.4         |
| 125                 | *7.0 ± 5.0         | 125                 | *9.2 ± 6.0         |
| Code | Disc content | Zone Diameter Mean values ± SD (mm) | Ref |
|------|--------------|-------------------------------------|-----|
| 11   |              |                                     |     |
| 6.25 | 0            | 12.5                                |     |
| 12.5 | 0            | 14                                   |     |
| 37.5 | n.s 13.0 ± 2.4 | 37.5                                |     |
| 62.5 | n.s 15.0 ± 2.2 | 62.5                                | [26] |
| 87.2 | n.s 19.0 ± 2.1 | 87.5                                | [26] |
| 112.5| *22.0 ± 3.0   | 112.5                               | N   |
| 125  | *23.0 ± 3.3   | 125                                 | [26] |
| 6.5  | 0            | 12.5                                |     |
| 37.5 | 0            | amx 20                              |     |
| 62.5 | *4.0 ± 2.2   | azit 15                             |     |
| 87.5 | *5.0 ± 2.0   | doxy 30                             |     |
| 112.5| *7.0 ± 2.0   | cep 30                              |     |
| 125  | *8.0 ± 3.0   | am 10                               |     |

N: in vitro susceptibility of this antibiotic *S. pyogenes* is included in the CSLI 2017 guideline.
Figure 4. Antibacterial susceptibility of species *E. coli* given as MICs values in µg/ml for compounds 9-15
Figure 5. Antibacterial susceptibility of species *P. mirabilis* given as MICs values in µg/ml for compounds 9-15
Figure 6. Antibacterial susceptibility of species *S. pyogenes* given as MICs values in µg/ml for compounds 9-15.
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
**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 completeness coated with silica 0.25 cm N-HR/UV254 obtained from Merck. IR spectra over a frequency spectrum of 4000–400 cm⁻¹ were recorded on the Shimatzu FT-IR 8400S spectrophotometer. ¹H-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-chloroacetyl chloride (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 °C ; (Yield: 2.8 g, 78%); νmax [KBr]: 2986 cm⁻¹ (C-H aliphatic) ; 1635 cm⁻¹ (C=O amide) ; 1452 cm⁻¹ (C-N) ; 707 cm⁻¹ (C-Cl).
2-chloro-1-(piperidin-1-yl)ethan-1-one 4.

White crystals, m.p., 202-203 °C; (Yield: 2.5g, 85%); $\nu_{\text{max}}$ [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 °C; (Yield: 2.7 g, 60%); $\nu_{\text{max}}$ [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 °C; (Yield: 4.1g, 83%); $\nu_{\text{max}}$ [KBr]: 3500 cm$^{-1}$ (N-H amide); 3040 cm$^{-1}$ (C-H aromatic); 2981 cm$^{-1}$ (C=O amide); 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 °C; (Yield: 3.9 g, 80%); $\nu_{\text{max}}$ [KBr]: 3346 cm$^{-1}$ (N-H amide); 3051 cm$^{-1}$ (C-H aromatic); 2941 cm$^{-1}$ (C=O amide); 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 °C; (Yield: 2.6 g, 90%); $\nu_{\text{max}}$ [KBr]: 3307 cm$^{-1}$ (N-H amide); 3074 cm$^{-1}$ (C-H aromatic); 2947 cm$^{-1}$ (C=O amide); 1656 cm$^{-1}$ (C=O amide); 1575 cm$^{-1}$ (C=C); 1545 cm$^{-1}$ (C-N); 743 cm$^{-1}$ (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 °C, 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$_2$Cl$_2$ (10 ml), treated with aqueous K$_2$CO$_3$ until neutralization, the organic layer washed with water, isolated and dried.
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 °C ; (Yield: 0.17 g, 50%); calculated. C, 60.31; H, 6.28;
N, 11.76. C12H15N2OCl Founded. C, 60.01; H, 6.02; N, 11.45; \( \nu_{\text{max}} \) [KBr]: 3132 cm\(^{-1}\)
(N-H) ; 3078 cm\(^{-1}\) (C-H aromatic) ; 2989 cm\(^{-1}\) (C-H aliphatic) ; 1679 cm\(^{-1}\) (C=O
amide) ; 1583 cm\(^{-1}\) (C=C) ; 1541 cm\(^{-1}\) (C-N) ; 690 cm\(^{-1}\) (C-Cl). \(^{1}\)H-NMR (DMSO-d\(_6\)): \( \delta \) 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\(_2\)CO-)

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

Yellow crystals, m.p., 188-189 °C ; (Yield: 0.29g, 85%); calculated. C, 58.42; H,
6.23; N, 5.24. C13H17NO3S Founded. C, 58.10; H, 6.02; N, 5.11; \( \nu_{\text{max}} \) [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\(_6\)): \( \delta \) 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\(_2\)CO-); 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 °C ; (Yield: 0.27g, 78%); calculated. C, 61.17; H, 6.73;
N, 11.08. C13H17NO3S Founded. C, 60.85; H, 6.90; N, 10.30; \( \nu_{\text{max}} \) [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\(_6\)): \( \delta \) 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\(_2\)CO-); 2.90 and 1.45 ppm (10H. 2m, piperidinyl
protons)

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

White crystals, m.p., 170-172 °C ; (Yield: 0.31g, 90%); calculated. C, 55.12; H, 6.0;
N, 4.94. C13H17NO3S Founded. C, 54.90; H, 6.10; N, 5.08; \( \nu_{\text{max}} \) [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\(_6\)): \( \delta \)
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\textsubscript{2}CO-); 3.70 and 2.50 ppm (8H, mm, morpholinyl protons)

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

White crystals, m.p., 204-207 °C ; (Yield: 0.27g, 78%); calculated. C, 57.93; H, 4.82; N, 9.65. C\textsubscript{14}H\textsubscript{14}N\textsubscript{2}O\textsubscript{3}S Founded. C, 58.20; H, 5.02; N, 9.50; v\textsubscript{max} [KBr]: 3298 cm\textsuperscript{-1} (N-H) ; 3031 cm\textsuperscript{-1} (C-H aromatic) ; 2977 cm\textsuperscript{-1} (C-H aliphatic) ; 1647 cm\textsuperscript{-1} (C=O amide) ; 1515 cm\textsuperscript{-1} (C=C) ; 1460 cm\textsuperscript{-1} (C-N) ; 1357 cm\textsuperscript{-1} ; 1174 cm\textsuperscript{-1} ; 580 cm\textsuperscript{-1} (S=O). ) ; \textsuperscript{1}H-NMR (DMSO-d\textsubscript{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\textsubscript{2}CO-); and 2.35 ppm (3H, s, CH\textsubscript{3}).

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

White crystals, m.p., 251-253 °C ; (Yield: 0.26 g, 76%); calculated. C, 57.93; H, 4.82; N, 9.65. C\textsubscript{14}H\textsubscript{14}N\textsubscript{2}O\textsubscript{3}S Founded. C, 57.60; H, 5.0; N, 9.61; v\textsubscript{max} [KBr]: 3344 cm\textsuperscript{-1} (N-H) ; 3080 cm\textsuperscript{-1} (C-H aromatic) ; 2977 cm\textsuperscript{-1} (C-H aliphatic) ; 1639 cm\textsuperscript{-1} (C=O amide) ; 1531 cm\textsuperscript{-1} (C=C) ; 1461 cm\textsuperscript{-1} (C-N) ; 1365 cm\textsuperscript{-1} ; 1103 cm\textsuperscript{-1} ; 557 cm\textsuperscript{-1} (S=O); ; \textsuperscript{1}H-NMR (DMSO-d\textsubscript{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\textsubscript{2}CO-); and 2.32 ppm (3H, s, CH\textsubscript{3}).

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

Brown crystals, m.p., 212-213 °C ; (Yield: 0.21 g, 62%); calculated. C, 60.37; H, 5.66; N, 8.80. C\textsubscript{16}H\textsubscript{18}N\textsubscript{2}O\textsubscript{3}S Founded. C, 60. 57; H, 5.42; N, 8.91; v\textsubscript{max} [KBr]: 3463 cm\textsuperscript{-1} (N-H) ; 3085 cm\textsuperscript{-1} (C-H aromatic) ; 2945 cm\textsuperscript{-1} (C-H aliphatic) ; 1677 cm\textsuperscript{-1} (C=O amide) ; 1596 cm\textsuperscript{-1} (C=C) ; 1544 cm\textsuperscript{-1} (C-N) ; 1336 cm\textsuperscript{-1} ; 1150 cm\textsuperscript{-1} ; 524 cm\textsuperscript{-1} (S=O) ; \textsuperscript{1}H-NMR (DMSO-d\textsubscript{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\textsubscript{2}CO-); and 2.45-2.35 ppm (9H, s, 3CH\textsubscript{3}).

*b- Microwave method*

The compounds 9-15 synthesized as previously stated [21] by combining equivalent quantities mentioned in above experiments, substituted aniline and \textit{para}-toluene sulfonate sodium salt with acetamides 3-8 in dry acetonitrile (7 ml) containing
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 CH₂Cl₂ (10 ml) and treated with aqueous K₂CO₃ 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, namely, 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 °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
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 °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 Et3N as a base.
Acknowledgment

The authors thank the Department of Chemistry, College of Applied Science, Samarra University, Iraq, for supporting facilities. The authors also thank Mr. Maroof S. Juma for technical assistance and Dr. Faesal G. Hussein for helpful comments.

References

1. Zogota, R.; Kinena, L.; Withers-Martinez, C.; Blackman, M. J.; Bobrovs, R.; Pantelejevs, T.; Jirgensons, A. Peptidomimetic plasmepsin inhibitors with potent anti-malarial activity and selectivity against cathepsin D. Eur. J. Med. Chem. 2019, 163, 344–352.

2. Li, L.; Zhao, P.; Hu, J.; Liu, J.; Liu, Y.; Wang, Z.; Chen, L. Synthesis, in vitro and in vivo antitumor activity of scopoletin-cinnamic acid hybrids. Eur. J. Med. Chem. 2015, 93, 300–307.

3. Naim, M. J.; Alam, M. J.; Nawaz, F.; Naidu, V. G. M.; Aaghaz, S.; Sahu, M.; and Alam, O. Synthesis, molecular docking and anti-diabetic evaluation of 2,4-thiazolidinedione based amide derivatives. Bioorg. Chem. 2017, 73, 24–36.

4. Tanwar, B.; Kumar, A.; Yogeeswari, P.; Sriram, D.; Chakraborti, A. K. Design, development of new synthetic methodology, and biological evaluation of substituted quinolines as new anti-tubercular leads. Bioorg. Med. Chem. Lett. 2016, 26, 5960–5966.

5. Kouatly, O.; Geronikaki, A.; Kamoutsis, C.; Hadjipavlou-Litina, D.; Eleftheriou, P. Adamantane derivatives of thiazolyl-N-substituted amide, as possible non-steroidal anti-inflammatory agents. Eur. J. Med. Chem. 2009, 44, 1198–1204.

6. Wang, M.; Song, X.; Jiang, J.; Xia, J.; Li, M. Binary amide-containing tung-oil-based Ca/Zn stabilizers: effects on thermal stability and plasticization performance of poly (vinyl chloride) and mechanism of thermal stabilization. Polym. Degrad. Stab. 2017, 143, 106–117.

7. Coleman, E. A. Applied Plastics Engineering Handbook, Plastics Additives. (2nd edition) 489–500, Elsevier. 2017.
8. Uppu, D. S.; Samaddar, S.; Ghosh, C.; Paramanandham, K.; Shome, B. R.; Haldar, J. Amide side chain amphiphilic polymers disrupt surface established bacterial bio-films and protect mice from chronic Acinetobacter baumannii infection. *Biomaterials*. **2016**, 74, 131–143.

9. Kojima, N.; maruko, D. Cleaning flux, cleaning solder paste, and solder joint. 10,259,083 (2019).

10. Ribeiro, R. F.; Pardini, L. C.; Alves, N. P.; Júnior, B.; Rios, C. A. Thermal Stabilization study of polyacrylonitrile fiber obtained by extrusion. *Polimeros*. **2015**, 25, 523–530.

11. Zannikos, F.; Lois, E.; Stournas, S. Desulfurization of petroleum fractions by oxidation and solvent extraction. *Fuel Process Technol*. **1995**, 42, 35–45.

12. Zarganes-Tzitzikas, T.; Neochoritis, C. G.; Dömling, A. Atorvastatin (Lipitor) by MCR. *Med. Chem. Lett*. **2019**, 10, 389–392.

13. Fabbri, L. M.; Calverley, P. M.; Izquierdo-Alonso, J. L.; Bundschuh, D. S.; Brose, M.; Martinez, F. J.; Rabe, K. F. Roflumilast in moderate-to-severe chronic obstructive pulmonary disease treated with longacting bronchodilators: two randomised clinical trials. *Lancet*. **2009**, 374, 695–703.

14. Gupta, K.; Mitra, S.; Kazal, S.; Saroa, R.; Ahuja, V.; Goel, P.; IV paracetamol as an adjunct to patient-controlled epidural analgesia with levobupivacaine and fentanyl in labour: a randomized controlled study. *BJA Br. J. Anaesth*. **2016**, 117, 617–622.

15. Karaman, M.; Budak, H.; Çiftci, M. Amoxicillin and gentamicin antibiotics treatment adversely influence the fertility and morphology through decreasing the Dazl gene expression level and increasing the oxidative stress. *Arch. Physiol. Biochem*. **2019**, 125, 447–455.

16. Kilic, M.; Seyhan, T. O.; Sungur, M. O.; Ekiz, N.; Bastu, E.; Senturk, M. The effects of subfascial wound versus epidural levobupivacaine infusion on postoperative pain following hysterectomy. *MinervaAnestesiol*, **2014**, 80(7), 769-78.

17. Kamiński, K.; Obniska, J.; Wiklik, B.; Atamanyuk, D. Synthesis and
anticonvulsant properties of new acetamide derivatives of phthalimide, and its saturated cyclohexane and norbornene analogs. Eur. J. Med. Chem. 2011, 46, 4634–4641.

18. Kumar, K. N.; Sreeramamurthy, K.; Palle, S.; Mukkanti, K.; Das, P. Dithiocarbamate and DBU-promoted amide bond formation under microwave condition. Tetrahedron Lett. 2010, 51, 899–902.

19. Nakamoto, K.; Tsukada, I.; Tanaka, K.; Matsukura, M.; Haneda, T.; Inoue, S.; Watanabe, N. Synthesis and evaluation of novel antifungal agents-quinoline and pyridine amide derivatives. Bioorg. Med. Chem. Lett. 2010, 20, 4624–4626.

20. Tang, J.; Wang, B.; Wu, T.; Wan, J.; Tu, Z.; Njire, M.; Ding, K. Design, synthesis, and biological evaluation of pyrazolo[1, 5-a] pyridine-3-carboxamides as novel antitubercular agents. Med. Chem. Lett. 2015, 6, 814–818.

21. Alsamarrai, A. S. H.; Abdulla, N. H.; Aldoori, M. K. Synthesis and Characterization of yl ) -N- ( Substituted Phenyl ) Acetamides Derivatives Anticipated to Inhibit HIV-1 Activity. Pharmaceutical and Phytopharmacological Research (eIJPPR). 2018, 8, 7–11.

22. Adharvana, C. M.; Syamasundar, K. "Polymer (PVP) supported ferric chloride: an efficient and recyclable heterogeneous catalyst for high yield synthesis of 1, 5-benzodiazepine derivatives under solvent free conditions and microwave irradiation." Catalysis communications. 2005, 6.1: 67-70.

23. De la Hoz, A.; Diaz-Ortiz, A.; Moreno, A. Microwaves in organic synthesis. thermal and non-thermal microwave effects. Chem. Soc. Reviews. 2005, 34(2), 164-178.

24. Özden S.; Atabey, D.; Yıldız, S.; Göker, H. Synthesis and potent antimicrobial activity of some novel methyl or ethyl 1H-benzimidazole-5-carboxylates derivatives carrying amide or amidine groups. Bioorg. Med. Chem. 2005, 13, 1587–1597.
25. Andrews JM, BSAC Standardized Disc Susceptibility Testing Method (version 4), *J Antimicrob Chemother*, 2005, 56, 60-76.

26. Clinical and Laboratory Standards Institute. M100. *Performance Standards for Antibacterial Testing*, Wayne PA: CLSI; -2017, 27th edition.

27. Trivedi, M. K.; Branton, A.; Trivedi, D.; Nayak, G.; , Charan, S. M.; Jana, S. Antimicrobial Susceptibility of Proteus mirabilis: Impact of Biofield Energy Treatment. *J Microb Biochem Technol*. 2016, 8(1), 25-29.

28. G, E.; Prena, M.; repetto, A.; ramagnoli, M.; Ripa, S.; Varaldo, P. E. Susceptibility of *Streptococcus pyogenes* from throat cultures to macrolide antibiotics and influence of collection criteria. *Clin. Microbiol. Infec.* 1997, 3(1), 58-62.

29. Benouda, A.; Sibile, S.; Ziane, Y.; Elouennass, M.; Dahani, K., Hassani, A. Place of *Streptococcus pyogenes* in the throat infection in Morocco and overview of its susceptibility to antibiotics. *Pathol Biol (Paris)*. 2009, 57(1), 76–80.

30. Nakae, M.; Murai, T.; Kaneko, Y.; Mitsuhashi, S. Drug resistance in *Streptococcus pyogenes* isolated in Japan (1974–1975). *Antimicrob. Agents Chemother*. 1977, 12, 427-428.

31. Silva-Costa, C.; Ramiirez M.; Melo-Crisino, J. Portuguense by a diversification of T and emm types among *streptococcus pyogenes* in Portugal. *Antimicrob agents Chemther*. 2005, surveillance group for the study of respiratory pathogens. Rapid inversion of the prevalence of macrolide resistance phenotype paralleled 49(5), 2109-2111.

32. Camara, M.; Dieng, A.; Boye, C. S. B. Antibiotic susceptibility of *streptococcus pyogenes* isolated from respiratory tract infections in Dakar, Senegal. *Microbiol. Insights*, 2013, 6, 71-75.

33. Hoelzer, K.; Cummings K. J.; Warnock, L. D.; Schukken, Y.H.; Siler, J. D.; Gröhn, Y.T.; Davis, M. A.; Busser, T.E.; Weidman, M. Agar disk diffusion and automated microbroth dilution produce similar antimicrobial susceptibility testing results for Salmonella serotypes Newport, Typhimurium, and 4, 5, 12: i-, but differ in economic cost. *Foodborne pathogens and disease*. 2011, 8(12) : 1281-1288.
