RESEARCH ARTICLE

The Potential Antibacterial Activity of a Novel Amide Derivative Against Gram-Positive and Gram-Negative Bacteria

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ABSTRACT

This study aimed to synthesize new compounds containing the amide group and test their antibacterial activity. The new amides have been synthesized, and ^{1}H, ^{13}C NMR, IR, and LC-MS have been used to identify the chemical structures. The compounds were tested for antibacterial activity against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli,* and *Pseudomonas aeruginosa*, and compound 3 showed higher activity against the tested organisms.

Keywords: Amide, Amoxicillin, Antibacterial, Ciprofloxacin, Gatifloxacin, Norfloxacin.

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INTRODUCTION

Fluoroquinolones are an antibacterial drug class that is used to treat a variety of bacterial illnesses in the clinic. Despite their potency, their vulnerability to central nervous system (CNS) adverse effects restricts their use. Modifications to the N atom of the C7 side chain were shown to improve absorption, activity, and side effects.[1] Fluoroquinolones have several advantages, including:[2] relative bioequivalence when administered orally or intravenously;[3] Food and Drug Administration (FDA) approval and indication for bone and joint infections caused by *Pseudomonas aeruginosa* and several other pathogens; and[4] broad-spectrum antimicrobial activity against *Staphylococcus aureus*, *P. aeruginosa*, and many other bacterial strains.[5] It exhibits bactericidal activity at clinically relevant dosages.[6-8] This class of antibiotics works by blocking the effects of cytoplasmic DNA gyrase or topoisomerase IV, both essential for bacterial DNA replication, and bacterial resistance is usually caused by amino acid substitution within these target enzymes. Reduced expression of essential outer membrane porins reduces the permeability of the outer membrane, which is a secondary resistance mechanism in Gram-negative bacteria. Protein F in the outer membrane is necessary for the passage of fluoroquinolones into the cell, and lowering protein F levels reduces permeability, resulting in lower fluoroquinolone intracellular concentrations.[9] Amides are found in pharmaceuticals, natural products, and biologically active molecules; amide bond formation processes are among the most significant transformations in organic chemistry and biochemistry. The amide group can be found in a wide range of medicines, intermediates, and natural compounds. It can also be found in a variety of industrial compounds, including polymers, detergents, and lubricants.[10] Carboxylic acid amides are important chemical molecules with numerous uses. It has been established that they have a wide spectrum of biological activities.[11,12] Anti-inflammatory, anticonvulsant, analgesic, antituberculosis, insecticidal, antifungal, and anticancer effects have all been linked to amide derivatives.[13,14]

Instrument

The NMR (^{1}H and ^{13}C) analyses were carried out using 400 MHz (Iran). The FTIR and LC-MS analysis were recorded at Al-Zahrawi University College (IRAQ).

Statistical Analysis

To express the results, we utilized mean standard error of the mean (SEM) and one-way analysis of variance (ANOVA) with Tukey's post hoc. We also tested for differences in activity between different compounds using $p = 0.05$. The analysis was carried out with the latest version of SPSS software, and the graphs were created with GraphPad Prism software v8.0.2.

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Preparation of Compounds 1-4

DCC and DMAP were added to a solution of acid substance (amoxicillin, ciprofloxacin, norfloxacin, and gatifloxacin) (0.0038 mM) dissolved in DMF solvent (50 mL). For 30 minutes, the mixture was stirred with a magnetic stirrer. Benzidine (0.0019 mM) was dissolved in DMF solvent and introduced drop by drop to the acid mixture in a round-bottomed flask. The resulting mixture was stirred for 12 hours with a magnetic stirrer. Compounds 1-4 were obtained after purification using ethanol recrystallization.[15]

Antimicrobial Activity

The disc diffusion assay was conducted for antibacterial test of the synthesized compounds 1-4. Compounds 1-4 were dissolved in dimethylsulfoxide (DMSO) with a range of concentrations from 15 to 35mg/mL. Filter papers with 1.6 mm were saturated with the solution of sample and negative control DMSO and placed on the surface of agar. The plates were incubated for 48 hours at 37°C. The diameter of zone inhibitions was measured in millimeters (mm) to evaluate the effect of new compounds against the microbial used.[16]

RESULTS AND DISCUSSION

Light yellow solid compound (**1**) was synthesized. At m/z 879.2926 $[M+H]^+$, a molecular ion peak conforms to the $C_{44}H_{46}N_8O_8S_2$ molecular formula. The In IR spectrum has a strong absorption at 1680 and 1690 cm^{-1}, referring to the carbonyl groups. In addition, the absorption peak at 3340 and 3260 cm-1 indicates the presence of NH_2 group. A strong absorption band corresponding to a hydroxyl group (O-H) at 3520 cm^{-1}. The ^{1}H–NMR (DMSO-d6, 400 MHz) spectrum of compound 1 appeared to be aromatic protons at 6.92-8.21 ppm (16 Ar-H, m). Other signals observed as singlets at δ 8.96 and 5.82 were identified to NH and NH_2, respectively. A singlet of CH_3 groups appeared at δ 1.28.

Yellow compound (**2**) was synthesized as a solid. At m/z 811.3511 $[M+H]^+$, the HRESIMS spectrum of compound 2 exhibited a molecular ion peak that conforms to the molecular formula of $C_{46}H_{44}F_2N_8O_4$. The FTIR spectrum of compound 2 exhibited absorption peak at 3210 cm^{-1} was revealed to the NH group. Strong absorption at 1628 and 1710 cm-1 refers to the carbonyl groups. ^{1}H–NMR (DMSO-*d6*, 400 MHz) spectrum of compound 2 displayed multiple signals at δ 1.17 to 4.33, which indicated to aliphatic protons. On the other hand, singlet signals appeared at δ 10.94, corresponding to the protons that attach to nitrogen atoms. The aromatic protons appeared at δ 6.68 to 9.02 as multiplate peaks.

Compound (**3**) was synthesized as a yellow solid. HRESIMS of compound 3 showed a significant molecular ion peak at m/z 787.3499 [M+H]+, which corresponds to the molecular formula of $C_{44}H_{44}F_2N_8O_4$. The FTIR spectrum of compound 3 showed an absorption band at 3220 cm-1 was revealed to the NH group. A strong absorption peak at 1628 and 1710 cm^{-1} was referred to as the carbonyl groups. The ^{1}H-NMR (DMSO-*d6*, 400 MHz) spectrum of compound 3 displayed a singlet peak at δ 11.07 which refers to NH of amid groups. The signals of aliphatic hydrogen appeared at δ 1.31-4.18, and other signals appeared as multiple signals at δ 6.72 to 9.21 allocated for aromatic protons.

Compound (**4**) was synthesized as a yellow solid. HRESIMS of compound 4 exhibited a molecular ion peak at m/z 871.3712 $[M+H]^+$, showing a possibility of the molecular formula $C_{21}H_{18}N_2O_2$, which was similar to the molecular weight of compound 4. Strong absorption peaks in fourier transform infrared spectroscopy (FTIR) spectrum of compound 4 at 1628 and 1710 cm-1, were referred to the carbonyl groups. However, the appearance of absorption peak at 3290 cm^{-1} was revealed to the NH group.

Preliminary examination to determine the most effective chemical, we evaluated new synthetic compounds and standers on different cell cultures bacteria (*B. subtilis, S. aureus, P. aeruginosa, and E. coli*). The antibacterial activity of amoxicillin, ciprofloxacin, norfloxacin, and gatifloxacin was tested using established reference drugs. The antibacterial activity of novel synthesized compounds was compared to that of reference compounds using the inhibitory zone (IZ) and minimum inhibitory concentration (MIC) methods.

Inhibitory Zone

The new compounds were more selective against all types of bacteria (Table 1 and Figure 5). Regarding the B. subtilis bacteria, compound-4 exhibited the best activity as compared with another new compound, followed by compound-2, compound-1, and the lower activity was Compound-3. There is an insignificant difference ($p > 0.05$) among the new compound except between Compound-3 and Compound-4 was there is a significant difference ($p < 0.05$) as we found. There is a significant difference ($p < 0.05$) between all-new synthesis compounds and all standers, with the exception of compound-3 and norfloxacin was an insignificant difference ($p > 0.05$) between them. See Table 1, Figures 1 to 5.

On the other hand, compound-3 exhibited the best activity against S. aureus. However, it exhibits low activity against both *P. aeruginosa and E. coli*. There is a significant difference ($p < 0.05$) between compound-1 and both compound-3 and

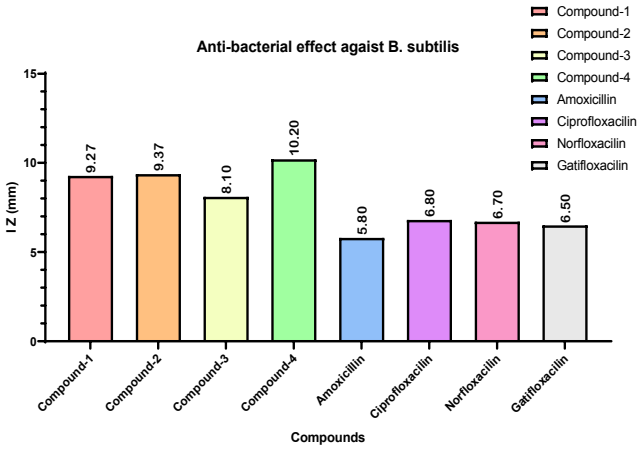

Figure 1: New synthesis and stander compounds' antibacterial activity vs. B. subtilis bacteria.

Table 1: The IZ values (mm) for new and reference compounds. The data express by ONE-ANOVA analysis.

	B. subtilis	*S. aureus*	*P. aeruginosa*	*E. coli*
Compounds	*Mean ± SEM*	*Mean ± SEM*	*Mean ± SEM*	*Mean ± SEM*
Compound-1	9.27 ± 0.29	8.20 ± 0.29	8.47 ± 0.32	10.37 ± 0.29
Compound-2	9.37 ± 0.45	9.30 ± 0.32	10.07 ± 0.20	11.10 ± 0.21
Compound-3	8.10 ± 0.40	10.63 ± 0.27	8.70 ± 0.12	9.70 ± 0.12
Compound-4	10.20 ± 0.32	9.37 ± 0.20	10.77 ± 0.20	10.17 ± 0.15
Amoxicillin	5.80 ± 0.31	6.40 ± 0.21	5.77 ± 0.18	6.23 ± 0.12
Ciprofloxacin	5.80 ± 0.20	6.60 ± 0.21	6.80 ± 0.06	6.10 ± 0.06
Norfloxacin	6.70 ± 0.29	6.50 ± 0.15	6.57 ± 0.09	6.20 ± 0.12
Gatifloxacin	6.50 ± 0.21	6.43 ± 0.13	6.57 ± 0.15	6.23 ± 0.12

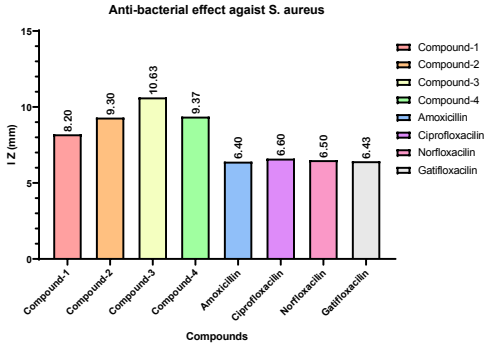

Figure 2: the antibacterial activity of new synthesis compounds and stander compounds vs. S. aureus bacteria.

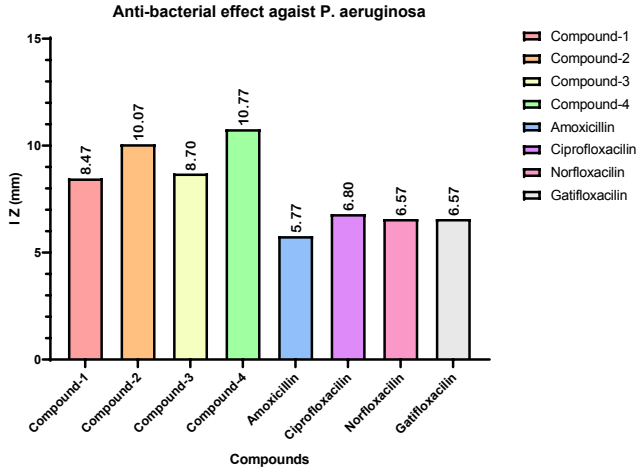

Figure 3: the antibacterial activity of new synthesis compounds and stander compounds vs. P. aeruginosa bacteria.

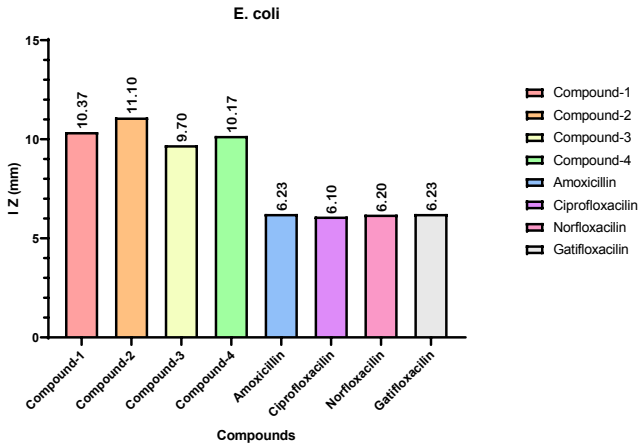

Figure 4: New synthesis and stander compounds' antibacterial activity vs. *E. Coli* bacteria.

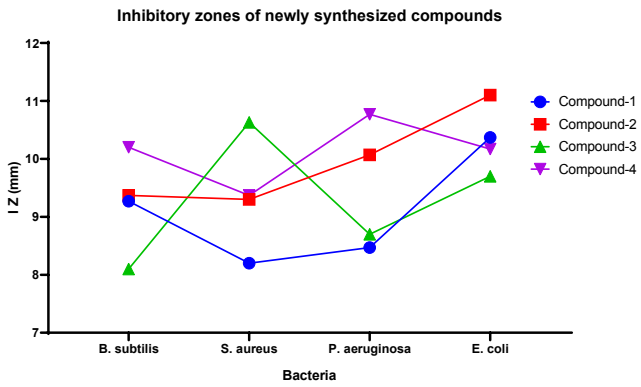

Figure 5: the antibacterial activity of all new synthesis compounds against four types of bacteria.

compound-4, there is a significant difference ($p < 0.05$) between compound-2 and compound-3, in addition to a significant difference ($p < 0.05$) between all-new synthesis compounds and all standers' compounds. There is an insignificant difference ($p > 0.05$) between compound-1 and compound-2 and also between compound-2 and compound-4. See Table-1, Figures 2 and 5.

The antibacterial activity of new compounds against *P. aeruginosa* showed that compound-4 has higher activity as compared with other new compounds. There is an insignificant difference ($p > 0.05$) between compound-1 vs. compound-3 and compound-2 vs. compound-4. There is an insignificant difference ($p < 0.05$) between all-new synthesis compounds and stander compounds. See Table-1, Figure 3, and 5. Regarding *E. coli*, compound-2 exhibits a higher activity. There is an insignificant difference ($p > 0.05$) between compound-1 vs. compound-2, compound-3, and compound-4,

Table 2: The MIC of the newly synthesized compound and standers. The data express by ONE-ANOVA analysis.

	B. subtilis	*S. aureus*	*P. aeruginosa*	*E. coli*
Compound	*Mean ± SEM*	*Mean ± SEM*	*Mean ± SEM*	*Mean ± SEM*
Compound-1	152.33 ± 8.45	196.67 ± 14.53	99.00 ± 3.21	151.67 ± 4.41
Compound-2	99.00 ± 3.21	200.00 ± 11.55	154.33 ± 3.38	154.00 ± 5.57
Compound-3	149.67 ± 3.76	200.00 ± 11.55	100.67 ± 1.20	152.33 ± 1.86
Compound-4	154.33 ± 2.60	154.00 ± 2.31	201.67 ± 2.19	201.00 ± 4.36
Amoxicillin	170.30 ± 2.31	265.40 ± 2.52	258.33 ± 4.41	258.33 ± 4.41
Ciprofloxacin	175.60 ± 0.88	259.40 ± 2.03	256.67 ± 5.24	246.67 ± 5.24
Norfloxacin	173.40 ± 1.73	253.10 ± 1.20	257.33 ± 4.33	237.33 ± 4.33
Gatifloxacin	177.20 ± 1.45	255.33 ± 0.33	259.67 ± 0.88	239.67 ± 0.88

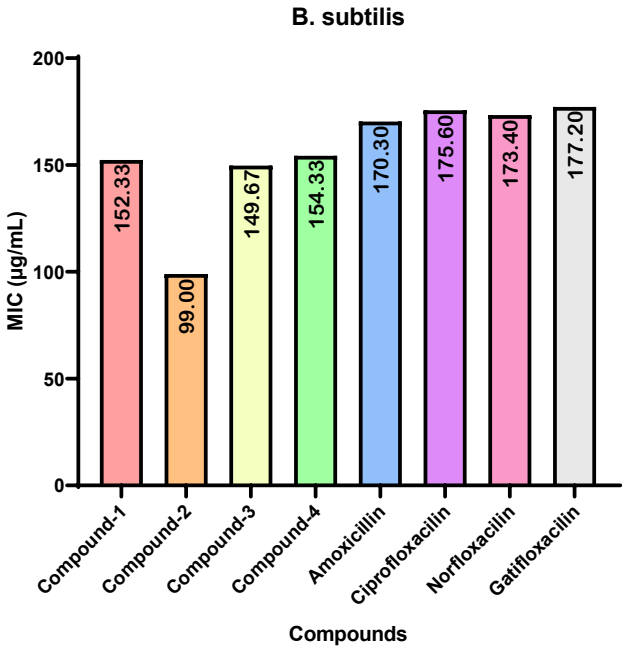

Figure 6: The MIC of all-new synthesis and stander compounds vs. B. subtilis bacteria.

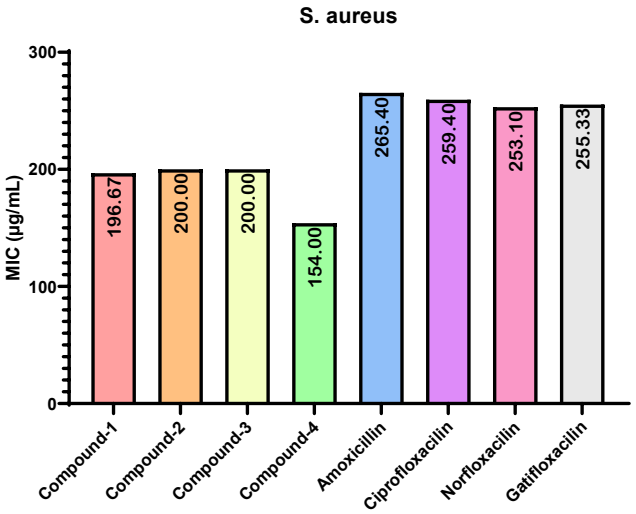

Figure 7: The MIC of all-new synthesis and stander compounds vs. S. aureus bacteria.

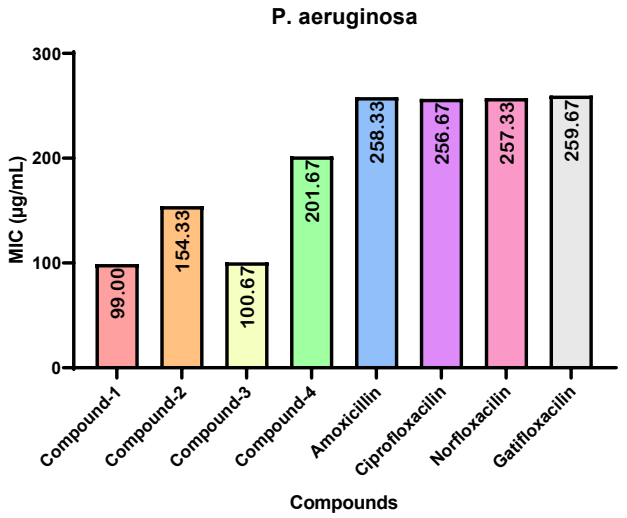

Figure 8: The MIC of all-new synthesis and stander compounds vs. P. aeruginosa bacteria.

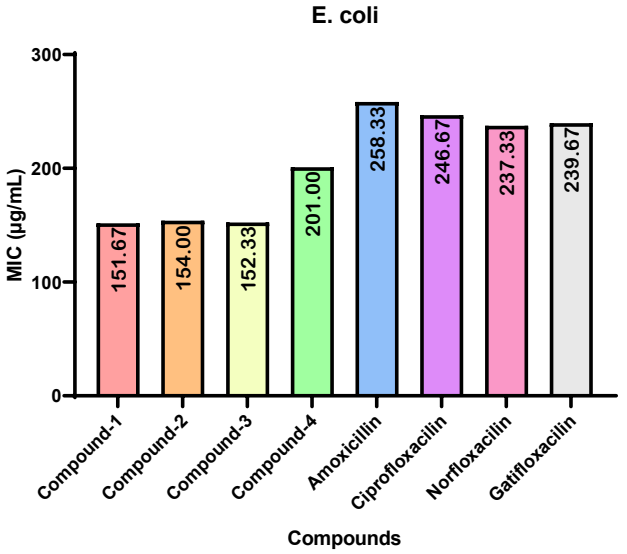

Figure 9: The MIC of all-new synthesis and stander compounds vs. E. Coli bacteria.

and between compound-3 and compound-4. However, there is a significant difference ($p < 0.05$) between compound-2 and both compound-3 and compound-4, in addition to a significant

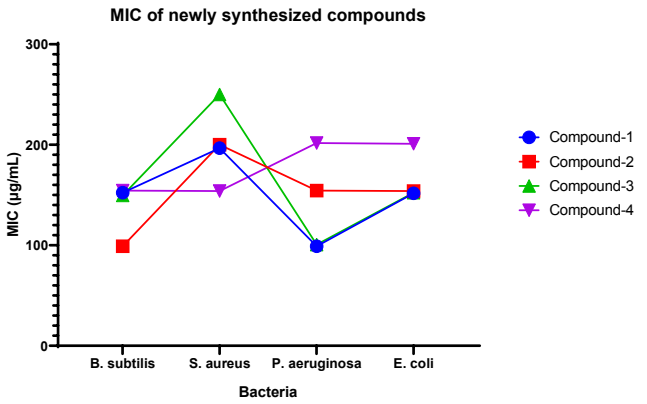

Figure 10: The MIC of all-new synthesis compounds against four types of bacteria.

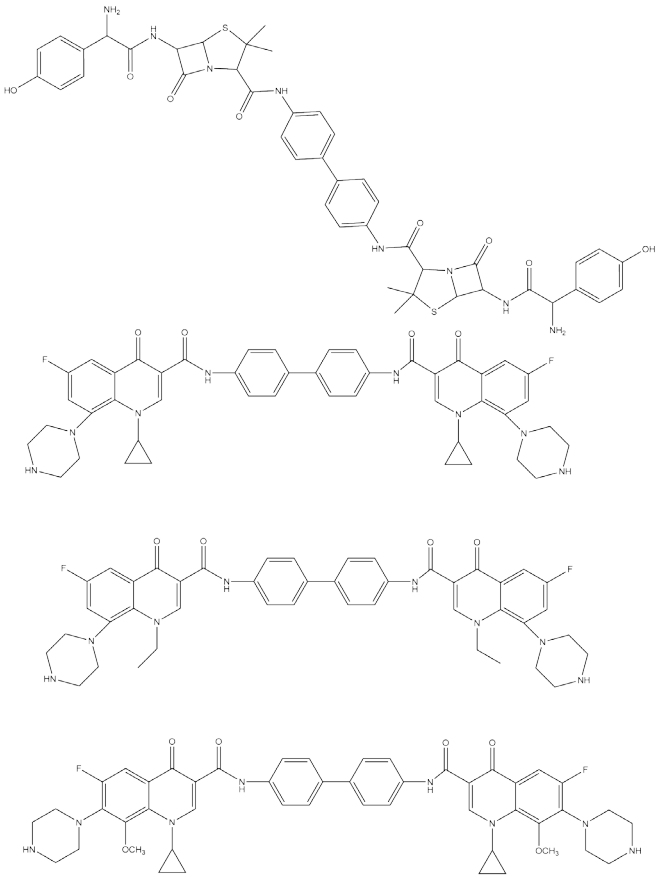

Figure 11: Synthesized compounds 1-4

difference ($p < 0.05$) between all-new synthesis compounds and stander compounds. (Table 1, Figures 4 and 5).

Minimum Inhibitory Concentration (MIC)

The new compounds exhibit different MIC when tested against all types of bacteria. Compound-2 exhibited the lowest MIC against *B. subtilis* bacteria, compound-4 shows the lowest against *S. aureus* bacteria, while when tested it against P. aeruginosa and *E. coli*, compound-1 exhibited the lowest one for both bacteria. There is a significant difference ($p < 0.05$) between all-new synthesis compounds and all standers. (Table 2 and Figure 10)

In B. subtilis bacteria, we found an insignificant difference ($p > 0.05$) between compound-1 and Compound-3, Compound-4, and amoxicillin. There is a significant difference ($p < 0.05$) between compound-2 and all other new synthesis compounds. See Table 2, Figure 6, and Figure-10. S. aureus bacteria, there is an insignificant difference ($p > 0.05$) between compound-1 and all other newly synthesized compounds. There is a significant difference ($p < 0.05$) between compound-4 and both compound-2 and compound-4. See Table 2, Figures 7 and 10. *P. aeruginosa* bacteria, there is an insignificant difference ($p > 0.05$) between compound-1 and compound-3. There is a significant difference ($p < 0.05$) between compound-4 and compound-2 with other new synthesized compounds. See Table 2, Figure-8, and Figure-10. *E. Coli* bacteria, there is an insignificant difference ($p > 0.05$) between compound-1 and both compound-2 and compound-3. There is a significant difference ($p < 0.05$) between compound-4 and other new synthesized compounds. See Table-2, Figures 9 and 10.

CONCLUSION

New amid compounds was effectively synthesized and tested for antibacterial action against Gram-negative and Gram-positive bacteria in this study. Extensive NMR, IR, and LC-MS analyses were used to deduce the structures of these substances. All the produced compounds were effective against *B. subtilis, S. aureus, P. aeruginosa, and E. coli.*

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