RESEARCH ARTICLE

Synthesis of Levofloxacin Derivatives with some Amines and their Complexes with Copper(II) Salts and Evaluation of their Biological Activity

Shaker M. Alwan[1*], Shayma L. Abdulhadi[2], Amera Abbas[2]

[1]*College of Pharmacy, Al-Mustafa University College, Moallem, Iraq*
[2]*Pharmaceutical Chemistry Department, College of Pharmacy, University of Baghdad, Baghdad, Iraq*

Received: 16th June, 2020; Revised: 26th July, 2020; Accepted: 25th August, 2020; Available Online: 25th September, 2020

ABSTRACT

Levofloxacin belongs to the fluoroquinolone family; it is a potent broad-spectrum bactericidal agent. The pharmacophore required for significant antibacterial activity is the C-3 carboxylic acid group and the 4-pyridine ring with the C-4 carbonyl group, into which binding to the DNA bases occur. In this work, we tried to show that by masking the carboxyl group through amide formation using certain amines to form levofloxacin carboxamides, an interesting activity is kept. Levofloxacin carboxamides on the C-3 group were prepared, followed by the formation of their copper complexes. The target compounds were characterized by FT-IR, elemental analysis. The antimicrobial activity of the target compounds was evaluated and showed satisfactory results compared with levofloxacin. This has indicated that the presence of the carbonyl of C-3 carboxyl moiety is not essential, as levofloxacin carboxamides showed interesting copper complexes indicating that they retain the activity of levofloxacin, since its activity depends on binding to DNA gyrase via magnesium binding.

Keywords: Carboxamide, Copper complexes, Fluoroquinolone, Levofloxacin.

International Journal of Drug Delivery Technology (2020); DOI: 10.25258/ijddt.10.3.18

How to cite this article: Alwan SM, Abdulhadi SL, Abbas A. Synthesis of levofloxacin derivatives with some amines and their complexes with copper(ii) salts and evaluation of their biological activity. International Journal of Drug Delivery Technology. 2020;10(3):408-413.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Fluoroquinolones are synthetic antibacterial agents mainly used for treating infections caused by gram-negative bacteria; however, several modifications were done to improve their spectrum of activity and pharmacokinetic properties.[1-5] In the 1980s, the second generation of the compounds, fluoroquinolones were developed which showed increased gram-positive activity and better pharmacokinetic and pharmacodynamic properties.[1,4,6] Fluoroquinolones are used extensively for a variety of indications worldwide. This resulted in emergence of resistance.[1,3,5,7-9] Fluoroquinolones are bactericidal drugs, cause bacterial cell death by interfering with DNA replication and transcription.[10,11] These drugs inhibit DNA-gyrase (topoisomerase II) and DNA topoisomerase IV,[12-14] by forming a drug-enzyme-DNA complex that stops bacterial replication.[15,16] The structural requirements for significant antibacterial activity consist of the 4-pyridone ring and 3-carboxylic acid group,[17] as seen in Figure 1.[18,19]

The affinity of quinolones to metal ions is important for their activity due to binding to DNA-enzyme-complex through magnesium ion,[20] as seen in Figure 2.[18,19]

Generally, inhibition of deoxyribonucleic acid (DNA) gyrase is connected to gram-negative bacteria and inhibition of DNA type IV topoisomerase connected to gram-positive bacteria.[21]

Resistance to some currently available quinolones led the way toward the synthesis of compounds with higher activities against organisms. Structural modifications of existing compounds are made,[22,23] and some of the metal

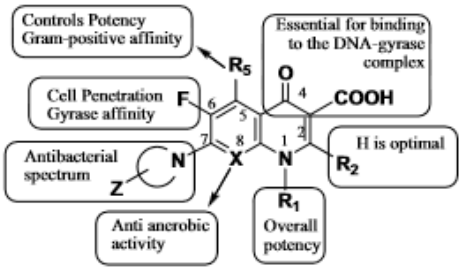


Figure 1: Fluoroquinolone structural features

**Author for Correspondence: universe.scientific@gmail.com*

complexes showed greater antimicrobial activity than the parent drugs.[22-25]

Quinolones form metal complexes acting as a bidentate ligand, unidentate ligand, or bridging ligand. Quinolones mainly are coordinated in a bidentate manner, through one of the oxygen atoms of the deprotonated carboxylic group and the ring carbonyl oxygen atom, as seen in Figure 3, and rarely coordinate through the two oxygen atoms of the carboxyl group, as seen in Figure 4.[26,27]

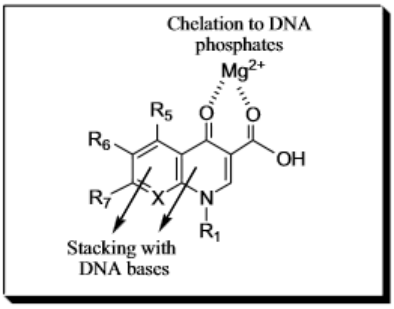


Figure 2: One binding model of quinolones to DNA

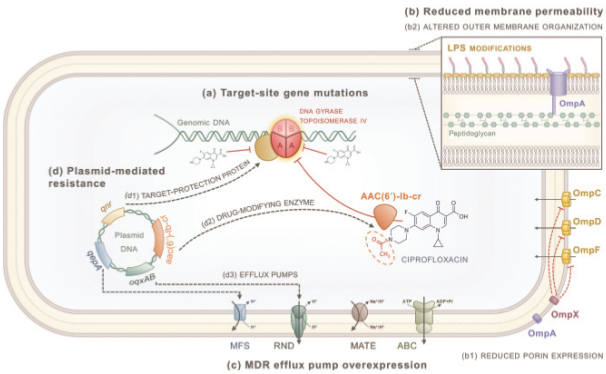


Figure 3: Mechanisms of quinolone resistance[26]

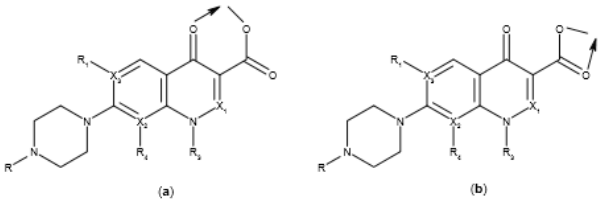


Figure 4: Quinolones main coordination modes

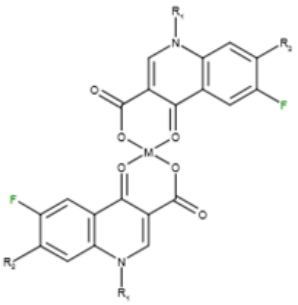


Figure 5: Fluoroquinolone chelates with divalent cations in 1:2 (metal:ligand) manner

Quinolones bind to divalent cations, such as, Mg^{2+}, Ca^{2+}, Cu^{2+}, Zn^{2+}, Fe^{2+}, and Co^{2+}, forming 1:1 or 1:2 (metal:ligand) chelates, or bind to trivalent cations ($A1^{3+}$ and Fe^{3+}), forming 1:1, 1:2, or 1:3 (metal:ligand) chelates.[28] It was found that the number of coordinated ligands in Cu(II)-ciprofloxacin complex depends on the pH. In acidic medium, a 1:1 complex is favored, while at higher pH, a 1:2 complex is favored, as seen in Figure 5.[28]

A copper(II) complex of norfloxacin showing the coordination of copper with C-4 carbonyl and hydroxyl group of C-3 carboxyl, as seen in Figure 6.[29]

Similarly, the ciprofloxacin complex with copper(II) proposed a square-planar structure, as seen in Figure 7.[30]

Levofloxacin form complexes with divalent metals 1:2, including copper(II) in the formula [M (levo)$_2$ (H_2O)$_2$]n H_2O.[27] Figure 8 illustrates the binding of ofloxacin with metals forming divalent complex.[22]

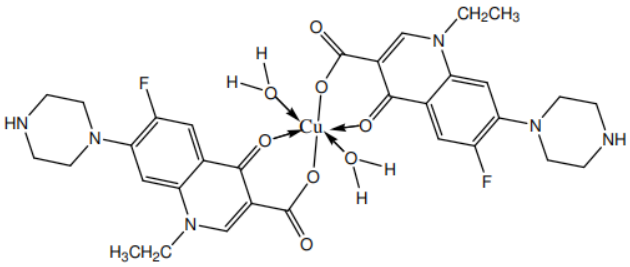


Figure 6: Norfloxacin copper(II) complex

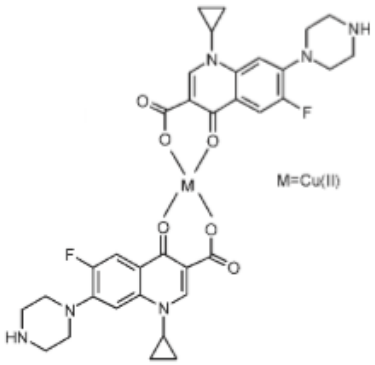


Figure 7: Ciprofloxacin copper(II) complex

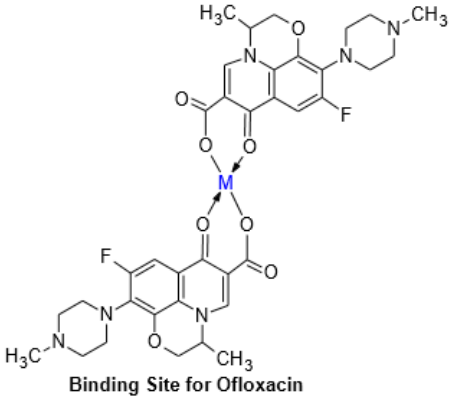


Figure 8: Ofloxacin metal complex

It was concluded that metal ions play important role in the interaction between quinolones and DNA.[31,32] Many chelates of quinolones showed equal or higher antimicrobial activity in comparison to parent compounds since the polarity of the metal ion is decreased by partial sharing of the positive charge with the ligand.[33] Delocalization of π electrons over the chelate ring is increased by chelation, which will result in increased lipophilicity and increased penetration of the complex to the cells.[34-36]

MATERIALS AND METHODS

- Reaction of levofloxacin with diethylamine, tertiary butylamine, glutamic acid, glycine.
- Preparation of metal complexes for levofloxacin carboxamides with divalent metal copper sulfate.
- Characterization of these copper complexes by spectral and elemental analysis.
- Antimicrobial screening for the synthesized copper complexes in comparison with the parent compound.

Chemicals

The chemical and solvents used were analytical grade. Levofloxacin was from Ajenta India, ethyl chloroformate from Sigma, Germany, glutamic acid from Fluka, Switzerland, diethylamine from Sigma-Aldrich, tertiary butylamine from Sigma-Aldrich, and triethylamine from BDH UK.

Chemical Synthesis

Synthesis of Carboxamides

Levofloxacin-primary amine carboxamide: Levofloxacin-diethylamine carboxamide (compound 1a) Levofloxacin-tert. butylamine carboxamide

N,N-diethyl-9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3,6,7-tetrahydro-5H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide

(compound 2a)

N-(*tert*-butyl)-9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3,6,7-tetrahydro-5*H*-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxamide

Levofloxacin (2.8 mmol, 1-gram) was dissolved in dry chloroform (25 mL) containing trimethylamine (2.8 nmol, 0.4 mL). The mixture was cooled in an ice bath to reach 0 to -5ºC, followed by a dropwise addition of ethylchloroformate (2.8 mmol, 0.3 mL) keeping the temperature below zero degree, and stirring was continued for 30 minutes, followed by addition of diethylamine (2.8 mmol, 0.3 mL). The mixture was stirred in ice for 2 hours, then at room temperature, overnight. Washing with 5% Na_2CO_3 solution three times, and extracted with chloroform. The chloroform layers were pooled together and washed with distilled water three times, dried with saturated solution of sodium chlorid, and then anhydrous $MgSO_4$. The chloroform layer was evaporated to get off white powder. The same procedure and quantities were done for the synthesis of levofloxacin-tert. butylamine carboxamide to get off white powder.

(9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3,6,7-tetrahydro-5H-[1,4]oxazino[2,3,4-ij]quinoline-6-carbonyl)glutamic acid

Levofloxacin-amino acid carboxamide: 37 levofloxacin-glutamic acid carboxamide (compound 1b)

Levofloxacin-glycine carboxamide (compound 2b)

(9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3,6,7-tetrahydro-5*H*-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carbonyl)glycine

Levofloxacin (10 mmol, 3.613 grams) was dissolved in dry chloroform (20 mL) containing triethylamine (10 mmol, 1.39 mL). The mixture was cooled in an ice bath to reach 0 to -5ºC, followed by a dropwise addition of ethyl chloroformate (10 mmol, 0.95 mL), keeping the temperature below zero degrees, and stirring continued for 30 minutes. A solution of glutamic acid (10 mmol, 1.471 grams) was added at once with vigorous stirring for 4 hours, at room temperature. The two layers were separated by a separatory funnel, followed by washing and drying to get off white powder. Same procedure was done for the synthesis of levofloxacin-glycine carboxamide, to get light brown powder.

Synthesis of Carboxamide Copper Complexes[38]

Levofloxacin-primary amine carboxamide copper complex (compounds 3a and 4a): Levofloxacin- diethylamine carboxamide (1.2 mmol, 0.5-gram) and KOH (1.2 mmol, 67 mg) were dissolved in ethanol (15 mL), and $CuSO_4.5H_2O$ (0.6-mmol, 150 mg) in 10 mL methanol was added to the first mixture, followed by reflux for 2 hours. Dark blue precipitate is formed, filtered, washed with ethanol, and distilled water. Same procedure and quantities were used for the synthesis of levofloxacin-tert.butylamine carboxamide copper complex to get brown color powder.

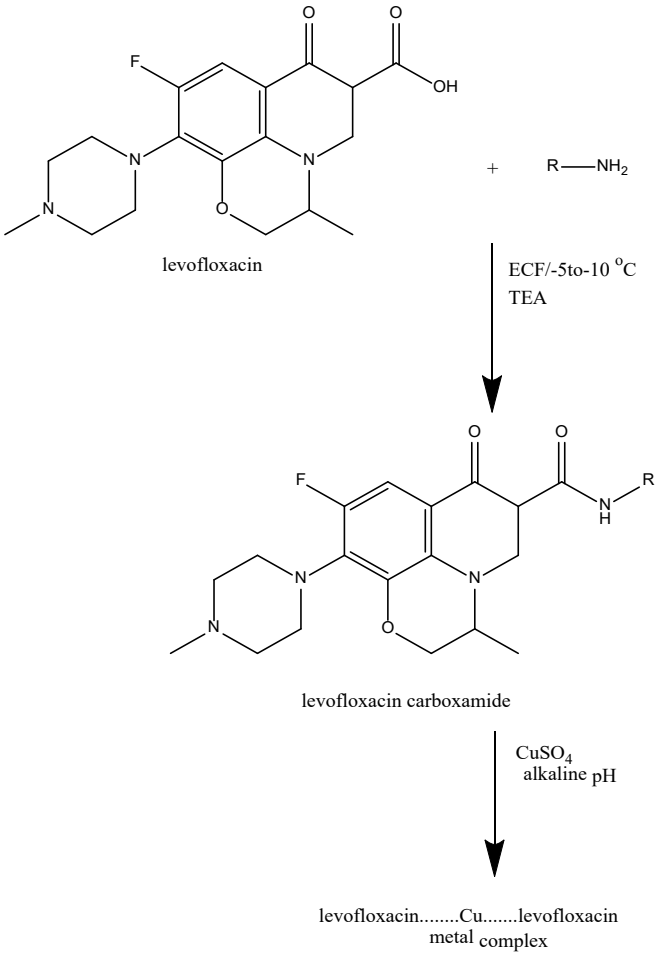
Levofloxacin-amino acid carboxamide copper complex (compounds 3b and 4b): Levofloxacin-glutamic acid carboxamide (1 mmol, 0.5-gram) and KOH (1 mmol, 56 mg) were dissolved in distilled water (20 mL), and $CuSO_4.5H_2O$ (0.5 mmol, 125 mg) in 20 mL distilled water was added to the first mixture, followed by reflux for 2 hours. Ethanol was added to get a precipitate, which was filtered and washed with ethanol several times to get dark green powder. Same procedure was used for the synthesis of levofloxacin-glycine carboxamide copper complex to get light blue powder, using the following quantities: (2.4 mmol, 1-gram) of the carboxamide, (2.4 mmol, 134.4 mg) KOH, and (0.5 mmol, 600 mg) $CuSO_4.5H_2O$.

Preliminary Antimicrobial Evaluation

The minimum inhibitory concentration (MIC) was done on gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, and yeast *Candida albicans*. The tested compounds were prepared in serial diluted concentrations (1,000, 500, 250, 125, 62.5, 31.26, 15.6, 7.8, and 3.9 µ/mL) in sterile nutrient broth, using Mueller Hinton agar. Each type of bacteria was spread on the agar surfaces by good streaking, and the agars were welled, then 100 µ of each concentration was added to the wells. For each type of bacteria, three plates of Mueller Hinton agar were used, and five wells were made in each one. The first plate contains the

Table 1: Physical properties of synthesized compounds

Compound	*Color*	*Yield (%)*	*Melting point*	*Molecular formula*	*Molecular weight*
1a	Off white powder	60	197–198	$C_{22}H_{29}FN_4O_3$	416.5
2a	Off white powder	78	157–160	$C_{22}H_{29}FN_4O_3$	416.5
1b	Yellow powder	30	205–207	$C_{23}H_{27}FN_4O_7$	490.49
2b	Light brown powder	71	218–220	$C_{20}H_{23}FN_4O_5$	418.43
3a	Dark brown powder	70	370 dec.	$C_{44}H_{62}F_2N_8O_8Cu$	932.57
4a	Dark green powder	62	210 dec.	$C_{44}H_{62}F_2N_8O_8Cu$	932.57
3b	Brown powder	50	370 dec.	$C_{46}H_{58}F_2N_8O_{16}Cu$	1,080.55
4b	Light blue powder	65	270 dec.	$C_{40}H_{50}F_2N_8O_{12}Cu$	936.43



Scheme 1: Chemical synthesis of levofloxacin carboxamides and their metal complexes; R = diethylamine, ter.butylamine, glycine, glutamic acid; ECF = ethylcloroformate; TEA = triethylamine

dilutions (15.6, 7.8, and 3.9 µ/mL), the fourth well was used for the positive control, and the fifth well was inoculated by sterile nutrient broth as a negative control, the second plate contains the dilutions (125, 62.5, and 31.26 µ/mL), and positive and negative control, and the third well contains the dilutions (1,000, 500, and 250 µ/mL), and the positive and negative controls.

RESULTS

The proposed structure of the complexes is seen in Figure 9, in which two molecules of levofloxacin carboxamide forming a complex with copper(II) cation. Table 1 shows the physical properties of the synthesized compounds, while Table 2 shows the antimicrobial activity, and Figure 10 shows the chart of the minimum inhibitory concentration (MIC) in µg/mL for synthesized compounds.

Table 2: Antimicrobial activity of synthesized compounds (MIC) in µg/mL

Bacterial strains / Antibiotics µg/ml	S.aureas	E.coli	K.pneumonia	P.aerogenosa
1a	125	500	125	31.2
3a	500	500	500	500
2a	62.5	125	62.5	500
4b	31.2	125	125	31.2
4a	500	125	62.5	125
2b	1000	1000	1000	250
ofloxacin	62.5	125	31.2	62.5

Keynotes: (MIC) minimum inhibitory concentration;
(*S. aureus*) *Staphylococcus aureus*; (*E. coli*) *Escherichia coli*;
(K. pneumoniae) Klebsiella pneumoniae;
(*P. aeruginosa*) *Pseudomonas aeruginosa*;
(*C. albicans*) *Candida albicans*

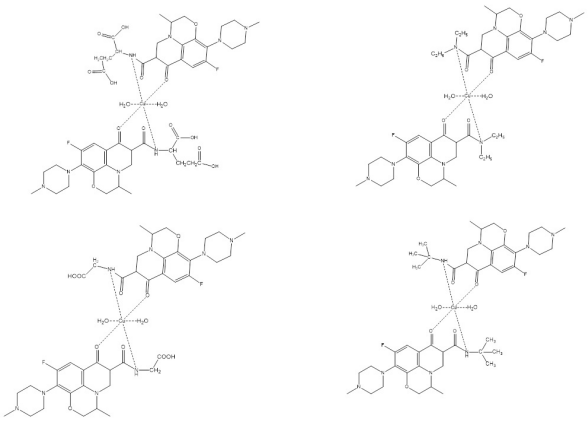


Figure 9: Proposed chemical structures of synthesized Cu(II) complexes

Characterization of the Target Compounds

Compound 3a

FT-IR spectrum (KBr, cm^{-1}): 1,600 ring keto, 1,649 C=O of tert.amide, 464 and 426, new bands of Cu-O and Cu-N,

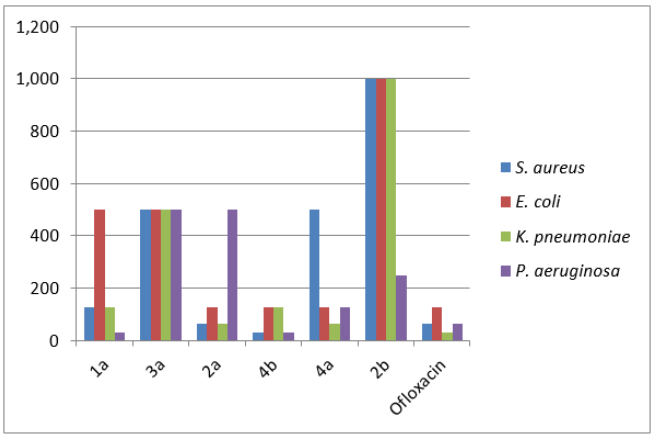


Figure 10: Chart shows MIC in µg/mL of compounds 1a, 2a, 3a, 4a, 2b, 4b

respectively, C=C 1,562. While, ligand 1,589 ring keto, 1,622 C=O of tert. amide, C=C 1,584 strong band.

Elemental CHNS analysis: calculated C 56.668, H 6.701, N 12.0156%; observed C 55.78, H 1.18, N 12.97%

Compound 4a

FT-IR spectrum (KBr, cm^{-1}): 1,622 ring keto, 1,716 C=O of sec. amide, 410 and 434, new bands of Cu-O and Cu-N, respectively. Elemental CHNS analysis: calculated C 56.668, H 6.701, N 12.0156%; observed C 54.9, H 1.36, N%

Compound 3b

FT-IR spectrum (KBr, cm^{-1}): 1,708 C=O stretching of carboxyl group, 1,647 C=O stretching of keto, 1,624 C=O stretching of sec.amide, new bands 489 and 447, of Cu-O and Cu-N, respectively.

Elemental CHNS analysis: calculated C 51.131, H 5.41, N 10.37; Observed C 44.48, H 4.42, N, 14.61%

Compound 4b

FT-IR spectrum (KBr, cm^{-1}): 1,695 C=O stretching of carboxyl group, 1,622 C=O stretching of keto, 1,581 C=O stretching of sec.amide, new bands 470 and 434, of Cu-O and Cu-N, respectively.

DISCUSSION

Compound 1a is less active towards *S. aureus*, *E. coli*, and *K. pneumoniae*, and more active toward *P. aeruginosa* than control. Compound 3a is less active toward bacteria tested than control. Compound 2a has the same activity toward *S. aureus* and *E. coli*, less activity toward *K. pneumoniae* and *P. aeruginosa* than control. Compound 4a has less activity toward *S. aureus*, *K. pneumoniae*, and *P.aeruginosa*, and the same activity toward *E. coli* as control. Compound 2b has less activity toward bacteria tested than control. Compound 4b has more activity toward *S. aureus* and *P. aeruginosa*, *the* same activity toward *E. coli*, and less activity toward *K. pneumoniae* than control. As seen from the antibacterial data, most of the compounds synthesized possess activity toward the selected bacteria, which means that the chemical modification at C3 of the carboxyl group by amide formation has retained the antibacterial activity of the compound, also, the copper(II) complexes 4a and 4b have antibacterial activity.

CONCLUSION

Quinolones are bactericidal agents. Their mechanism of action targets bacterial DNA synthesis, and the modification in the structure by formation of amide at C-3 carboxyl group, followed by complex formation with Cu(II) ion, led to the production of new derivatives with reasonable and promising activity. The synthesized compounds act as bidentate ligands since they have an oxygen atom of the ring carbonyl group and the nitrogen atom of the amide group. Compound 1a showed more activity against *P. aeruginosa*. Compound 2a showed comparable activity against *S. aureus* and *E. coli* to that of control, while its copper complex (4a) has the same activity toward *E. coli* as control. Compound 4b showed greater activity toward *S. aureus* and *P. aeruginosa*, and the same activity toward *E. coli*, compared to control. These results give a promising path for synthesizing new compounds in this field to overcome resistant species of bacteria in the future.

ACKNOWLEDGMENT

The authors are grateful to Dr. Luma Al-Ubaidy, for her help to accomplish the antimicrobial study.

REFERENCES

1. Aldred KJ, Kerns RJ, Osheroff N. Mechanism of quinolone action and resistance. Biochemistry. 2014;53:1565–1574.
2. Hernández A, Sanchez MB, Martínez JL. Quinolone resistance: much more than predicted. Frontiers in microbiology. 2011 Feb 11;2:22.
3. Kim ES, Hooper DC. Clinical importance and epidemiology of quinolone resistance. Infect Chemother. 2011;46:226–238.
4. Fabrega A, Madurga S, giralt E, Vila J. Mechanism of action of and resistance to quinolones. Microb Biotechnol. 2009;2:40–61.
5. Redgrave LS, Sutton SB, Webber MA, Piddock LJ. Fluoroquinolone resistance: mechanisms, impact on bacteria, and role in evolutionary success. Trends Microbiol. 2014;22:438–445.
6. Bisacchi GS. Origins of the quinolone class of antibacterials: an expanded "Discovery Story". J Med Chem. 2015;58:4874–4882.
7. Hooper DC, Jacoby GA. Topoisomerase inhibitors: fluoroquinolone mechanisms of action and resistance. Cold Spring Harb Perspect Med. 2016;1:6:a025320.
8. Fair RJ, Tor Y. Antibiotics and bacterial resistance in the 21st century. Perspect Medicin Chem. 2014;6:25–64.
9. CDC.Antibiotic Resistance Threats in the United States, 2013. Atlanta: Centers for Disease Control and Prevention. 2013;1:44.
10. Cozzarelli NR. DNA gyrase and the supercoiling of DNA, Science. 1980;207:953–960.
11. Mitscher LA. Bacterial topoisomerase inhibitors: Quinolone and pyridone antibacterial agents. Chem. Rev. 2005;105:559-592.
12. Blondeau JM. Fluoroquinolones: Mechanism of action, classification, and development of resistance, Surv. Ophthalmol. 2004;49:S73–S78.
13. Drlica K, Zhao X. DNA gyrase, topoisomerase IV, and the 4-quinolones, Microbiol. mol. Biol. Rev. 1997;61:377–392.
14. Joanna F, Jarosław S. Modifications of quinolones and fluoroquinolones: hybrid compounds and dual-action molecules, Chemical monthly. 2018;149:1199–1245.

15. Hooper DC. Mechanisms of action and resistance of older and newer fluoroquinolones, Clin. Infect. Dis. 2000;31:S24–S28.
16. Maxwell A. The molecular basis of quinolone action, J. Antimicrob. Chemother. 1992;30:409–414.
17. De Sarro A, De Sarro G. Adverse reactions to fluoroquinolones: an overview on mechanistic aspects, Current Med. Chem. 2001;8: 371-384.
18. Domagala JM, Heifetz CL, Hutt MP, Mich TF, Nichols JB, Solomon M, Worth DF. 1-Substituted 7-[3[(ethylamino) methyl]-1-pyrrolidinyl]-6,8- difluoro1,4-dihydro-4-oxo-3-quinolinecarboxylic acids: New quantitative structure-activity relationships at N1 for the quinolone antibacterials, J. Med. Chem. 1988;31:991-1001.
19. Chu DTW, Fernandes PB, Claiborne AK, Pihuleac E, Nordeen CW, Maleczka RE and Pernet AG. Synthesis and structure-activity relationships of novel arylfluoroquinolone antibacterial agents, J. Med. Chem. 1985;28:1558-1564.
20. Palù g, Valisena S, Ciarrocchi g, gatto B and Palumbo M. Quinolone binding to DNA is mediated by magnesium ions, Proc. Natl. Acad. Sci. USA. 1992;89:9671-9675.
21. Hooper D, Quinolones. In: Mandell gL, Bennett JE, Dolin R, Mandell, Douglas, and Bennett's Principles and practice of infectious diseases, 5th ed. Churchill Livingstone, Philadelphia. 2000;1:404-423.
22. Akinremi CA, Obaleye JA, Amolegbe SA, Adediji JF, Bamigboye MO. Biological activities of some Fluoroquinolones-metal complexes, Int J Med Biomed Res. 2012;1(1):24-34.
23. Kumar A. Studies and synthesis of new drugs formed with fluoroquinolones and metal ions, Int. J. of Life Sciences. 2018;6(2):446-452.
24. Mausin K, Mahtab A, Divya J. Ciprofloxacin metal complexes and their biological activities: A review, The Pharma Innovation Journal. 2017;6(5):73-78.
25. Maria JF, Isabel S, Mariana F. Fluoroquinolone-metal complexes: A route to counteract bacterial resistance, Journal of Inorganic Biochemistry. 2014;138:129-143.
26. Susana C, Patrícia P, Michel H, Jose L. C and gilberto I. Mechanisms of quinolone action and resistance: where do we stand, Journal of Medical Microbiology. 2017;66:551–559.
27. Valentina U. Metal Complexes of Quinolone Antibiotics and Their Applications: An Update, molecules. 2013;18:11153-11197.
28. Turel I, Bukovec N, Farkas E. Complex formation between some metals and a quinolone family member (ciprofloxacin). Polyhedron. 1996 Jan 1;15(2):269-275.
29. Singh R, Debnath A, Masram DT, Rathore D. Synthesis and Biological Activities of Selected Quinolone-Metal Complexes, Research Journal of Chemical Sciences. 2013;3(6):83-94.
30. Chohan ZH, Supuran CT, Scozzafava A. Metal binding and antibacterial activity of ciprofloxacin complexes. Journal of Enzyme Inhibition and Medicinal Chemistry. 2005 Jun 1;20(3):303-307.
31. Sissi C, Andreolli M, Cecchetti V, Fravolini A, Gatto B, Palumbo M. Mg2+-mediated binding of 6-substituted quinolones to DNA: relevance to biological activity. Bioorganic & medicinal chemistry. 1998 Sep 1;6(9):1555-1561.
32. Robles J, Martin-Polo J, Alvarez-Valtierra L, Hinojosa L, Mendoza-Díaz G. A theoretical-experimental study on the structure and activity of certain quinolones and the interaction of their Cu (II)-complexes on a DNA model. Metal-based drugs. 2000;7(6):301-311.
33. Temitayo Olufunmilayo A, Isaac Ayoola O, Adeleke Clement A, Grace Olufunmilayo O, Olayinka O, Ezekiel Olugbenga A, Adebowale Olusoji A. Synthesis, characterization and antimicrobial activities of some metal (II) amino acids' complexes. Advances in Biological Chemistry. 2012 Aug 22;2012.
34. Tümer M, Köksal H, Sener MK, Serin S. Antimicrobial activity studies of the binuclear metal complexes derived from tridentate Schiff base ligands. Transition Metal Chemistry. 1999 Aug 1;24(4):414-420.
35. Imran M, Iqbal J, Iqbal S, Ijaz N. In vitro antibacterial studies of ciprofloxacin-imines and their complexes with Cu (II), Ni (II), Co (II), and Zn (II). Turkish journal of biology. 2007 Jun 13;31(2):67-72.
36. Patel NH, Parekh HM, Patel MN. Synthesis, physicochemical characteristics, and biocidal activity of some transition metal mixed-ligand complexes with bidentate (NO and NN) Schiff bases. Pharmaceutical Chemistry Journal. 2007 Feb 1;41(2):78-81.
37. Awatif AE, Shaker MA. Synthesis characterization and evaluation of Antimicrobial and Antitumor activities of new derivatives of certain fluroquinolones, a thesis. 2013.
38. Mustapha AN, Ndahi NP, Paul BB, Fugu MB. Synthesis, characterization and antimicrobial studies of metal (II) complexes of ciprofloxacin. Journal of chemical and Pharmaceutical Research. 2014;6(4):588-593.