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SYNTHESIS OF QUINOLONE DRUG ANALOGUES AND COPPER COMPLEXES: COMPARISON OF BIOLOGICAL ACTIVITIES

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Abstract

In recent research, we have correlated the chemistry of metals and esters in a single molecular framework by synthesizing new ester ligands derived from the esterification of Quinolone drug with three different aliphatic and aromatic alcohols. Further, these ester ligands were chelated with Cu (II) metal sulfates. Structure elucidation and chemical characterization were done through spectral (^{1}HNMR, FTIR, ^{13}CNMR) and CHNS analysis. *In vitro* biological evaluation revealed that 5b against *Bacillus pumilus* and *Staphylococcus aureus,* 3b for *Pseudomonas aeruginosa*, 3c & 5c against *Escherichia coli* showed excellent antibacterial action. The derivative 3c with IC_{50}=1.25±0.54 against urease enzyme was found as the most potent derivative among all the synthesized compounds even more than the parent drug. The 5c gave the highest score (74%) for DPPH (1, 1 diphenyl-2-picrylhydrazyl) radical scavenging activity. So, it is concluded that the most active derivatives can be served as a lead compound for the treatment of gastroenteritis, appendicitis, and crohn's disease as well as for other bacterial infections.

Index Terms: Esters, Metals, Spectral, Enzyme Inhibition, Gastroenteritis, Bacterial

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1. INTRODUCTION

Antimicrobial resistance is becoming a major health threat due to mutation in various microorganisms. According to CDC, 35,000 deaths have been recorded annually because of antibiotic-resistant infections (1), (2). The main reason of quinolone resistance is mutation in aminoacid residues of topoisomerase IV or gyrase enzyme which is important site of interaction for quinolne antibiotics. Recent research shows that cell uptake of free quinolone is totally different from that of quinolone metal complexes. So, it supports the permeability of metal chelates as a lead for future development of quinolone resistant microorganisms (3). The increase in the bulk at C7 of fluoroquinolones will reduce the phenomenon of efflux pump which aids in combating resistance (4).

A novel group of composite products, MOFs (metal-organic framework) synthesized by organic ligands and metal ions, had proven numerous applications in energy storage, adsorption, and catalytic oxidation (5-9). In the last few decades, metal complexes synthesis and their biological evaluation have gained extreme importance in the research world (10, 11). Esters drugs are also playing a vital role in different chemicals, pharmaceuticals, and natural products (12).

As a result of coordination, the ligand might increase its bioactivity relationships, lipophilic behavior, and antimicrobial action (13). The metal complex should have high stability against heat and hydrolysis, good water solubility, and a neutral charge for diffusion from biological membranes without the expenditure of energy (14). The oral absorption of the drug can be enhanced by synthesizing esters in the form of prodrugs (15). For achieving the applications of both esters and metal complexes, we synthesized the copper complexes of Norfloxacin derived ester ligands which can serve as novel metallic drugs for curing different diseases with enhanced potential. Ligands which can serve as novel metallic drugs for curing different diseases with enhanced potential. The structures of synthesized derivatives are given belowin **Figure 1.**

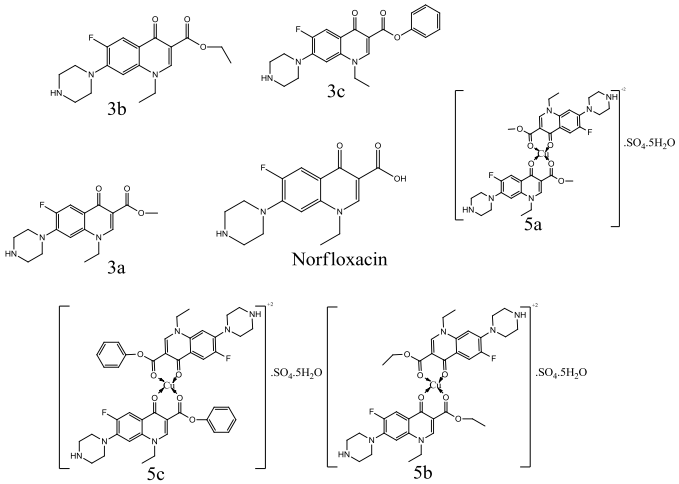

Figure 1: **Structures of synthesized derivatives3a-3c and 5a-5c**

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2. MATERIALS AND METHODS

Norfloxacin **1** was purchased from Sigma-Aldrich while methanol, ethanol, phenol coded as (**2a-c),** and copper sulfate **3** were purchased from Merck international. All the chemicals used during the project were of analytical grade. Thin layer chromatography was used for Compound purification and reaction. The solubility of novel compounds was assessed in DMSO, dichloromethane, chloroform, distilled water, methanol, and ethanol. Gallen Kamp was used for the analyzing melting point. The thermo scientific flash 2000 CHNS analyzer was used for the assessment of amount of carbon, hydrogen, and nitrogen. The spectroscopic analysis was done by Bruker high performance digital FT-Nuclear Magnetic Resonance spectrometer advance III (400/100 MHZ) and Bruker FTIR (Tensor model 27). ^{1}HNMR and ^{13}CNMR were recorded using deuterated DMSO solvent on a δ value scale as downfield chemical shift measured in ppm against TMS as internal reference. IR spectra of synthesized compounds were recorded within wavelength of 400-4000 cm $^{-1}$.

Norfloxacin **1** (1mM) was reacted with aromatic (1mM) and aliphatic (1mM) alcohols **2a-c** with sulphuric acid as a catalyst. This reaction mixture was refluxed for four hours and after that, it was filtered. The reaction process was analyzed through TLC. All the synthesized derivatives (**3a-c)** were processed further for vacuum drying in an oven. The resultant products were recrystallized with ethanol for purification.

The copper complexes **5a-c** of the synthesized derivatives **3a-c** were prepared by magnetically stirring the 1:1 molar ratio of 20ml of methanolic solution of the ligand with 20ml methanolic solution of copper sulfate **4**. It was stirred for about 6 hours in a water bath. The volume of the final mixture was finally reduced to its half. The different colored complexes were filtered and vacuum-dried. The reaction was monitored through TLC from time to time. Recrystallization was done with ethanol to assure the purity of our products **5a-c**.

The physical characterization and spectral studies of all synthesized derivatives are given below.

3a, IUPAC Name: methyl 1-ethyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate, yield 70% as cream color powder, slightly soluble in dichloromethane and chloroform but soluble in DMSO-D_6, distilled water, and methanol; melting point 171℃; molecular weight 333.36; molecular formula $C_{17}H_{20}FN_3O_3$.

Elemental analysis($C_{17}H_{20}FN_3O_3$): C, 61.25; H, 6.05; N, 12.61, (found) C, 61.30; H, 6.10; N, 12.65; FT-IR v(cm-1), 3060, 2954 (C−H), 1654 (C=O), 1623, 1487 (C=C), 1186 (C−N), 3241 (N−H), ^{1}H NMR (DMSO-d_6, ppm) δ: 1.31-132 t (3H, CH_3), 3.34-3.35 q (4H, CH_2), 3.60-3.62 t (2H, CH_2), 2.42-2.45 t (2H, CH_2), 2.48-2.50 m (2H, CH_2), 7.53 s (1H, CH), 7.56 s (1 H, NH), 7.61 s (1H, CH), 8.12 s (1H, CH), 3.92 s (3H, CH_3) ^{13}C NMR (DMSO-d_6, ppm) δ: 145.4 (C1), 92.2 (C2), 177.3 (C3), 145.4 (C4), 122.3 (C5), 147.0 (C6), 157.3 (C7), 86.9 (C8), 157.3 (C9), 160.9 (C10), 61.8 (C11), 14.1 (C12), 70.4 (C13), 79.0 (C14), 79.4 (C15), 79.9 (C16), 25.7 (C17).

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3b, IUPAC Name; ethyl 1-ethyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate, yield 68% as yellow crystals, slightly soluble in dichloromethane and chloroform but soluble in DMSO-d_6, distilled water, and methanol; melting point 168℃; molecular weight 347.38; molecular formula $C_{18}H_{22}FN_3O_3$.

Elemental analysis ($C_{18}H_{22}FN_3O_3$): C, 62.23; H, 6.38; N, 12. (Found) C, 62.28; H, 6.33; N, 12.15, FT-IR v(cm-1), 3058, 2971 (C−H), 1794 (C=O), 1622, 1486 (C=C), 1186 (C−N), 3242 (N−H), ^{1}H NMR (DMSO-d_6, ppm) δ: 1.31-133 t (3H, CH_3), 4.49-4.52 q (2H, CH_2), 3.00-3.02 m (4H, CH_2), 3.04-3.06 t (2H, CH_2), 2.81-2.84 t (2H, CH_2), 2.95-2.97 m (2H, CH_2), 7.00 s (1H, CH), 7.11 s (1 H, NH), 7.22 s (1H, CH), 7.50 s (1H, CH), 2.72 s (3H, CH_3), ^{13}C NMR (DMSO-d_6, ppm) δ: 143.0 (C1), 114.8 (C2), 165.9 (C3), 140.4 (C4), 122.7 (C5), 140.3 (C6), 156.7 (C7), 115.4 (C8), 156.6 (C9), 166.0 (C10), 61.5 (C11), 14.7 (C12), 116.0 (C13), 52.9 (C14), 61.4 (C15), 114.8 (C16), 61.5 (C17), 19.7 (C18).

3c, IUPAC Name; phenyl 1-ethyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate, yield 71% as a cream color powder; slightly soluble in dichloromethane and chloroform but soluble in DMSO-d_6, distilled water, and methanol; melting point 183℃; molecular weight 395.43; molecular formula $C_{22}H_{22}FN_3O_3$.

Elemental analysis for ($C_{22}H_{22}FN_3O_3$): C, 66.82; H, 5.61; N, 10.63, (Found), 66.80; H, 5.64; N, 10.68, FT-IR v(cm-1), 3123, 2903 (C−H), 1699 (C=O), 1614, 1480 (C=C), 1245 (C−N), 3322 (N−H), ^{1}H NMR (DMSO-d_6, ppm) δ: 1.31-133 t (3H, CH_3), 2.41-2.42 m (4H, CH_2), 3.48-3.48 t (2H, CH_2), 3.51-3.52 t (2H, CH_2), 4.12-4.15 m (2H, CH2), 5.81 s (1H, CH), 1.91 s (1 H, NH), 7.94 s (1H, CH), 8.40 s (1H, CH), 8.87 s (1H, OH), 7.68-7.70 m (5H, CH), ^{13}C NMR (DMSO-d_6, ppm) δ: 134.7 (C1), 119.7 (C2), 160.9 (C3), 128.6 (C4), 128.4 (C5), 145.4 (C6), 144.3 (C7), 119.6 (C8), 130.7 (C9), 157.8 (C10), 61.7 (C11), 14.1 (C12), 70.4 (C13), 19.8 (C14), 19.7 (C15), 74.9 (C16), 145.4 (C17), 128.7 (C18), 129.5 (C20), 129.6 (C21), 129.4 (C22).

5a, yield 63 %; as brown crystals; slightly soluble in dichloromethane and water but soluble in DMSO and methanol; melting point 176℃; molecular weight 916.40; molecular formula $C_{34}H_{50}CuF_2N_6O_{15}S$.

Elemental analysis for ($C_{34}H_{50}CuF_2N_6O_{15}S$): C, 44.56; H, 5.50; N, 9.17; S, 3.50, (found) C, 44.51; H, 5.55; N, 9.12; S, 3.53, FT-IR v(cm-1), 3060, 2954 (C−H), 1654 (C=O), 1623, 1487 (C=C), 1186 (C−N), 3241 (N−H), ^{1}H NMR (DMSO-d_6,, ppm) δ: 1.31-1.33 t (6H, CH3), 3.34-3.35 m (8H, CH2), 3.62-3.64 t (4H, CH2), 7.42-7.46 t (4H, CH2), 7.50-7.52 m (4H, CH2), 7.56 s (2H, CH), 7.59 s (2H, NH), 7.65 s (2H, CH), 8.17 s (2H, CH), 7.98 s (6H, CH3) ^{13}C NMR (DMSO-d_6,, ppm) δ: 145.7 (C1,1'), 92.5 (C2,2'), 177.5 (C3,3'), 147.1 (C4,4'), 122.7 (C5,5'), 147.5 (C6,6'), 157.8 (C7,7'), 86.2 (C8,8'), 157.3 (C9,9'), 160.9 (C10,10'), 61.5 (C11,11'), 14.7 (C12,12'), 70.7 (C13,13'), 79.4 (C14,14'), 79.4 (C15,15'), 79.1 (C16,16'), 25.1 (C17,17').

5b, yield 59% as a greenish powder; slightly soluble in dichloromethane and water but soluble in DMSO and methanol; melting point 186 ℃; molecular weight 944.45; molecular formula; $C_{36}H_{54}CuF_2N_6O_{15}S$.

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Elemental analysis for ($C_{36}H_{54}CuF_2N_6O_{15}S$): C, 45.78; H, 5.76; N, 8.90; S, 3.40, (found) C, 45.76; H, 5.79; N, 8.93; S, 3.44, FT-IR v(cm-1), 3058, 2971 (C−H), 1794 (C=O), 1622, 1486 (C=C), 1186 (C−N), 3242 (N−H), ^{1}H NMR (DMSO-d_6,, ppm) δ: 1.29-131 t (6H, CH3), 4.47-4.49 m (4H, CH2), 3.03-3.05 m (8H, CH2), 3.8-3.09 t (4H, CH2), 6.85-6.88 t (4H, CH2), 6.97-6.99 m (4H, CH2), 7.03 s (2H, CH), 7.17 s (2 H, NH), 7.26 s (2H, CH), 7.51 s (2H, CH), 7.78 s (6H, CH3), ^{13}C NMR (DMSO-d_6,, ppm) δ: 142.9 (C1,1'), 114.5 (C2,2'), 165.7 (C3,3'), 140.3 (C4,4'), 122.5 (C5,5'), 140.1 (C6,6'), 156.5 (C7,7'), 115.2 (C8,8'), 156.1 (C9,9'), 166.3 (C10,10'), 61.2 (C11,11'), 14.1 (C12,12'), 116.3 (C13,13'), 52.7 (C14,14'), 61.2 (C15,15'), 114.5 (C16,16'), 61.7 (C17,17'), 19.2 (C18,18').

5c, yield 50% as yellow crystal; slightly soluble in dichloromethane and water but soluble in DMSO and methanol; melting point 166℃; molecular weight 1040.54; molecular formula $C_{44}H_{54}CuF_2N_6O_{15}S$.

Elemental analysis for ($C_{44}H_{54}CuF_2N_6O_{15}S$): C, 50.79; H, 5.23; N, 8.08; S, 3.08, (found) C, 50.77; H, 5.20; N, 8.05; S, 3.12, FT-IR v(cm-1), 3123, 2903 (C−H), 1699 (C=O), 1614, 1480 (C=C), 1245 (C−N), 3322 (N−H), ^{1}H NMR (DMSO-d_6,, ppm) δ: 2.31-2.33 t (6H, CH3), 2.38-2.40 m (8H, CH2), 3.45-3.47 t (4H, CH2), 3.50-3.52 t (4H, CH2), 4.21-4.23 m (4H, CH2), 5.85 s (2H, CH), 1.93 s (2H, NH), 7.90 s (2H, CH), 8.43 s (2H, CH), 7.68-7.70 m (5H, CH), 7.73-7.75 m (5H, CH), ^{13}C NMR (DMSO-d_6,, ppm) δ: 134.6 (C1,1'), 119.5 (C2,2'), 160.7 (C3,3'), 128.4 (C4,4'), 128.5 (C5,5'), 145.1 (C6,6'), 144.1 (C7,7'), 119.6 (C8,8'), 130.6 (C9,9'), 157.6 (C10,10'), 61.5 (C11,11'), 14.1 (C12,12'), 70.1 (C13,13'), 19.6 (C14,14'), 19.2 (C15,15'), 74.7 (C16,16'), 145.3 (C17,17'), 128.5 (C18,18'), 129.4 (C20,20'), 129.7 (C21,21'), 129.2 (C22,22').

The performance of the urease inhibition assay was done according to Ejaz et al. The absorbance of the 96-well plate was recorded at 625nm. The calculation of enzyme inhibition percentage was done through the following formula (16).

$$\text{Inhibition}(\%) = \frac{\text{Control (Absorbance)} - \text{test (Absorbance)}}{\text{Control (Absorbance)}} \times 100$$

Where Control is the total enzyme action in absence of an inhibitor and test is the activity along with the test compound.

Antibacterial activity was performed according to the previously reported agar diffusion method against four different bacterial strains The minimum concentration of test compounds that completely prevents bacterial growth on the agar plate following incubation of overnight period represents MIC (17). Antioxidant activity was performed according to previously reported method(18). The standard used was ascorbic acid. The blank one was the sample without a test or standard compound. The high antioxidant activity will be revealed by low absorbance and vice versa. The scavenging activity was assessed using the formula given below. % age of antioxidant activity = Control drug absorbance- test sample absorbance/ control drug absorbance X 100 All results were further processed through Origin Pro (Software) and documented as mean ± SD. One-way ANOVA was utilized for the calculation of experimental results.

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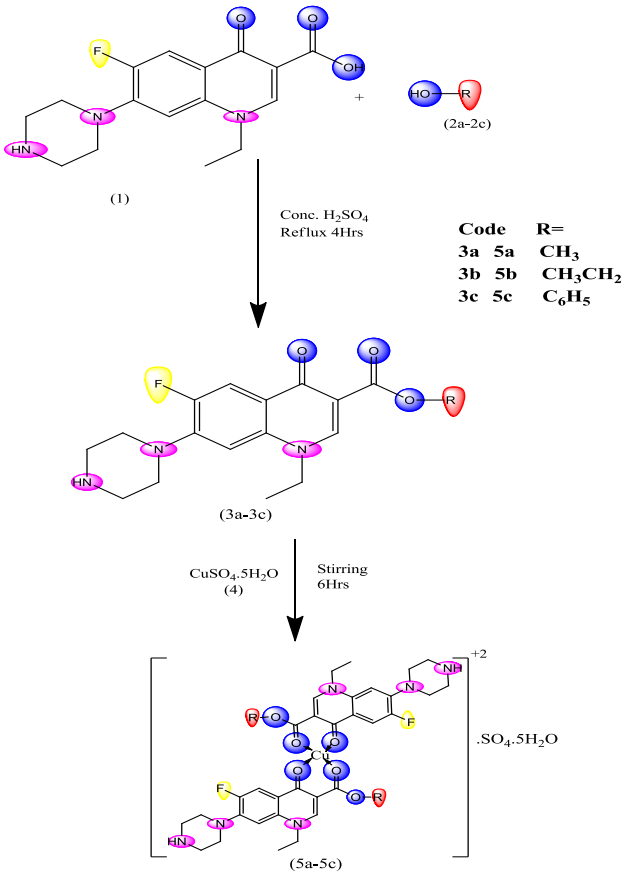
3. RESULTS AND DISCUSSION

Norfloxacin (1mM) was reacted with aromatic (1mM) and aliphatic (1mM) alcohols with sulphuric acid as a catalyst. This reaction mixture was refluxed for four hours and after that, it was filtered. All the synthesized derivatives were vacuum dried and recrystallized with ethanol. Then, copper complexes were prepared by magnetically stirring the 20ml methanolic solution of already synthesized derivatives with 20ml methanolic solution of copper sulfate for 6 hours in a water bath. The volume of the final mixture was reduced to half volume and vacuum-dried. The whole reaction was analyzed through TLC Recrystallization was done with ethanol to assure the purification. Here**, scheme 1** shows the scheme for the synthesis of ester ligands and copper complexes.



Scheme 1: Synthesis of ester ligands (3a-c) and copper complexes (5a-c) of Norfloxacin

The synthesized metal complexes exhibit significant antiurease activity. The urease is important in catalyzing urea into ammonia and carbon dioxide [44]. The elevation or deprivation in body pH, due to ammonia production, can disturb different organ functions. The ureolytic bacteria can create serious issues like kidney stones, hepatic encephalopathy, pyelonephritis, and finally hepatic coma (19, 20). Due to the immense

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importance of urease in the pharmacological field, these enzymes are being targeted now for proper cure of various diseases caused by urease-dependent bacterial strains like *Helicobacter Pylori*, *Proteus mirabilis* and *Staphylococcus saprophyticus* and some strains of *Escherichia coli* (21). We performed the *in vitro* urease inhibition activity on our newly synthesized derivatives to correlate the significance of our compounds in different pathophysiological conditions. The assessment of urease enzyme inhibition activity of all synthesized derivatives is enlisted in **Table 1** with Thiourea used as a standard.

Table 1: Urease Inhibitory activity of compounds 3a-3c and 5a-5c

Code	IC_{50}±SEM (µM)	Compound Code	IC_{50} (µM)
Thiourea [a]	4.24±0.13	**Norfloxacin [b]**	5.04±0.63
3a	4.13±0.31	5a	5.11±0.11
3b	2.14± 0.32	5b	2.83 ± 0.18
3c	1.25±0.54	**5c**	3.75±0.24

Values are the mean ±SEM for triplicate experiments [a]: reference inhibitor. [b]: parent drug.

Most of the ester derivatives exhibited significant inhibitory potential against urease with IC_{50} values of 1.25±0.54 to 5.11±0.11 as compared to thiourea ($IC_{50=}$ 4.24±0.13) used as inhibition standard and parent drug with IC_{50} values of 5.04±0.63. The derivative 3c (1.25±0.54) and 5b (2.83±0.18) had significant antiurease potential even more than the standard drug. The highest antiurease activity (IC_{50}=1.25±0.54) was exhibited by the 3c. The inhibitory action of these six novel derivatives is also remarkable.

So, it means that the above-mentioned derivatives can easily cure resistant microbial infections caused by urease-dependent microorganisms specifically against some species of *Cryptococcus neoformans* (yeast pathogen) and *Proteus vulgaris*. So, these derivatives can be used for various nosocomial infections, gastroenteritis, appendicitis, and Crohn's disease (22)

The results of the antibacterial activity of Norfloxacin with its synthesized derivatives and ceftriaxone sodium against four strains in MIC µg/ml are compiled in **Table 2**.

Table 2: Antibacterial activity of compounds 3a-c and 5a-c *against Bacillus pumilus, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli*

CODE	*B. pumilus* MIC (µg/ml)	*S. Aureus* MIC (µg/ml)	*P. Aerugenosa* MIC (µg/ml)	*E. Coli* MIC (µg/ml)
Ceftriaxone sodium [a]	**0.062**	**0.025**	**0.043**	**0.078**
Norfloxacin [b]	0.231	0.082	0.043	0.231
3a	0.154	0.231	0.031	0.093
3b	0.231	0.093	0.025	0.154
3c	0.457	0.341	0.031	0.062
5a	0.231	0.082	0.062	0.082
5b	0.093	0.078	0.031	0.341
5c	0.571	0.082	0.043	0.062

a: reference inhibitor b: parent drug.

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Compiling the results of inhibitory action against *Bacillus pumilus*, the 3a and 5b were found to be more effective than their parent drug. The highest activity was exhibited by 5b with a MIC value of 0.093µg/ml which was comparable with the standard. In history, methicillin-resistant *Staphylococcus aureus* had been considered responsible for a variety of community and hospital-acquired infections like pneumonia, bacteremia, endocarditis, soft tissue, and skin infection (23). The synthesized derivatives exhibited excellent results, so they can be used for the cure of the above-mentioned diseases. The greatest bactericidal action was produced by 5b (MIC 0.078µg/ml) thus elaborating its maximum antibacterial activity among the synthesized compounds. Against *Pseudomonas aeruginosa,* the highest antibacterial activity of 3b with MIC 0.025µg/ml was observed among ester derivatives. All ester derivatives showed greater bactericidal action against *Escherichia coli* as compared to the parent drug. The 3c and 5c showed remarkable antibacterial action. So, the synthesized derivatives have shown excellent antibacterial activity even more than parent and ceftriaxone sodium.

The standard used for antioxidant assay was ascorbic acid. The DPPH radical scavenging action of the parent drug and its derivatives are presented in tabular form below in **Table 3**.

Table 3: Antioxidant activity of compounds 3a-3c and 5a-5c

Drug 1mg/ml	%age inhibition ±SEM	Drug 1mg/ml	%age inhibition ±SEM
Ascorbic acid [a]	91.36 ± 0.75	Norfloxacin [b]	49.8 ± 0.75
3a	43.36 ± 0.32	5a	51.36 ± 0.34
3b	43.49 ± 0.51	5b	63.29 ± 0.32
3c	35.23± 0.32	5c	74.13± 0.42

[a]: positive control [b]: parent drug.

Antioxidant studies reveal the free radical scavenging action of antioxidant molecules and are mostly used for the evaluation of pure compounds (24). The oxidative stress created by a high concentration of reactive oxygen species can lead to disruption of the cytoskeleton and mutation of DNA sequence thus actively participating in bacterial death (25). The 5a (51%), 5b (63%), and 5c (74%) showed more antioxidant activity than the parent drug. Copper complexes have been found effective against various inflammatory conditions, microbial infections, and viral diseases because of multiple mechanism of action. They are revealed as potent antitumor agents as well. They might act through DNA intercalation, reactive oxygen species (ROS) synthesis, DNA degradation, enzyme inhibition and others. Most of the copper complexes produce adducts with glutathione in cell environment which may synthesize coordination compound of Cu (I). This can lead to the formation of ROS (26). So, because of high redox action, the biological efficiency of copper complexes is enhanced. The possible mechanism for improvement in biological activities of copper complexes than the initial derivatives of Norfloxacin is because of induction of ROS and good enzyme inhibition.

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5. CONCLUSIONS

Recent research was done to evaluate the newly synthesized ester and ester-based copper complexes of Norfloxacin through urease enzyme inhibition assay, bactericidal action, and antioxidant potential. The spectral studies validate the synthesis of the designed novel compounds. By considering all the results observed from *in vitro* studies, it is concluded that the majority of the compounds showed greater biological activities than the parent. Because of their marvelous antiurease activity, they can be served as lead compounds for the treatment of diseases like gastroenteritis, appendicitis, and crohn's disease caused by urease containing microorganisms. The antioxidant assay revealed the excellent radical scavenging action of the 5c complex (74%) which is far greater than the parent drug (49%). Both esters and ester-based metal complexes showed remarkable biological action but if we compare both series then it is concluded that the novel synthesized ester-based copper complexes exhibited better biological activity and can be further considered as a lead compound in the field of antibiotics and antiulcer drugs.

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