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Synthesis, characterization, and antimicrobial activity investigations of ruthenium (II)–bipyridine complexes of ciprofloxacin derivatives

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Abstract

A series of ruthenium (II) complexes derived from the reaction between cis-bis (2,2′-bipyridine) dichloro ruthenium (II) dihydrate and enaminone derivatives of ciprofloxacin were synthesized and fully characterized using elemental analysis, cyclic voltammetry and different spectroscopic techniques (Uv–vis, FTIR, NMR, mass spectroscopy, and X-ray photoelectron spectrometry (XPS)). The isolated compounds were tested for their antibacterial and antifungal activities against gram-negative and gram-positive bacteria. The FTIR data revealed that ciprofloxacin derivatives act as bidentate ligands through the pyridone carbonyl and the carboxylate oxygen atom. The UV–visible data showed that the charge transfer CT band is blue shifted upon the coordination of the ciprofloxacin derivatives compared to the CT band of the parent complex. The XPS results revealed the characteristic peaks of $Ru_{3p3/2}$ and $Ru_{3p1/2}$ as well as $Ru_{3d5/2}$ and $Ru_{3d3/2}$, which confirmed the assembly of the ruthenium (II) ciprofloxacin derivative complexes. Cyclic voltammetry data showed that the ciprofloxacin enaminone derivatives have a similar reduction potential for the Ru (II)/Ru (III) redox couple, and it revealed that the coordination of the ruthenium (II) ion altered the redox property of the ligands and enhanced their electron transfer rate. The electrochemical and the UV–visible results suggest that the ciprofloxacin derivative ligands are π-acceptor ligands. Further, the complexes showed higher antibacterial activities than the parent ciprofloxacin antibiotic and did not show antifungal activities among the tested fungi strains.

KEYWORDS

antimicrobial, ciprofloxacin, cyclic voltammetry, Ru (II) complexes, XPS

1 | INTRODUCTION

The study of the interactions between drugs and metal ions is considered an active research area. Therefore, the complexes of various metal ions with fluoroquinolones have been prepared and explored for their biological activities.[1–5] The quinolone in these complexes coordinates with the metal ion as a bidentate ligand via the ring's carbonyl group and the oxygen atoms of the carboxylate group. It was noticed that in these complexes, the divalent cations are preferred over the trivalent cations, such as iron (III) and ruthenium (III).[6]

Among the quinolones, ciprofloxacin, which coordinates to the metal ion via the carbonyl and the carboxylate oxygen atoms, exhibits both identical or greater bioavailability, greater plasma concentration, and an increase in tissue penetration. Molecular modifications of ciprofloxacin are made to further improve its biological activities.[2] The coordination of metal ions to ciprofloxacin and its derivatives enhanced their antibacterial activity.[4,7,8]

Ruthenium (II) might also bind to ciprofloxacin ligands via the N,N′-coordination site, or via the O,O′-coordination site. The chelation via O, O′ site is reported for ruthenium (II)–arene frameworks,[9–11] and recently, Ziga Ude,[12] synthesized and characterized a half-sandwich organo-ruthenium (II) complex, [Ru(η6-p-cymene)(CipA-H) Cl], which has the 7-(4-[decanoyl] piperazin-1-yl)-ligand (CipA), as a derivative of ciprofloxacin. The x-ray crystallography data showed that the metal ion binds to the ciprofloxacin ligand via the O, O′-site. The complex is highly cytotoxic and revealed potential antitumor activity against different human cancer cell lines, similar to cisplatin, the well-known anticancer drug. However, the complex is also cytotoxic against the cell lines that are resistant to cisplatin and oxaliplatin, which suggests a different mode of action by this complex. Moreover, the complex has a moderate and a dose-dependent antibacterial activity against two *Escherichia coli* strains that are resistant to antibiotics. Accordingly, this complex has dual-biological reactivities, an anticancer and anti-bacterial property.

A unique half-sandwich ruthenium (II) complex of aminomethyl (diphenyl)-phosphine ligands, as well as a ciprofloxacin derivative, were also synthesized and their chemotherapeutics properties were investigated. The complexes showed cytotoxicity in *vitro* and have IC_{50} values lower than cisplatin.[13] The platinum-based drug is one of the best known drugs in the treatment of various kinds of human cancers, but it has severe side effects and cell resistance. Thus, exploring alternative metal complexes of chemotherapeutic properties with less or no side effects is required. Ruthenium compounds have been reported as antitumor metallotherapeutic agents,[14,15] and they display biological selectivity while overcoming cell resistance encountered by cisplatin drugs. Therefore, they are considered promising compounds that can be used in the design and in the development of oncotherapeutic drugs due to their low cytotoxicity and genotoxicity, and to their high biological activity.[16,17] The presented study is aimed to synthesize and characterize ruthenium (II) complexes of ciprofloxacin-enaminone derivatives and to examine their biological activity along with their electrochemical properties.

1.1 | Significance of the study

The synthesis of novel antimicrobial and antitumor compounds is considered to be an active research area. Several infectious diseases caused by the presence or the overgrowth of pathogens currently do not have a cure, and more research is required. Ciprofloxacin is a highly effective and wide spectrum antibiotic. Its antimicrobial activity might be enhanced by the coordination of metal ions or by the incorporation of an active functional group. Therefore, the synthesis of new derivatives of ciprofloxacin and studding their metal complexes are considered to be important toward our understanding of the rules that govern their biological reactivities and chemical properties.

2 | MATERIALS AND METHODS

2.1 | Materials

The following chemicals were purchased from Sigma-Aldrich: dimethyl formamide dimethyl acetate (94%), acetophenone (99%), 2-acetylfuran (99%), 4-nitroacetophenone (98%), 4-methylacetophenone (95%), 2-acetylpyrrole (99%), 4-chloroacetophenone (97%), *p*-xylene (99%), ethanol absolute (99.8%), methanol (99.9%), sodium metal (99.9%), and ammonium hexafluorophosphate (95%). The following chemicals were purchased from Merck Group: diethylether (99.7%), dimethylformamide (99%), dioxane (99.8%), acetonitrile (99.9%), acetic acid, and meluller hinton agar. The following chemicals were purchased from Fluka: dimethylsulphoxide, sodium hydroxide (98%), and ciprofloxacin (98.0%). *N,N* dimethyl formamide (99.5%) was purchased from Scharlau. Cis-bis (2,2′-bipyridine) dichlororuthenium (II) dihydrate was prepared from $RuC1_3.3H_2O$ and bipyridine, both purchased from Sigma-Aldrich, using the method described by Sullivan *et al* (1978). Enaminone (E) and ciprofloxacin derivatives ligands (CFE) were synthesized as described previously.[18] Filter paper discs (No.1) was pursued from Whatman ® Inc. The following antibiotic susceptibility testing discs were purchased from Himedia: ceftazidime, gentamycin, erythromycin and penicillin G. Potato dextrose agar purchased from Oxoid.

2.2 | Instrumentation

Elemental analysis was performed using Elementar Vario Micro Cube CHNS analyzer. The ^{1}HNMR spectra were recorded on a Burker DPX 400- and 600-MHz instrument

using tetramethyl silane (TMS) as internal reference. Infrared spectra were recorded on a Jasco FTIR-6300 spectrophotometer. Samples were prepared as KBr discs and measurements were taken at the range 4000 to 400 cm^{-1}. The UV–vis spectra were recorded using Varian Cary-5 double beam spectrometer. Mass spectroscopy was performed on high resolution gas chromatography mass spectrometer-double focusing sector (GC–MS–DFS). Ionization was accelerated with Cs ions with energies of −70.1 eV. 3-nitrobenzyl alcohol was used as the matrix in the fast atomic bombardment (FAB) mass spectroscopy. X-ray photoelectron spectrometry (XPS) measurements were conducted on a VG ESCALAB 200 spectrometer using MgKα radiation 1253.6 eV and operating at 300 W (15 kV, 20 mA). All binding energy values were determined with respect to C 1 s line (284.6 eV) obtained from adventitious carbon. Depth profiling was done with an Ar ion gun with 5 kV energy and 1-mA current. Cyclic voltammetry (CV) experiments were carried out using BASi Epsilon 100w potentiostat. A single compartment cell was used, and the electrode system consisted of glassy carbon working electrode, platinum wire auxiliary electrode, and Ag/AgCl reference electrode. The supporting electrolyte solution was $TBAPF_6$ in DMSO or 0.1 M $TBAPF_6$ in acetonitrile depending on the solubility of the compound. All of the CV measurements were conducted after purging the solution with nitrogen gas. The concentration of the complexes was 10^{-4} M and prior to any measurements, the potential was calibrated against ferrocene couple (0.44 V Fc^{+}/Fc vs. Ag/AgCl reference electrode) and the parent Ru $(bpy)_2.Cl_2.2H_2O$ complex.

Melting points were measured using Electrothermal 9100. pH measurements were made using Orion 420APlus. The synthesized compounds were dried using an Isotemp 281A vacuum oven.

2.3 | Synthesis of enaminone (E) ciprofloxacin derivative ligands

The enaminone-derivatives (E1–E6) were synthesized as described previously,[18] in which N, N-dimethyl formamide dimethylacetal (DMF–DMA) (0.1 mole, 13 mL) was dissolved in ethanol (10 mL), then an equivalent amount of the corresponding phenone derivatives (0.1 mol) was added. The reaction mixture was refluxed for 48 h, cooled to room temperature and its volume was reduced. The enaminone-ciprofloxacin derivatives (CFE1–CFE6) were also synthesized as previously explained,[18] in which ciprofloxacin (CF) (5–10 mmol) was dissolved in distilled water/dioxane mixture (5:10, v/v) then the enaminone (E1–E6) (5–10 mmol) was added to it. The reaction mixture was refluxed for three hours, then the product was filtered out, washed with ethanol (3 × 25 mL) and distilled water (3 × 25 mL), and crystallized using DMF.

2.4 | Synthesis of ruthenium (II) enaminone ciprofloxacin derivatives complexes

Ruthenium (II) complexes of the ciprofloxacin derivatives were synthesized according to the following general procedure: ciprofloxacin derivative (CFE1–CFE6) (0.08 mmol) was dissolved in DMF-ethanol (4:14, v/v) with continuous stirring, then NaOMe (0.08 mmol) was added. Cis-bis (2,2′-bipyridine) dichlororuthenium (II) dihydrate (0.08 mmol) was dissolved in ethanol (14 mL) and stirred for one hour at 50°C, then slowly added to the ciprofloxacin derivative solution. The reaction mixture was refluxed for five to six hours and a saturated aqueous solution of NH_4PF_6 (2 g NH_4PF_6 in 60-mL water) was added slowly to precipitate the complex. The reaction mixture was left overnight, and the precipitated complex was filtered off and washed with water (3 × 25 mL) and diethyl ether (3 × 25 mL). The complex was further purified by dissolving it in acetonitrile and filtering off the impurities. Anal. Calcd. for **[Ru (bipy)$_2$(CFE1)] PF_6.$(H_2O)_3$**: C, 51.49; H, 4.23; N, 9.14%. Found: C 51.44; H, 4.432; N, 9.16%.^{1}H NMR (400 MHz, $CDCl_3$) δ 1.1 (m, 2H), δ 1.45 (d, 2H), δ 2.9 (m, 2H), δ 3.2 (m, 3H), δ 3.4 (m, 2H), δ 3.7 (m, 4H), δ 7.1 (m, 1H). δ 7.2 (m, 1H), δ 7.5 (m, 5H), δ 7.7 (m, 3H), δ 7.9 (m, 5H), δ 8.1 (m, 2H), δ 8.3 (m, 1H), δ 8.5 (m, 3H), δ 8.8 (m, 1H), δ 9.0 (m, 1H), δ 9.1 (m, 1H). ESI (+)MS (*m/z*) $[M + (bipy)_2(CFE1)]^+$ calcd. 873.9, found 874.4, M. P 222–231°C. (yield: 53%). Anal. Calcd. for **[Ru (bipy)$_2$(CFE2)]PF_6.$(H_2O)_5$**: C, 50.27; H, 4.58; N, 8.73%. Found: C, 50.05; H, 4.409; N, 8.87%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.1 (d, 2H), δ 1.35 (m, 2H), δ 3.2 (s, 1H), δ 3.3 (m, 1H), δ 3.4 (m, 2H), δ 3.7 (m, 3H), δ 7.1 (m, 1H),7.2 δ (m, 1H). δ 7.5 (m, 4H), δ 7.65 (m, 1H), δ 7.8 (m, 1H), δ 7.89 (m, 2H), δ 7.95 (m, 1H), δ 8.1 (m, 2H), δ 8.3 (m, 1H), δ 8.5 (m, 2H), δ 8.75 (m, 1H), δ 9.1 (m, 1H). ESI(+)MS (*m/z*) $[M + (bipy)_2(CFE2)]^+$ calcd. 978.02, found 978.0, M. P 215–226°C (yield: 51%). Anal. Calcd. for **[Ru (bipy)$_2$(CFE3)]PF_6.(H2O)$_5$**: C, 47.3; H, 4.23; N, 8.58%. Found: C, 47.92; H, 4.234; N, 8.83%. ESI(+)MS (*m/z*) $[M + (bipy)_2(CFE3)]^+$ calcd. 908.4, found 908.4. M. P 226–235°C (yield: 52%). Anal. Calcd. for **[Ru (bipy)$_2$(CFE4)]PF_6.$(H_2O)_2$**: C, 50.23; H, 3.85; N, 10.19%. Found: C, 50.2; H, 4.103; N, 10.2%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.15 (d, 2H), δ 1.35 (m, 2H), δ 3.3 (m, 3H), δ 3.55 (m, 2H), δ 3.7 (m, 2H), δ 7.1 δ (m, 1H), δ 7.6 (m, 3H), δ 7.8 (m, 3H), δ 8.0 (m, 1H), δ 8.1

(m, 2H), δ 8.2 (m, 1H), δ 8.35 (m, 3H), δ 8.5 (m, 1H), δ 8.7 (m, 1H). ESI (+)MS (*m/z*) $[M + (bipy)_2(CFE4)]^+$ calcd. 918.91, found 919.0. M. P ^{0}C 251–257°C (yield: 55%). Anal. Calcd. for **[Ru (bipy)$_2$(CFE5)]PF$_6$.(H$_2$O)$_3$**: C, 49.72; H, 4.10; N, 9.22%. Found: C, 49.45; H, 4.538; N, 9.23%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.1 (s, 2H), δ 1.35 (m, 2H), δ 3.25 (s, 1H), δ 3.35 (m, 4H), δ 3.6 (m, 3H), δ 7.12 δ (m, 1H). δ 7.2 (m, 1H), δ 7.55 (m, 3H), δ 7.65 (m, 1H), δ 7.75 (m, 2H), δ 7.85 (m, 2H), δ 8.15 (m, 2H), δ 8.35 (m, 2H), δ 8.8 (d, 1H), δ 9.07 (m, 2H). ESI (+) MS (*m/z*) $[M + (bipy)_2(CFE5)]^+$ calcd. 863.9, found 864.1, M. P 252–260°C (yield: 48%).. Anal. Calcd. for **[Ru (bipy)$_2$(CFE6)]PF$_6$.(H$_2$O)$_4$**: C, 48.94; H, 4.29; N, 10.38%. Found: C, 49.3; H, 4.338; N, 10.34%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.1 (d, 2H), δ 1.35 (m, 2H), δ 3.15 (s, 2H), δ 3.3 (m, 3H) δ 3.65 (m, 2H), δ 7.05 (m, 1H). δ 7.2 (m, 1H), δ 7.5 (m, 4H), δ 7.8 (m, 2H), δ 8.05 (m, 2H), δ 8.25 (m, 2H), δ 8.45 (m, 2H), δ 8.75 (d, 1H), δ 9.03 (m, 2H). ESI (+)MS (*m/z*) $[M + (bipy)_2(CFE6)]^+$ calcd. 934.95, found 935.0, M. *P* > 300°C m.p (yield: 51%).

2.5 | Biological study

2.5.1 | Antibacterial testing

A Kirby–Bauer disc diffusion method,[19,20] was followed to determine the antibacterial activity of the synthesized ciprofloxacin enaminone derivative ligands and their ruthenium (II) complex compounds against three gram-negative (*E. coli* ATCC 25922*, Klebsiella pneunomiae and ESBL positive K. pneunomiae*) bacteria and one gram-positive bacterium (*Staphylococcus aureus*, ATCC 25923). *K. pneunomiae ESBL* strain that used in this study was identified and confirmed to be *K. penumoniae* by using the Vitek 2 GNI (BioMerieux, Marcy L'Etoile, France). *ESBL* production was screened by using the integrated ESBL screen on the Vitek AST-N020 cards (BioMerieux, Marcy L'Etoile, France). The test was performed under sterile conditions. Ceftazidime and cephotaxime antibiotics were used as positive controls in the case of the gram-negative bacteria, while gentamicin and erythromycin antibiotics were used as positive controls in the case of the gram-positive bacterium. The tested compounds were dissolved in acetonitrile (ACN). The concentrations of the tested ruthenium (II) ciprofloxacin derivative complexes ranged from 10–20 mg mL^{-1} and at least 2–3 sterilized filter paper disks were made for each compound. The preparation was made by dissolving the correspondent mass (mg) of the complex, for example 2.5 and 5.0 mg, in 250-μL ACN. The solution was sonicated for about 2 minutes to ensure complete dissolution. Disks saturated with only ACN solvent were also made to confirm that the solvent ACN has no effect tested strains. The prepared disks were allowed to air dry for 24–48 h and carefully placed on an agar inoculated with the specific bacteria and incubated in an L-C incubator (Barnstead Labline) at 37°C for 24 to 36 h. The inhibition zone surrounding each disk was measured in millimeters and used to calculate the mean of the inhibition zones. The assignment of the activity levels was made based on the following code (included in the booklet provided by Himedia where the controls antibiotics were purchased): gram negative strain (<14 mm resistant, 15–22 mm intermediate, >22 mm sensitive), gram positive strain (<12 mm resistant, 13–14 mm intermediate, >14 mm sensitive).

2.6 | Antifungal testing

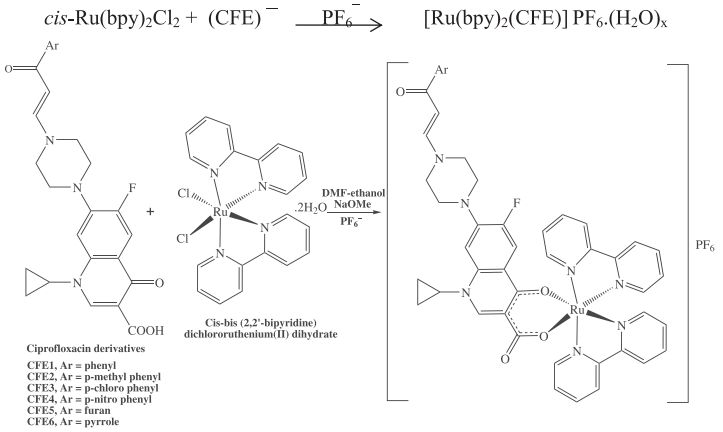
The antifungal activity of the ciprofloxacin enaminone derivatives ligands and their ruthenium (II) complexes were tested against the following fungi strains: *Aspergillus flavus, Aspergillus niger, Alternaria alternata*, *Fusarium solani*, and *Ttichophyton rubrum*. The fungal strains were isolated from salt-marsh soil in Kuwait and were identified macroscopically and microscopically for characteristic morphology. In a typical experiment, the ligands and their complexes (15 mg) were dissolved in DMF or acetonitrile (1.5 mL) and sonicated to ensure complete dissolution. A 100 μL of this solution was then dispensed into a conical flask containing potato dextrose agar media at 42–60°C and mixed well, then poured into Petri dishes (triplicate), and allowed to solidify. A control plate was also prepared by adding a 100 μL of the solvent into a conical flask of the media. A 100 μl of the fungus inoculum was then inoculated into the compound's plate. The plates were kept at ambient temperature for 2 weeks and were observed regularly.

3 | RESULTS AND DISCUSSION

3.1 | Synthesis of ruthenium (II) ciprofloxacin enaminone derivatives

The synthesis and the characterization of ciprofloxacin enaminone derivative ligands (CFE1–CFE6) used in the preparation of the ruthenium (II) complexes were reported previously;[18] however, their electrochemical and XPS properties are examined in this study. The general reaction pathway of the synthesis is carried out by the addition of *cis*-Ru $(bpy)_2Cl_2$ to the deprotonated ciprofloxacin derivative ligands using 1:1 molar ratio in the

SCHEME 1 Synthesis of ruthenium (II) ciprofloxacin enaminone complexes



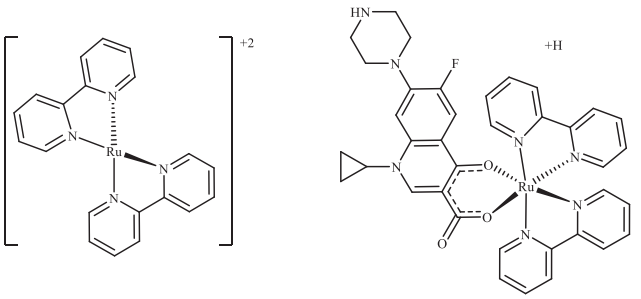


FIGURE 1 Common fragments in the synthesized Ru (II) ciprofloxacin complexes

presence of PF_6^- ion according to the following reaction and as shown in Scheme 1.

$$cis-\mathrm{Ru(bpy)_2Cl_2} + (\mathrm{CFE})\xrightarrow{\mathrm{PF_6^-}}\left[\mathrm{Ru(bpy)_2(CFE)}\right]\mathrm{PF_6}(\mathrm{H_2O})_x$$

The elemental analysis data of the complexes confirmed their expected stoichiometry and the percentage yield varied from 43%–63%. The complexes were dark red and their melting points ranged from 215–300°C. They were insoluble in aqueous and some organic solvents, but soluble in acetonitrile and DMF.

3.2 | Mass spectra

The mass spectra of the synthesized ruthenium (II) complexes are shown in Figures S1–S6. All of the complexes mass spectra have the parent ion peak $[M\text{-}PF_6]^+$ and the fragments $[Ru\ (bpy)_2(CFE)]^+$ and $[Ru\ (bpy)_2]^{2+}$, as shown in Figure 1. The spectra confirmed the presence of the expected masses of the complexes.

Most of the mass spectra of the ruthenium (II) ciprofloxacin derivative complexes have the enaminone fragment, which is bonded to ciprofloxacin during the synthesis of the derivative ligands.

3.3 | FTIR spectra

The FTIR spectra of the synthesized ruthenium (II) ciprofloxacin enaminone complexes are shown in Figures S7 and S8, and Table 1 summarizes the results. The vibrational band that is due to the $(C{=}O)_{carb}$ stretching frequency of the carboxylate group, which is present in the spectra of the ligands around 1703–1729 cm^{-1},[18] is absent from the spectra of the complexes, indicating the coordination of the Ru (II) ion at this site of the ligand. The pyridone carbonyl stretch $\nu(C{=}O)_p$ of the complexes are present in the range of 1624–1629 cm^{-1}. Two characteristic bands are present in the range 1550–1581 and 1343–1371 cm^{-1} that could be assigned to the asymmetric $\nu\ (COO^-)_{asym}$ and symmetric $\nu\ (COO^-)_{sym}$ carboxylate ion stretching vibrations, respectively. The difference in the stretching modes of the carboxylate ion ($\Delta\nu_{COO^-} = \nu\ (COO^-)_{asym} - \nu\ (COO^-)_{sym}$) can be used to determine the coordination mode of the caboxylato ligands. The Δ value falls in the range 195–211 cm^{-1} (Table 1), which indicates a monodentate coordination mode of the carboxylato group of the ciprofloxacin derivative ligands.[21,22] These changes in the FTIR spectra suggest that the ciprofloxacin derivative ligands were coordinated to the Ru (II) via the pyridone oxygen and one carboxylato oxygen. The FTIR spectra of the complexes display a medium broad absorption band at 3430–3433 cm^{-1}, which can be attributed to the ν(O-H) stretching frequency of a coordinated water molecule.[22,23]

3.4 | ^{1}H NMR spectra

The ^{1}HNMR spectrum of the ruthenium (II) complexes of ciprofloxacin derivatives reveal the disappearance of the carboxylate hydrogen atom, which supports the coordination of the ruthenium ion via the carbonyl and the carboxylate atoms of the ciprofloxacin derivatives. On the

TABLE 1 FTIR spectra diagnostic bands (in cm^{-1}) of the ruthenium (II) ciprofloxacin derivative complexes

Compound	ν (C=O) carboxylic	ν (C=O) pyridone	Carboxylate ion		
			Asymmetric ν $(COO^-)_{asym}$	Symmetric ν $(COO^-)_{sym}$	[a]Δν $_{COO^-}$
CFE1	1703	1632			
[Ru (bipy)$_2$(CFE1)]PF_6.$(H_2O)_3$		1627	1581	1370	211
CFE2	1709	1628			
[Ru (bipy)$_2$(CFE2)]PF_6.$(H_2O)_5$		1628	1576	1369	207
CFE3	1703	1632			
[Ru (bipy)$_2$(CFE3)]PF_6.$(H_2O)_5$		1626	1575	1368	207
CFE4	1726	1625			
[Ru (bipy)$_2$(CFE4)]PF_6.$(H_2O)_2$	1726 $_{weak}$	1629	1550	1343	208
CFE5	1715	1632			
[Ru (bipy)$_2$(CFE5)]PF_6.$(H_2O)_3$		1624	1575	1366	209
CFE6	1724	1627			
[Ru (bipy)$_2$(CFE6)]PF_6.$(H_2O)_4$		1627	1566	1371	195

[a]$\Delta\nu = \nu(COO^-)_{asym} - \nu(COO^-)_{sym}$.

other hand, the propyl group and the piperazinyl groups retained the same chemical shifts throughout the complexes. The aromatic protons of pyridone and the aryl rings overlapped with the bipyridine protons. The ^1H NMR spectra are shown in Figures S9–S13.

Both the FTIR and 1HNMR spectra of the ruthenium (II) complexes of ciprofloxacin derivatives suggest that the ciprofloxacin derivative ligands were coordinated to the Ru (II) via the pyridone oxygen and one carboxylato oxygen indicating that the ligand behaves as mono-negative bidentate ligand as a result of the deprotonation of carboxylic acid group.

3.5 | UV–visible spectra

The UV–visible spectra of the ruthenium (II) complexes of ciprofloxacin derivatives are shown in Figures S14–S19 and Table 2 summarizes the results. The spectra show a broad band at 514–519 nm, which is assigned as metal to ligand charge transfer (MLCT) transition,[24] in which an electron located in a metal-centered *d*-orbital is promoted into a ligand–centered π*-orbital. The UV–visible spectra of the parent complex, cis-bis (2,2′-bipyridine) dichlororuthenium (II), has its MLCT bands at 550 nm, which is "blue" shifted in the synthesized complexes. The increase in the energy of the electronic transition in the ruthenium (II) ciprofloxacin derivative complexes indicate a stabilization in the molecular orbitals of the

TABLE 2 UV–visible absorption bands of the ciprofloxacin enaminone ligands CFE1–CFE6[a], ruthenium (II) ciprofloxacin derivatives complexes of RuCFE1–RuCFE6[b], and the parent cis-bis (2,2′-bipyridine) dichloro ruthenium (II) dihydrate (in nm)

Compound	λ_{max}			
cis-Ru (bpy)$_2Cl_2$	242	296	377	550
CFE1	283	341		
[Ru (bipy)$_2$(CFE1)]PF_6.$(H_2O)_3$	246	295	339	519
CFE2	281	344		
[Ru (bipy)$_2$(CFE2)]PF_6.$(H_2O)_5$	247	294	343	517
CFE3	281	346		
[Ru (bipy)$_2$(CFE3)]PF_6.$(H_2O)_5$	250	284	344	514
CFE4	284	368		
[Ru (bipy)$_2$(CFE4)]PF_6.$(H_2O)_2$	249	294	376	515
CFE5	280	346		
[Ru (bipy)$_2$(CFE5)]PF_6.$(H_2O)_3$	247	294	345	519
CFE6	282	340		
[Ru (bipy)$_2$(CFE6)]PF_6.$(H_2O)_4$	246	295	339	517

[a]Measured in DMF.
[b]Measured in acetonitrile.

complex, and it is a characteristic of a π-acceptor ligand. Thus, ciprofloxacin enaminone derivative ligands are classified as π-acceptor ligands based on the UV–visible and the electrochemistry data (see below). The bands at 246–250, 284–295, and 339–376 nm can be attributed to ligands π- π* electronic transitions. The RuCFE4 complex shows the highest value of this transition, which is an indication of the influence of the enaminone's substituent, the *p-nitro* group, compared to the other substituents.

3.6 | X-ray photoelectron spectroscopy

The investigated ciprofloxacin enaminone derivative ligands (CFE1–CFE6) consist of C, O, N, and F atoms, while their ruthenium (II) complexes consist of the same atoms in addition to a second source of the P and F atoms, which is the counter ion PF_6^-. The XPS spectra of the free ciprofloxacin enaminone derivatives and their ruthenium (II) complexes have been measured. The XPS of the isolated complexes are shown in Figure S20 and the binding energy (BE) values with the peak assignments are given in Table S1. Relative to the spectra of the free ligands, ruthenium (II) complexes have the same binding energies for C1s, O1s, F1s, and N1s. In addition, the XPS spectrum of RuCFE3 complex, exhibits one peak at 200.4 eV owing to the binding energy of the Cl2p. On the other hand, the XPS spectra of all the complexes exhibit a characteristic peak at 135.83–136.89 eV, which belongs to P2p. In addition, the spectra of the complexes display two series of peaks at 461.92–462.12 and 483.60–484.10 eV, which are assigned to $Ru_{3p3/2}$ and $Ru_{3p1/2,}$ respectively, with a binding energy difference (ΔE = BE $Ru_{3p1/2}$ − BE $Ru_{3p3/2}$) of 21.64–22.15 eV. Also, the XPS spectra show another two series of bands at 280.41–280.57 and 284.60–284.78 eV, that are assigned to $Ru_{3d5/2}$and $Ru_{3d3/2,}$ respectively, with a binding energy difference (ΔE = BE $Ru_{3d3/2}$ − BE $Ru_{3d5/2}$) of 4.12–4.32 eV. The information derived from the XPS results is in accordance with those reported for Ru (II) ions.[25] The peaks assigned to $Ru_{3p3/2}$ and $Ru_{3p1/2}$ as well as $Ru_{3d5/2}$and $Ru_{3d3/2}$ with their binding energy differences, confirmed the formation of the ruthenium (II) ciprofloxacin derivative complexes. In addition, it has been observed that the peak assigned to $Ru_{3d3/2}$ overlaps with the peak of C1s at 284.7 eV.

3.7 | Cyclic voltammetry

Cyclic voltammetry (CV) was carried out to investigate the redox properties of the synthesized ciprofloxacin derivative ligands CFE1–CFE6, Figures S21–S24, and their ruthenium (II) complexes RuCFE1–RuCFE6, Figures S21–S24. The cyclic voltammograms were recorded in the potential range +2800 mV to −2800 mV. Table S2 summarizes the obtained electrochemical data of the ciprofloxacin derivative ligands and their ruthenium (II) complexes.

The CV data of the complexes showed one redox wave in the 0- to +2000-mV region (positive region Figures S23 and S24), which is assigned to the Ru (II)/Ru (III) redox couple (∼600 mV, vs. Ag/Ag^+). Since the peak-to-peak separation (ΔE_p) of this wave is shifted with the scan rate, and the peak current (I_p) varies linearly with the square root of the scan rate (Figure S25), this wave is described as a quasi-reversible electron transfer process.[26,27] Among the synthesized complexes, this wave did not show significant changes, which indicates that there is no effect of the enaminone substituents on the redox potential value of the ruthenium metal in these complexes. However, the rate of the electron transfer of this redox wave is different between the complexes. The values of the ΔE_p vary between 63 and 88 mV (Table S2), and the ratio of I_{pa}/I_{pc} varies between 1.2 and 3.2 (Table 3) are also characteristics of a quasi-reversible electron transfer process. This also showed variations in the electron transfer rate between the complexes. The peak separation ΔE_p is the greatest for RuCFE2 (88 mV) and RuCFE4 (87 mV) complexes, emphasizing the greatest decrease in the reversibility of the electron transfer process in these complexes, which is attributed to the different enaminone substituents. RuCFE2 complex has the methyl substituent and RuCFE4 has the nitro substituent. Moreover, the current response, I_{pa}/I_{pc}, is the highest for the RuCFE6 complex, which indicates a faster electron transfer for this complex. RuCFE6 complex has the pyrrole substituent, which increased the inductive property of the complex. The differences in the values of ΔE_p and I_p among the ruthenium (II) ciprofloxacin complexes demonstrate the influence of the enaminone

TABLE 3 Anodic current I_a, cathodic current I_c and currents ratio at scan rate 200 mV s^{-1} for the ruthenium (II) ciprofloxacin enaminone derivatives complexes, RuCFE1–RuCFE6

Complex	I_{pc}	I_{pa}	I_{pa}/I_{pc}
RuCFE1	1.3	3.2	2.5
RuCFE2	2.1	3.2	1.5
RuCFE3	1.8	3.9	2.1
RuCFE4	2.4	2.9	1.2
RuCFE5	2.8	3.3	1.2
RuCFE6	1.0	3.2	3.3

substituents on the reversibility of the electron transfer process and its rate in these compounds.

Compared to the parent complex, Ru $(bpy)_2Cl_2$, which has a redox potential at ~315 mV (vs Ag/Ag^+) for the Ru (II)/Ru (III) couple, this wave is shifted to higher positive values, which indicates that the ruthenium (II) ciprofloxacin complexes are more difficult to be oxidized compared to the parent complex, implying that the ciprofloxacin ligands withdrew the electron density from the metal center, and hence are classified as good π-acceptor ligands, which is also suggested from the UV–visible data that showed a blue shift in the charge transfer (CT) band of the complexes compared to the parent complex.

The CV of the ligands in the 0- to +2000-mV regions showed irreversible oxidation wave (~1200 mV, vs. Ag/Ag^+), in which ligand CFE1, which has the phenyl substituent, and the lowest oxidation potential value, is thus considered the easiest one to be oxidized among the investigated ciprofloxacin derivative ligands.

The negative region of the CV (0 to − 2800 mV) of the complexes showed multiple quasi-reversible electrochemical waves (Figures S23 and S24), which is most likely due to the reduction and/or the oxidation of the coordinated ligands, since their CVs have similar waves, but are irreversible. Ruthenium (II) ion coordination to the ciprofloxacin derivative promoted the reversibility of the electron transfer process (Figure S21) and hence improved the electrical conductivity of the ligands. These results demonstrate the influence of the metal ion on the redox properties of the ligands and vice versa. Moreover, the values of the redox potentials of these electrochemical waves are different for each complex (Table S2), which illustrate the effect of the enaminone substituents on the redox properties of the complexes. For example, RuCFE6 complex has the highest $E_{1/2}$ value, − 2080 mV, with the smallest peak-to-peak separation ΔE_p of 103 mV. Thus, this complex has the highest reducing power and the highest electron transfer rate compared to the other investigated complexes. As explained above, RuCFE6 complex has the pyrrole substituent that influences its electrochemical property, consequently influencing its biological activity (see below). Both RuCFE5 and RuCFE2 also have high $E_{1/2}$ values (− 2063 and − 2070 mV, respectively), but the ΔE_p value of the former (137 mV) is less than that of the later (170 mV), and therefore the rate of the electron transfer is higher in the RuCFE5 complex than in RuCEF2 complex. The substituent in RuCFE5 is furan and in RuCEF2 is *p*-methylphenyl. Thus, pyrrole, furan, and methylphenyl substituents have greater effect on the electrochemistry of the complexes than the phenyl and the *p*-chlorophenyl substituents.

3.8 | Biological activity of the ruthenium (II) complexes

The antibacterial activity of the ruthenium (II) complexes of ciprofloxacin derivatives was investigated against gram positive, *S. aureus*, and gram negative, *E. coli, K. pneunomiae*, and *ESBL* positive *K. pneunomiae*, bacteria. The solvent ACN has no effect on the antibacterial activity of the tested bacteria. Table 4 summarizes the inhibition zone obtained by each tested organism. Inspection of the data revealed that all of the ruthenium (II) complexes are active against *S. aureus*, and only the complexes RuCFE1, RuCFE5 and RuCFE6 are active against *E. coli*. Intermediate activity against *E. coli* was observed for RuCFE2, RuCFE3, and RuCFE4.The complex RuCFE6 has the highest activity against *S. aureus* and *E. coli*, and as explained above, it has the highest electron transfer rate and the highest negative redox potential among the examined complexes, which is attributed to the presence of the pyrrole substituent. Moreover, pyrrole is known for its antibacterial activity.[28] All of the complexes are inactive against *K. pneunomiae* and *ESBL* positive *K. pneunomiae*. This is possibly due to different resistance mechanisms among these gram-negative strains.

The coordination of ruthenium (II) ion improves the antibacterial activity of some of the investigated ciprofloxacin derivative ligands against *S. aureus*. For example, ligands CFE1 and CFE3 are inactive against *S. aureus*, whereas their ruthenium (II) complexes are active. Even though there are slight differences in the concentrations between the ligands (8.0 mg mL^{-1})[18] and their ruthenium (II) complexes (10 mg mL^{-1}), the zone of inhibition remained almost the same with higher concentrations. Thus, the binding of the metal ion to the ciprofloxacin derivatives influenced their antibacterial activity. Furthermore, upon comparing the values of the inhibition zone, the antibacterial activity of copper (II) complexes of ciprofloxacin derivative ligands[18] is more than that of the ruthenium (II) complexes of the same ligands. This demonstrates the effect of the metal ion "type" on the antibacterial activity of the ligands. Moreover, copper (II) complexes are active against all tested gram-negative bacteria, in contrast to the ruthenium (II) complexes, who are active only against *E. coli*. In addition, none of the tested compounds showed activity against the fungi strains within the concentrations of the examined compounds. The inhibition zones of the tested bacteria are shown in Figures S26–S29.

TABLE 4 Zone of inhibition[a] (in mm) for ruthenium (II) ciprofloxacin derivatives complexes[b] against the tested bacteria

Sample	Concentration	*Staphylococcus aureus* (G^+)	*Escherichia coli* (G^-)	*Klebsiella pneunomiae* (G^-)	ESBL *Klebsiella pneunomiae* (G^-)
Gentamycin[c]	10 mcg	30	—	—	—
Erythromycin[c]	15 mcg	19	—	—	—
Cefotaxime[c]	30 mcg	—	27	16	14
Ciprofloxacin	5 mcg	21	21	21	21
CFE1[d]	8 mg mL^{-1}	NZH[e]	20	—	—
CFE2[d]	8 mg mL^{-1}	21	22	—	—
CFE3[d]	8 mg mL^{-1}	NZH	13	—	—
CFE4[d]	8 mg mL^{-1}	16	21	—	—
CFE5[d]	8 mg mL^{-1}	20	25	—	—
CFE6[d]	8 mg mL^{-1}	26	24	—	—
Ru (II)complex of CFE1	10 mg mL^{-1}	22	24	NZC	NZC
	20 mg mL^{-1}	22	24	NZC	NZC
Ru (II) complex of CFE2	10 mg mL^{-1}	19	21	NZC	NZC
	20 mg mL^{-1}	18	20	NZC	NZC
Ru (II) complex of CFE3	10 mg mL^{-1}	19	16	NZC	NZC
	20 mg mL^{-1}	18	15	NZC	NZC
Ru (II) complex of CFE4	10 mg mL^{-1}	20	18	NZC	NZC
	20 mg mL^{-1}	16	15	NZC	NZC
Ru (II) complex of CFE5	10 mg mL^{-1}	21	21	NZC	NZC
	20 mg mL^{-1}	22	23	NZC	NZC
Ru (II) complex of CFE6	10 mg mL^{-1}	23	25	NZC	NZC
	20 mg mL^{-1}	25	25	NZC	NZC

[a]Average of triplicate measurements. Activity levels: gram negative strain (<14 mm resistant/inactive, 15–22 mm intermediate sensitivity/activity, >22 mm sensitive/active), gram positive strain (<12 mm resistant/inactive, 13–14 mm intermediate sensitivity/activity, >14 mm sensitive/active).
[b]Complexes were dissolved in ACN and triplicate disks were made for each complex. All disks were dried completely prior to incubation with the bacteria strains at 37°C for 24 h.
[c]For gram positive strain gentamycin and erythromycin antibiotics were used as positive control, and for gram negative strain cefotaxime antibiotic was used as positive control, blank sterilized disk and disk with solvent only were used as negative controls.
[d]From reference 18.
[e]NZC = No zone of clearance.

4 | CONCLUSIONS

Six new ruthenium (II) complexes of ciprofloxacin enaminone derivative ligands have been synthesized and characterized. Elemental analysis, mass spectroscopy, FTIR, cyclic voltammetry, UV–Visible spectroscopy, ^{1}H NMR, and XPS were used to determine their structures and to explore their properties. FTIR spectra revealed that the ciprofloxacin derivatives coordinate to the Ru (II) ion as bidentate ligands via its pyridone carbonyl and its carbroxylato oxygen atom. The complexes were shiny dark-red compounds of high melting points and were insoluble in aqueous solution. The UV–visible spectra of the complexes showed a band in the visible region at 514–519 nm, which is assigned as metal to ligand charge transfer (MLCT) transition. In comparison to the parent complex, this band is blue shifted in the newly prepared ruthenium (II) ciprofloxacin complexes, suggesting an increase in the stability of the molecular orbitals, which is a characteristic of a π-acceptor ligand. Cyclic voltammetry results showed one quasi-reversible wave in the positive region at ∼600 mV, which is assigned to the Ru (III)/Ru (II) couple and was similar among the complexes. In comparison to the parent complex, this reduction is shifted to a higher positive potential value, which indicates a decrease in the electron density on the metal center. The values of ΔE_p and I_{pa}/I_{pc} of the complexes are different, which is contributed to the different rate of the electron transfer process and are related to the enaminone substituent of the ligands. The UV–visible and the cyclic voltammetry results suggest that the ciprofloxacin derivative ligands are π-acceptor ligands. The

XPS results showed the characteristic peaks of $Ru_{3p3/2}$ and $Ru_{3p1/2}$ as well as $Ru_{3d5/2}$and $Ru_{3d3/2}$ along with their binding energy values, which confirmed the formation of the ruthenium (II) ciprofloxacin complexes. The newly synthesized compounds have promising activity against gram-positive and gram-negative bacteria. All of the ruthenium (II) complexes were active against *S. aureus* and only RuCFE1, RuCFE5 and RuCFE6 complexes were active against *E. coli*. The complexes were inactive on *K. pneunomiae* and *ESBL positive K. pneunomiae* bacteria, which may be due to the different resistance mechanisms performed by the tested gram-negative bacteria strains. Some of the complexes enhanced the antibacterial activity of the ligands. CFE1 and CEF3 ligands were inactive against *S. aureus*, however the coordination of the Ru (II) ion influenced their antibacterial activity. The ligands and their ruthenium (II) complexes did not show antifungal activities against the tested fungus strains.

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AUTHOR CONTRIBUTIONS

Dhuha Al-Wahaib: Conceptualization; methodology; visualization. **Ali El-Dissouky:** Supervision; visualization. **Nada Abrar:** Investigation; methodology; resources. **Tarek Khalil Akel:** Conceptualization; investigation; methodology; visualization.

CONFLICT OF INTEREST

The authors declare that no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the supporting information of this article.

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