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# Proceedings

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## Pure and Applied Chemistry International Conference 2020

# Chemistry for Catalyzing Sustainability and Prosperity

**February 13 - 14, 2020**

IMPACT Forum, Muang Thong Thani
Nonthaburi , Thailand

# Proceedings

**The Pure and Applied Chemistry International**

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**"Chemistry for Catalyzing Sustainability and Prosperity"**

**February 13-14, 2020**

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# Synthesis, spectral characterization and biological activities of the mixed ligand copper(II) complexes containing guanidine derivatives and antibiotic, ciprofloxacin

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The mixed ligand complexes of copper(II) chloride containing guanidine derivatives and ciprofloxacin (Hcip), [$CuL^1$(cip)]Cl (**1**) and [$CuL^2$(cip)]Cl (**2**), have been synthesized and physicochemically characterized. In these complexes, ciprofloxacin acts as the deprotonated bidentate ligand coordinated with the copper(II) center through oxygen atoms of the carboxylate and pyridine groups. The electronic spectra of new complexes indicate the square-planar geometry with $CuN_2O_2$ chromophore. Their DNA cleaving ability toward plasmid pBR322 DNA has been investigated by gel electrophoresis. The results show that the DNA cleavage of the complexes probably involves the generation of reactive oxygen species in the oxidative mechanism with the cleaving activity of **2** > **1**. The antibacterial activity of the complexes has been screened tested on Gram-negative (*E. coli* and *Pseudomonas aeruginosa* PAO01) and Gram-positive (*Bacillus cereus*) microorganisms in terms of minimum inhibitory concentration (MIC). Both complexes show better antibacterial activity against all tested bacteria than the starting compounds ($[CuL^1Cl_2]_2$ and $[CuL^2Cl_2]_2$). Especially, complex **2** displays greater biological activity against *E. coli* than **1** and ciprofloxacin. The cytotoxicity of the complexes further determined by Resazurin Microplate assay (REMA) reveals that only **2** can inhibit the proliferation of small cell lung cancer (NCI-H187) with better cytotoxicity than the corresponding starting complex.

## 1. Introduction

The copper(II) complexes with diverse organic ligands have been the subject of research studies to develop as new therapeutic agents.[1-3] Amidino-*O*-methylurea and its derivative are one of the interesting ligand systems. The biological activities of the copper(II) complexes containing these ligands in binding to DNA, DNA cleavage, anticancer as well as antibacterial activities have been reported by our research group.[4-6] However, it necessary to be continually developed to improve both anticancer and antibacterial efficiency of the complexes. These evidences encourage us to synthesize the new compounds based on amidino-*O*-methylurea by the addition of ciprofloxacin as the second ligand.

Ciprofloxacin (Hcip, Fig. 1) is an antibacterial drug used to treat diverse infections caused by Gram-negative bacteria, such as urinary tract infections, lower respiratory tract infections, bone and joint infections and typhoid fever.[7] In addition, ciprofloxacin can act as bidentate ligand and coordinate with transition metal through the pyridone oxygen and one carboxylate oxygen. Several binary and ternary complexes of metal ions $Mn^{2+}$, $Fe^{3+}$, $Co^{2+}$, $Ni^{2+}$, $MoO_2^{2+}$ and $Cu^{2+}$ with deprotonated ciprofloxacin have been studied in an attempt to investigate the structure, spectroscopic and biological properties.[8-10]

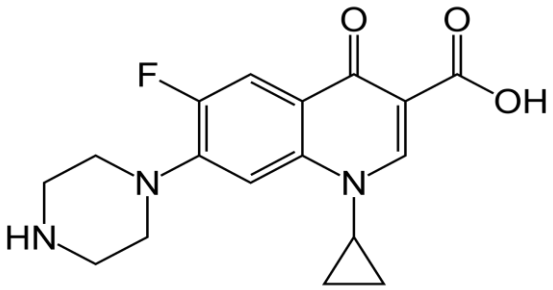


**Figure 1.** Ciprofloxacin (Hcip).

In this paper, we describe the synthesis, characterization of two new mix-ligand copper(II) complexes of amidino-*O*-methylurea and derivative with ciprofloxacin. Data on the biological activities of the synthesized complexes acting as an artificial nuclease, anticancer and antibacterial agents are also included.

## 2. Materials and Methods

### 2.1 Materials and physical measurements

Copper(II) chloride anhydrous, benzylamine, sodium dicyanamide and ciprofloxacin monochloride monohydrate were purchased from Sigma-Aldrich. The organic solvents and DI water with HPLC grade were purchased from Univar. Tris-HCl buffer (1 M) pH 7.0, 25X TAE (Tris-Acetate-EDTA) buffer and ethidium bromide (10 mg $mL^{-1}$) and agarose were purchased from Bio Basic INC. Plasmid pBR322 DNA (50 μg) was obtained from BBI Life Sciences. All chemical reagents were used as received and without further purification.

Elemental analysis (C, H, N) was performed with a CHNS/O elemental analyzer, Thermo Flash 2000. FT-IR spectra (4000 – 400 $cm^{-1}$) were recorded on a FT-IR spectrophotometer, Perkin Elmer, Spectrum One. Electronic absorption spectra were recorded on a UV-Vis-NIR scanning spectrophotometer, Shimazu, UV-3101PC (for the solid phase) and UV-Vis spectrophotometer, Jasco, V-730 (for the methanolic solution phase). Mass spectra were obtained on a liquid chromatography-micrOTOF mass spectrometer (LC-MS), Bruker. Gel electrophoresis was done on a horizontal electrophoresis system, Major Science and photographed under UV light on a Benchtop UV Transilluminator, UVP BioDoc-It Imaging System.

### 2.2 Synthesis of the copper(II) complexes of guanidine derivatives

The blue copper(II) complexes $[CuL^1Cl_2]_2$ and $[CuL^2Cl_2]_2$ were used as a starting complexes and prepared following the procedures as described in previous work.[5]

### 2.3 Synthesis of the mixed-ligand complexes (1 and 2)

The complex $[CuL^1Cl_2]_2$ (0.0501 g, 0.2 mmol) for **1** or $[CuL^2Cl_2]_2$ (0.0681 g, 0.2 mmol) for **2** in methanol (20 mL) was added to ciprofloxacin (cip) (0.1544 g, 0.4 mmol) deprotonated by 1 M NaOH (0.4 mL, 0.4 mmol). The resulting mixtures were adjusted the pH to 7.0 with 1 M NaOH and stirred at ambient temperature for 2 h. The excess solid was filtered off. The filtrate was kept overnight at low temperature. The purple product was obtained, filtered, washed with cold water and dried in a vacuum desiccator.

*$[Cu(L^1)(cip)]Cl{\cdot}2H_2O$* ***(1)***, Yield: 60%. M.p. 210 – 212 °C. Elemental analysis found (%): C, 41.62; H, 5.71; N, 17.72; calculated for $C_{20}H_{29}N_7O_6FCl$ (MW. = 581) (%): C, 41.27; H, 4.99; N, 16.85. FT-IR (KBr, $cm^{-1}$): 3486, $\nu_{as}(NH_2)$; 1630 $\nu_s(NH_2)$; 1623, $\nu$(CO); 1587 $\nu_{as}(CO_2)$; 1377, $\nu_s(CO_2)$. $ESI^+$ (*m*/*z*): 509, $[CuL^1(cip)]^+$.

*$[Cu(L^2)(cip)]Cl{\cdot}2H_2O$* ***(2)***, Yield: 72%. M.p. 238 – 241 °C. Elemental analysis found (%): C, 47.98; H, 5.56; N, 15.08; calculated for $C_{27}H_{35}N_7O_6FCl$ (MW. = 671) (%): C, 48.28; H, 5.21; N, 14.61. FT-IR (KBr, $cm^{-1}$): 3501, $\nu_{as}(NH_2)$; 1624 $\nu$(CO); 1583 $\nu_{as}(CO_2)$; 1381, $\nu_s(CO_2)$. $ESI^+$ (*m*/*z*): 599, $[CuL^2(cip) - H]^{2+}$.

### 2.4 DNA cleavage assay

Nuclease activity of the complexes was investigated by gel electrophoresis. Tris-

buffer (5 mM Tris-HCl/50 mM NaCl) containing 15% DMSO was used as a solvent. The samples were prepared by mixing plasmid pBR322 DNA (4361 bp, 0.2 μg) with the complexes (100 – 1000 μM) in the buffer (10 μL) and incubated at 37 °C for 24 h. A loading dye (2 μL) was added into the samples after incubation and subjected into 0.8% agarose gel immersed in a 1X TAE running buffer. The gel was electrophoresed at 50 V for 1 h, stained with EB for 5 min and photographed under UV light. To explore the possible DNA cleavage mechanism, $H_2O_2$ (10 μM) was further added into the samples containing plasmid pBR322 DNA and the complexes, and then incubated at 37 °C for 1 h.

**2.5 *In vitro* antimicrobial test**

Antibacterial activities of the complexes against two Gram-negative bacteria, *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* PAO1, and one Gram-positive bacteria, *Bacillus cereus* were investigated by optical density (OD) measurement at 600 nm (for *E. coli* and *Pseudomonas aeruginosa*)[11] and Resazurin Microplate assay (REMA)[12] (for *Bacillus cereus*). Amikacin and ofloxacin were used as positive control for *E. coli* and *Pseudomonas aeruginosa*, and vancomycin was used that for *Bacillus cereus*. The 0.5% DMSO was used as negative control for all tested bacteria. Bacterial growth was observed by $OD_{600}$ measurement using microplate reader. Percentage of bacterial inhibition was calculated by Eq. (1).

$$\% \text{ Inhibition} = [1- (OD_T/OD_C)] \times 100 \quad (1)$$

Where $OD_T$ and $OD_C$ represent the mean OD unit of cells treated with test compound and that treated with 0.5% DMSO, respectively. Similarly, fluorescence signals for REMA assay were measured using SpectraMax M5 multi-detection microplate reader (Molecular devices, USA) at the excitation and emission wavelengths of 530 and 590 nm, respectively. Inhibition percentage of the bacterial growth was calculated by Eq. (2).

$$\% \text{ Inhibition} = [1- (F_{UT}/F_{UC})] \times 100 \quad (2)$$

Where $F_{UT}$ and $F_{UC}$ are the mean fluorescence unit from the treated and untreated conditions by the copper(II) complexes, respectively. The MIC values defined as the lowest concentration of compound exhibiting 90% inhibition of bacterial growth after exposure incubation were also determined. This assay was performed in triplicate.

**2.6 *In vitro* anticancer test**

Anticancer activities of the complexes against 5 human cancer cell lines including KB (ATCC CCL-17), MCF-7 (ATCC HTB-22), NCI-H187 (ATCC CRL-5804), HepG2 (ATCC HB-8065) and CaCo2 (ATCC HTB-37) were investigated by Resazurin Microplate assay (REMA).[12] The anticancer activity of the copper(II) complexes was expressed as 50% inhibitory concentration ($IC_{50}$) determined from dose-response curves using the SOFTMax Pro software (Molecular devices, USA). The plotted data were obtained from 6 concentrations of 2-fold serially diluted samples.

## 3. Results & Discussion

### 3.1 General properties of the mixed-ligand complexes

The mixed-ligand complexes (**1** and **2**) were synthesized by the reaction of ciprofloxacin with the blue starting complexes $[CuL^1Cl_2]_2$ or $[CuL^2Cl_2]_2$ in a 2:1 mole ratio according to the proposed mechanism:

$$2Hcip{\cdot}HCl{\cdot}H_2O + 2NaOH \rightarrow 2cip^- + 2NaCl + 4H_2O \quad (3)$$

$$[CuL^{1/2}Cl_2]_2 + 2cip^- \rightarrow 2[CuL^{1/2}(cip)]Cl + 2Cl^- \quad (4)$$

$$2Hcip{\cdot}HCl{\cdot}H_2O + [CuL^{1/2}Cl_2]_2 \rightarrow 2[CuL^{1/2}(cip)]Cl + 2NaCl \quad (5)$$

Initially, ciprofloxacin was deprotonated by NaOH to obtain $cip^-$ form (3) and continually reacted with $[CuL^{1/2}Cl_2]_2$ (4). The overall

reaction as shown in (5). The change in color from blue to purple was observed after reacting with ciprofloxacin, indicating that the reactions have taken place. A by-product NaCl was removed by washing with water and checked by the $AgNO_3$ solution. All resulting complexes are soluble mainly in methanol, DMSO and slightly soluble in water. Additionally, they show high melting points, suggesting the stability in air. Elemental data (C, H and N) of the complexes are in good agreement with theoretical expectation.

### 3.2 Spectroscopic studies of the mixed-ligand complexes

The spectroscopic data of the complexes are summarized in Table 1. Figure 2 shows infrared spectra of **1**, **2**, ciprofloxacin, $[CuL^1Cl_2]_2$ and $[CuL^2Cl_2]_2$. The peak at 1709 $cm^{-1}$ assigned to the stretching vibrations of carboxylic group ($\nu(C=O)_{carb}$) of free ciprofloxacin is disappeared on complexation with the starting compounds and replaced by two strong characteristic bands at 1587 and 1377 $cm^{-1}$ for **1**, and 1583 and 1381 $cm^{-1}$ for **2** corresponding to asymmetric $\nu_{as}(CO_2)$ and symmetric $\nu_s(CO_2)$ stretching vibrations, respectively. The frequency separation ($\Delta\nu = \nu_{as}(CO_2)-\nu_s(CO_2)$) is used to determine the coordination mode of ciprofloxacin (Table 1). It is found in the range of 200 – 230 $cm^{-1}$ suggesting an unidentate bonding nature for the carboxylate group of ciprofloxacin.[13] In addition, the pyridone stretching $\nu(C=O)_p$ vibration at 1623 $cm^{-1}$ of ciprofloxacin is slightly shifted upon complexation. This result confirms that ciprofloxacin coordinates to the copper(II) center as chelating ligand through the carboxylate and pyridone oxygen atoms. Moreover, the vibrational bands of the $L^1$ or $L^2$ ligands are observed in the infrared spectra of **1** and **2**.

The mixed-ligand complexes (**1** and **2**) in the solid and methanolic solution phases displayed the similar d-d absorption band at ~18,000 $cm^{-1}$ indicating the stability of the square planar geometry of these compounds which is probably unchanged and not affected by the of solvent molecules. Moreover, the changed color from the blue starting complexes to the purple complexes **1** and **2** suggested that the environment around the copper(II) centers of **1** and **2** differs from that of their starting compounds.[5]

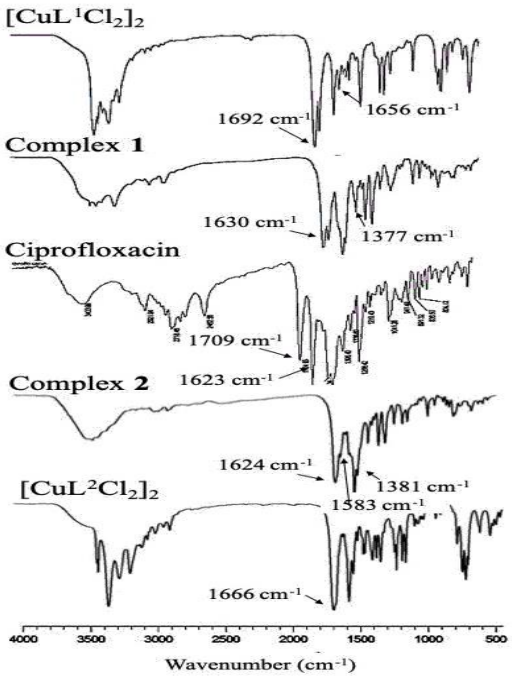


**Figure 2.** Overlayered FT-IR spectra of $[CuL^1Cl_2]_2$, $[CuL^2Cl_2]_2$, ciprofloxacin, **1** and **2**.

**Table 1.** Spectroscopic (FT-IR, electronic absorption and mass) measurement data of the complexes.

| Compound | FT-IR bands ($cm^{-1}$) | | | | Electronic absorption ($cm^{-1}$) | |
|---|---|---|---|---|---|---|
| | $\nu(C=O)$ | $\nu_{as}(CO_2)$ | $\nu_s(CO_2)$ | $\Delta^a$ | in solid | in solution |
| **1** | 1630 | 1587 | 1377 | 210 | 17 746 | 17 699 |
| **2** | 1624 | 1583 | 1381 | 202 | 17 921 | 17 794 |
| **Ciprofloxacin** | 1623 | | | | | |

[a] $\Delta = \nu_{as}(CO_2) - \nu_s(CO_2)$.

Mass spectra of the complexes (Figure 3) display the parent peaks of the molecular ions including $[CuL^1(cip)]^+$ (*m/z* 509) for **1** and $[CuL^2(cip)\text{-}H]^{2+}$ (*m/z* 599) for **2**. In addition, the peaks of other molecular ions observed in Figure 3a are probably generated during the ionization process.

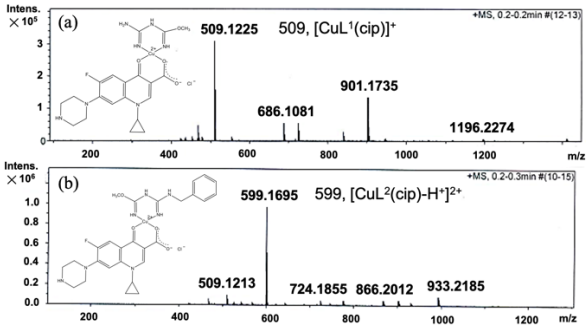


**Figure 3.** ESI+ mass spectra of the complexes (a) **1** and (b) **2**.

### 3.3 Cleavage of plasmid pBR322 DNA

Electrophoretic diagram (Figure 4) reveals that with enhancement of the complex concentration, only the extent of nicked Form II increased due to single-strand scission of supercoiled Form I initiated from 200 μM for **1** (lane 3a) and 100 μM for **2** (lane 2b). It suggests that DNA cleavage activity of **2** > **1**. In addition, $H_2O_2$ (10 μM) which itself does not cause any DNA degradation (Figure 5, lane 2) was added to investigate the possible DNA cleavage mechanism. Both complexes show much more efficiency in cleaving DNA in the presence of $H_2O_2$ as observed by the linear Form III (lane 4) and a tiny piece of DNA that looks like a smear (lanes 5-9 and 11-16). Possible DNA cleavage mechanism of copper(II) complexes in the presence of $H_2O_2$ under aerobic environment has been proposed to be an oxidative pathway.

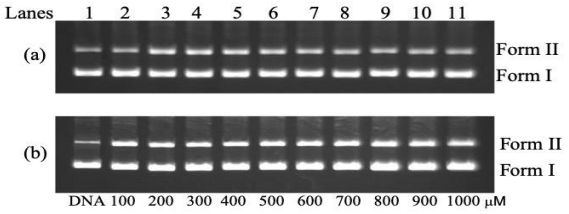


**Figure 4.** DNA cleavage of (a) **1** and (b) **2** toward pBR322 DNA.

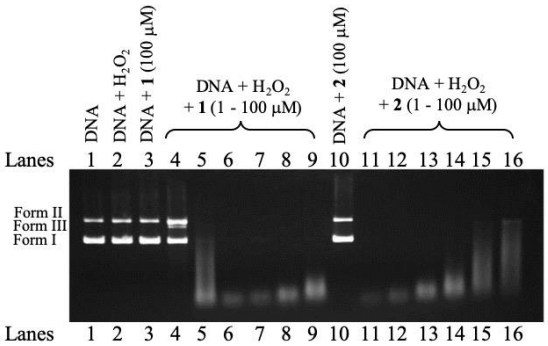


**Figure 5.** DNA cleavage of **1** and **2** toward pBR322 DNA in the present of $H_2O_2$.

### 3.4 *In vitro* antimicrobial activity

Antimicrobial activity of **1** and **2** further tested against *E. coli*, *Pseudomonas* and *Bacillus cereus* bacteria. The results reveal that **1** and **2** are active to all tested microorganisms while the starting complexes do not show inhibitory activity (Table 2). Both **1** and **2** are more potent against *E. coli* than *Pseudomonas aeruginosa* and *Bacillus cereus.* In particular complex **2** expresses significantly greater antimicrobial activity against *E. coli* than ciprofloxacin. These results confirm that the existence of ciprofloxacin in the copper(II) complexes can enhance the bactericidal activity.

**Table 2.** Antimicrobial activity expressed by MIC values for **1** and **2** and the related compounds.

| Compound | MIC [a] (μg mL$^{-1}$) ± S.D. | | |
|---|---|---|---|
| | *E. coli* | *Pseudomonas aeruginosa* | *Bacillus cereus* |
| **1** | 0.01323 ± 0.01069 | 0.16257 ± 0.0056 | 6.25 |
| **2** | 0.00637 ± 0.0057 | 0.0977 | 6.25 |
| $[CuL^1Cl_2]_2$ | Inactive | Inactive | Inactive |
| $[CuL^2Cl_2]_2$ | Inactive | Inactive | Inactive |
| Ciprofloxacin | 0.01627 ± 0.00704 | 0.0977 | 6.25 |

[a] MIC means the lowest concentration of the complexes which prevent visible growth of bacteria.

**Table 3.** Anticancer activity for **1** and **2** and the related compounds against five cancer cell lines.

| Compound | $IC_{50}$ [a] (μg $mL^{-1}$) | | | | |
|---|---|---|---|---|---|
| | KB [b] | MCF-7 [b] | NCI-H187 [b] | HepG2 [b] | CaCo2 [b] |
| **1** | Inactive | Inactive | Inactive | Inactive | Inactive |
| **2** | Inactive | Inactive | 36.71 | Inactive | Inactive |
| $[CuL^1Cl_2]_2$ | Inactive | Inactive | 49.42[6] | Inactive | Inactive |
| $[CuL^2Cl_2]_2$ | 22.51[6] | Inactive | 47.63[6] | 33.39 | 68.19 |

[a] $IC_{50}$ means the concentration of an inhibitor where the response (or binding) is reduced by 50%.
[b] Human cancer cell lines: KB (oral cavity cancer); MCF-7 (breast cancer)); NCI-H187 (small cell lung cancer); HepG2 (hepatocarcinoma) and CaCo2 (caucasian colon adenocarcinoma).

### 3.5 *In vitro* antiproliferative activity

The results of the anticancer activity of the complexes listed in Table 3 show that the copper(II) complexes containing ciprofloxacin are almost inactive against all tested human cancer cell lines excepting NCI-H187 for complex **2** with better activity than it starting compound $[CuL^2Cl_2]_2$. The reason is probably due to the complex structure of ciprofloxacin that can increase anticancer activity with more specific cancer cell lines, especially, the small cell lung cancer (NCI-H187).

## 4. Conclusion

In conclusion, we have demonstrated that ciprofloxacin can significantly increase the biological activity of **1** and **2**, particularly, antimicrobial activity against *E. coli*. It points to the possibility of further development of these two complexes as new antibacterial agents.

## Acknowledgements

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