Available Online

J. Sci. Res. **11** (3), 351-363 (2019)

**JOURNAL OF SCIENTIFIC RESEARCH**

www.banglajol.info/index.php/JSR

# Synthesis, Characterization and Biological Evaluation of Ciprofloxacin-*p*-nitrobenzoylated and Its Transition Metal Analogues

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Received 14 November 2018, accepted in final revised form 16 May 2019

**Abstract**

Six novel transition metal complexes of ciprofloxacin-*p*-nitro benzoyl derived from ciprofloxacin have been synthesized to find out their medicinal evaluation. It has been characterized by different techniques, i.e., UV-Vis, IR, NMR and mass spectrometry together with elemental analysis and molar conductivity. All the compounds were screened for their antimicrobial activities by the disc diffusion method. Cytotoxicity was also made against brine shrimp lethality assay. The antimicrobial activity of the analogues compared with the parent was evaluated against three Gram-positive, seven Gram-negative bacterial strains and three fungi. The synthesized compounds demonstrated a variety of antibacterial profiles among which most of the analogues showed a comparable or better activity compared to the ciprofloxacin. Moreover, unlike ciprofloxacin, most of the derivatives were also found to show antifungal activity. Interestingly, all the derivatives possessed an enhanced activity in comparison to the ciprofloxacin against *Candida albicans*. Regarding cytotoxicity, most of the derivatives showed to a greater degree cytotoxic agent compared to ciprofloxacin.

*Keywords*: Ciprofloxacin- *p*-nitro benzoylation; Transition metal complex; Antibacterial; Antifungal; Cytotoxicity.


doi: http://dx.doi.org/10.3329/jsr.v11i3.38843 J. Sci. Res. **11** (3), 351-363 (2019)

## 1. Introduction

Ciprofloxacin, 1-cyclopropyl-6-fluoro-l, 4-dihydro-4-oxo-7-(l-piperazinyl)-3-quinoline carboxylic acid ($C_{17}H_{18}FN_3O_3$) is a second generation broad spectrum belongs to fluoroquinolone antibiotic. It acts by inhibiting DNA gyrase [1-3]. Ciprofloxacin has been permitted to treat a lot of bacterial infections such as urinary tract infections, prostatitis, shigellosis, gonorrhea, continuous ambulatory peritoneal dialysis infections, diabetic foot infection, acute sinusitis, skin structure infections, bone and joint infections, infection

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diarrhea, also effected against bacillus anthrax [4-8], typhoid fever, etc. The World Health Organization states that it is one of the most regularly prescribed antimicrobial drugs [9]. It has as well shown anti-tumor activity against P388 leukemia [10]. Structure-activity relationship, mechanism of action, resistance and clinical aspects of some fluoroquinolones antibacterial activity have been reported [11]. A series of nadifloxacin derivatives have been synthesized and were found to show activity against hospital infections of multi-drug-resistance with vancomycin-resistant *Staphylococcus aureus* [12]. Effects of skeletal modification of ciprofloxacin on antimicrobial and cytotoxic activities have been observed some of its derivatives having antifungal properties [13]. Ciprofloxacin has been incorporated into a new series of Schiff base of 1, 2, 4-triazole via Mannich reaction and got comparable antibacterial results than ciprofloxacin [14]. NH-derivatives of ciprofloxacin have been prepared and showed enhanced activities against Gram-negatives bacteria compared to ciprofloxacin [15]. Metal complexes stay a significant resource for creating chemical range in the fields of biological, pharmaceutical and medicinal chemistry as antitumor and antimicrobial agents [16,17]. Moreover, a variety of compounds are introduced into the body by food or drugs, which may potentially correlate with metals and thus affecting their biological effect [18]. Many metal complexes have powerful antimicrobial activities and are already in frequent day-to-day use in the medicinal field such as metal clusters for the treatment of anti-HIV drugs, zinc oxide antiseptic creams, bismuth drugs for the cure of ulcers and silver bandages for blazes. The metal-based drugs are permitted to treat with drug-resistant bacteria and a range of viral diseases [19,20]. Most of the ciprofloxacin biological research has been focused on the functionality at the C-7 position or other functional groups but synthesis and biological research of metal complexes of ciprofloxacin or ciprofloxacin derivatives are not enough found in the literature. An initiative has been taken to substitute of the 2° amine of the piperazine moiety of ciprofloxacin and replaced with *p*-nitro benzoyl group to form ciprofloxacin- *p*-nitro benzoyl derivative, **2** and consequently, converted to its metal complex with inorganic salts, **3-8** for biological evaluation (Scheme-1).

In the present study, enlightens the synthesis, structure conformation and evaluation of biological activities, i.e., antibacterial, antifungal and cytotoxicity of ciprofloxacin- *p*-nitro benzoylation derivative and its transition metal complexes.

## 2. Materials and Methods

### 2.1. *General*

Gonoshasthaya Antibiotic Ltd, Savar, Dhaka, Bangladesh gifted ciprofloxacin hydrochloride. All the synthetic works were carried out by using laboratory reagents and analytical grade solvents whenever necessary. The solvents and reagents were purified and dried according to a standard procedure. The progress of all reactions was monitored by TLC, which was performed on aluminum sheets pre-coated with silica gel 60F254 to a thickness of 0.25 mm (Merck). The mobile phase was acetonitrile:conc. $NH_3$ solution:

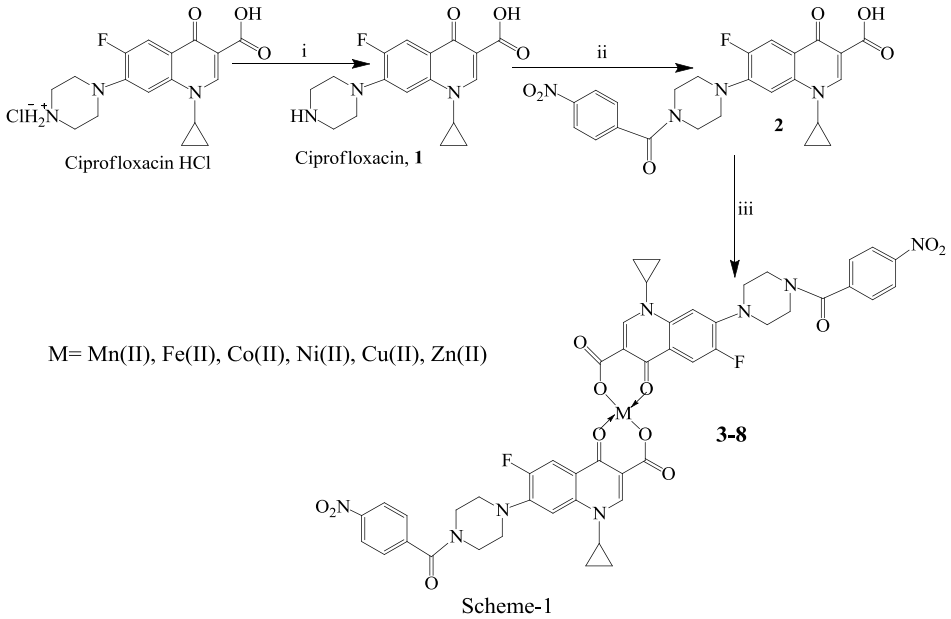
$CH_3OH$:$CH_2Cl_2$ (10:20:40:40). The chromatograms were visualized under ultraviolet light, 254 nm or iodine vapors. The purity of the compounds was examined by HPLC on an LC-20 AT liquid chromatography equipped with UV detector SPD-20A at 278 nm and column oven CTO-10ASvp, using a mobile phase of acetonitrile and phosphoric acid (2.45 g/L solution) in the ratio 13:87 and pH was adjusted at required (pH 3.0) with triethylamine. HPLC column was 250×4.6 mm length with 10 µL injection system. The column temperature was maintained at 40 °C during analysis, with flow rate of 1.5 mL/ min. The compounds were purified by re-crystallization using suitable solvents. The melting points of the synthesized compounds were determined in open capillaries using Veego VMP-1 apparatus and expressed in °C and are uncorrected. Molar conductance measurement made on a Multimeter (conductivity, TDS, DO, pH and temperature) in DMSO and the model of the instrument is Inolab Multi 9310P, WTW, Germany, and Serial No.15040594. The UV-Vis spectra of the compounds were recorded on a Shimadzu UV-1601PC spectrometer using DMSO as solvent $\lambda_{max}$ are expressed in nm. The IR spectra of the compounds were recorded on a Shimadzu FT-IR-8400s spectrometer using KBr pellet technique is expressed in $cm^{-1}$. $^1$H-NMR and $^{13}$C- NMR spectra were recorded on a Bruker DRX-400 (400 MHz FT-NMR) using DMSO solvent and TMS as an internal standard. Mass spectra were obtained using a Shimadzu LC-MS (ESI) 2010A spectrophotometer. Either protonated ions $(M + H)^+$ or sodium adducts $(M + Na)^+$ were used for empirical formula confirmation at the Department of Nano Fusion Technology, Organic Optoelectronic Material Lab., Pusan National University, South Korea. Elemental analyses (C, H, and N) were performed using a Carlo Erba NA-1500 analyzer. The *in vitro* antimicrobial activities of the analogues were carried out by disc diffusion method, and all the bacterial and fungal strains were collected as a pure culture from Vaccine Research Laboratory, Gonoshasthaya Kendra, Savar, Dhaka. Cytotoxicity measured by the brine shrimp lethality assay from the Department of Chemistry, Jahangirnagar University, Savar, Dhaka, Bangladesh.

### 2.2. *Regeneration of ciprofloxacin and general procedure for the synthesis of ciprofloxacin derivatives*

A solution of ciprofloxacin hydrochloride (5 g, 13.60 mmol) in water (50 mL) was treated with an excess of 5% aqueous sodium carbonate solution resulting in the formation of white precipitates, which were filtered through suction filter and left to dry as a neutral ciprofloxacin, **1** (4.2 g, 94%). This precipitate was used as starting material for the benzoylation reaction. Ciprofloxacin was 4-nitrobenzoylated, **2** with 4-nitro benzoyl chloride in 5% aqueous NaOH solution, and it was subsequently converted to its metal complexes **3-8** using Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Zn(II) inorganic salts (Scheme-1).

2.2.1. *Synthesis of p-nitrobenzoyl derivative of ciprofloxacin, 7-[-4-(-4-nitro-benzoyl)-piperazin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1, 4-dihydroquinoline -3-carboxylic acid, **2***

Ciprofloxacin (1.786 g, 5.39 mmol) was dissolved in 5% aqueous NaOH (10 mL) and was treated with finely powdered 4-nitrobenzoylchloride (1.00 g, 5.39 mmol) and the mixture was shaken vigorously in a stopper test tube. The reaction mixture was then acidified with dilute HCl after which the crystalline product was obtained. It was collected by filtration, washed with 60% ethanol and dried under vacuum in a desiccator. The physical properties, spectral data, and elemental analysis are given in sections 3.1.2 and 3.1.3.




Scheme 1. Synthesis of *p*-nitrobenzoyl derivative of ciprofloxacin.

2.2.2. *Synthesis of metal (II) complexes of ciprofloxacin derivative, **3-8***

The 4-nitrobenzoylation derivative**, 2** (ligand) was dissolved in 5% aq. NaOH solution. Then metal salts were added in mole ratio, (ligand, L: metal= 2:1) to the ligand solutions. The pH of each reaction mixture was adjusted to 8. The solutions were stirred for 24 hours through a crystal appeared after one or two hrs. Each of the reaction masses was filtered off, washed and dried under vacuum in a desiccator.

*Metal complex **3** (Mn(II) metal complex of ligand **2**)*: Ligand, **2** (0.50 g, 1.04 mmol); $MnSO_4$, $H_2O$ (0.088 g, 0.52 mmol); yield 0.360 g, 69.20 %.
*Metal complex **4** (Fe(II) metal complex of ligand **2**)*: Ligand, **2** (0.50 g, 1.04 mmol); $FeSO_4$, 7 $H_2O$ (0.166 g, 0.53 mmol); yield 0.402 g, 76.14 %.
*Metal complex **5** (Co(II) metal complex of ligand **2**)*: Ligand, **2** (0.36 g, 0.748 mmol); $CoSO_4$, 7 $H_2O$ (0.106 g, 0.376 mmol); yield 0.306 g, 80.76 %.

*Metal complex **6** (Ni(II) metal complex of ligand **2**)*: Ligand, **2** (0.60 g, 1.248 mmol); $NiCl_2$, 6 $H_2O$ (0.149g, 0.626 mmol); yield 0.460 g, 73.80%.
*Metal complex **7** (Cu(II) metal complex of ligand **2**)*: Ligand, **2** (0.360 g, 0.748 mmol); $CuSO_4$, $5H_2O$ (0.15g, 0.840 mmol); yield 0.370 g, 78.85 %.
*Metal complex **8** (Zn(II) metal complex of ligand **2**)*: Ligand, 2 (0.30 g, 0.62 mmol); $ZnSO_4$, $7H_2O$ (0.09g, 0.31 mmol); yield: 0.254 g, 79.43 %.

The metal complexes were insoluble in water, ethanol, methanol, chloroform, acetone, ether, ethylene glycol, 2-propanol, carbon tetrachloride, cyclohexanone, dichloromethane and soluble in dimethyl sulfoxide or hot methanol but decomposed in diluted solutions of all strong acids. Physico-analytical data, IR and UV-Vis spectral data of the metal complexes are given in Table 1, Table 2 and Table 3 respectively.

### 2.3. *Antimicrobial activity (In vitro)*

#### 2.3.1. *Antibacterial studies*

The antimicrobial activity of the derivatives was determined by the disc diffusion method [21,22] against Gram-positive, Gram-negative bacteria and antifungal strains. The organisms were accumulated as pure cultures. The experiments were carried out in triplicate using ciprofloxacin as standard and the results have been shown as mean ± SD. For the antibacterial study, 100 μg/mL stock solution of ciprofloxacin and its derivatives were prepared in hot methanol. Commercially available filter paper discs were drenched in the prepared drug and analogues solution, dried and applied on the surface of solid culture media (Nutrient agar), which had been streaked with standardized bacterial inoculums and incubated at 37 °C for 24 h. This method is based on the determination of an inhibited zone comparative to the bacterial susceptibility to the antibacterial present in the disc.

The compounds were screened for their antibacterial activity and compared with the parent against three different Gram-positive strains (*Staphylococcus aureus, Streptococci, Bacillus* spp) and seven Gram-negative strains, (*E. coli, Klebsiella pneumoniae, Pseudomonas* spp, *Salmonella* spp, *Salmonella typhi, Shigella dysenteriae,* and *V. cholera*). The results are mentioned in Tables 4 and 5.

#### 2.3.2. *Antifungal studies*

For the antifungal assay, 100 μg/mL stock solution of ciprofloxacin and its derivatives were prepared in hot methanol. The stock solutions were diluted to three different concentrations, i.e. 20, 40 and 60 μg/mL. Commercially available filter paper discs were impregnated with the prepared solutions of the drugs and its derivatives, dried and applied on the surface of the agar plate over which a culture of microorganism was already streaked. After 48 h of incubation at 37 °C, the clear zone of inhibition around the disc was determined; this is proportional to the fungal susceptibility for the fungal agent present in the disc. The results have been shown as mean ± SD.

Ciprofloxacin and its derivatives were screened for their antifungal action against the fungi; *Candida albicans, Fusarium solani* and *Aspergillus fumigatus* and compared with the parent as well as an antifungal drug miconazole nitrate. The results of antifungal activity are listed in Table 6.

### 2.4. *Cytotoxicity bioassay (in vitro)*

Cytotoxicity measurements by $LC_{50}$ calculation: The cytotoxic activity of the synthesized compounds was measured by brine shrimp lethality assay [23]. For determining cytotoxic activity 4.0 mg of each compound was dissolved in 10 mL of DMSO to get the first concentration 400 µg/mL and diluted to 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.781 and 0.0390 µg/mL using DMSO with the help of micropipette. An equal amount of the vincristine sulfate was dissolved in DMSO to get a preliminary concentration of 400 µg/mL from which solution with decreasing concentration was made by serial dilutions using DMSO to get 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.781 and 0.0390 µg/mL. Brine shrimps (*Artemia salina*) were hatched using brine shrimp eggs in a conical shaped vessel (1 L), filled with sterile artificial seawater (prepared using sea salt 38 g/L and adjusted to pH 8.5 using 1N NaOH) under constant aeration for 48 h. After hatching, active nauplii free from eggshells were collected from a brighter portion of the hatching chamber and used for the assay. Ten nauplii were drawn through a glass capillary and placed in each vial containing 4.5 mL of brine solution. In each experiment, 0.5 mL of the sample was added to 4.5 mL of brine solution and maintained at room temperature, 25 °C for 24 h under the light and surviving larvae were counted. The median lethal concentration $LC_{50}$ of the test samples was obtained by a plot of percentage of the shrimps killed against the logarithm of the sample concentration. The best-fit line was obtained from the graph. The readings were taken in triplicate. The anticancer drug vincristine sulfate was used as the positive control and DMSO as the negative control for the experiment. $LC_{50}$ results of the compounds are shown in Table 7.

## 3. Results and Discussion

### 3.1. *Chemistry*

#### 3.1.1. *Ligand compound, 2*

Ciprofloxacin was converted to *p*-nitrobenzoylated derivative, **2** with *p*-nitrobenzoyl chloride by Schotten-Baumann reaction with 73.60 % yield. The infrared spectra give a new stretching amidic C=O absorption band at 1664 $cm^{-1}$ as well as vanished the 2° amino N-H stretching band of ciprofloxacin at 3350 $cm^{-1}$ which indicated the presence of amidic C=O group in the molecule. In addition, the two new stretching bands at 1514 $cm^{-1}$ and 1315 $cm^{-1}$ supported the incorporation of the $NO_2$ group in the derivative which confirmed 2° amino group of ciprofloxacin had benzoylation with *p*-nitrobenzoyl chloride (sections 3.1.2 and 3.1.3).

3.1.2. *Physical properties, elemental analysis and spectral data of derivative, **2***

Physical constants, elemental analysis and MS: The product was obtained as white crystals; yield 1.90 g, 73.60 %; m.p. 225-226 °C; TLC $R_f$ 0.65; HPLC system had purity 99.34 %. *Anal*. calcd. for $C_{24}H_{21}FN_4O_6$: C, 60.00; H, 4.41; N, 11.66; Found: C, 61.38; H, 4.44; N, 11.51; ESI-MS m/z calcd. for $C_{24}H_{21}FN_4O_6$+ ($Na^+$) = 503.1343; Found: 503.1303.

3.1.3. *IR and NMR spectral data*

IR (KBr, ν $cm^{-1}$): 3420 (O-H str.); 3055 (C-H str., aromatic); 2922 (C-H str., $CH_2$); 1718 (C=O, COOH); 1664 (C=O, amide), 1627 (C=O str., conjugated pyridone); 1514, 1315 (N=O str., Ar-$NO_2$); 1490 (C-N str.); 1338 (C-O str.); 1267 (C-F str.); $^1$H-NMR (DMSO-$d_6$, 400 MHz): δ 11.02 (s, 1H, H-14, COOH); 8.67 (s, 1H, H-2, aryl H); 8.23 (dd, 2H, H-4', H-8', *p*-nitro benzoyl); 8.10 (dd, 2H, H-5', H-7', *p*-nitro benzoyl); 7.92 (d, 1H, $J_{HF}$= 13.2 Hz, H-5, aryl H); 7.58 (d, 1H, $J_{HF}$= 4.2 Hz, H-8, aryl H); 3.84 (m, 1H, H-11, cyclopropane); 3.43 (s, 4H, H-16, H-20 piperazinyl H); 3.17 (s, 4H, H-17, H-19, piperazinyl H); 1.31 (m, 2H, H-12, cyclopropane); 1.18 (m, 2H, H-13, cyclopropane); $^{13}$C-NMR (DMSO-$d_6$, 100 MHz): δ 212.11 (C-4, C=0 quinolone); 167.63 (C-2', amide); 166.64 (C-14, COOH); 154.32 (C-6); 149.54 (C-6', *p*-nitro benzoyl); 148.82 (C-2); 146.10 (C-7); 139.9 (C-10); 131.04 (C-3'); 128.01(2C, C-5', C-7', *p*-nitro benzoyl); 123.92 (2C, C-4', C-8', C *p*-nitro benzoyl); 111.73 (C-9); 107.53 (C-5); 107.3 (C-3); 99.15 (C-8); 36-39 (4C, C piperazin); 8-14 (3C, C cyclopropane)**.**

Further, we confirmed the benzoylation reaction with *p*-nitrobenzoyl chloride by NMR data. The $^1$H-NMR spectrum of **2** showed new two bands 2H dd in the aromatic region δ 8.10 and 8.23 for ortho and meta position protons of *p*-nitrobenzene ring that confirmed the introduction of a *p*-nitrobenzene ring in the derivative **2**. $^{13}$C spectrum is also consistent with this finding, C=O amidic carbon appeared at δ 167.63 and the *p*-nitrobenzoyl aromatic carbons appeared at δ123.92, 128.01, 131.04 and 149.54 where two pairs of carbon being equivalent overlapped with each other that again confirmed a *p*-nitrobenzene ring in the derivative **2**. The derivative, mp 225 °C showed m/z peak at 503.1303 appropriate for (M+Na), $C_{24}H_{21}$ $FN_4O_6Na$ and the elemental analysis results were in good agreement with the derivative. The above results clearly confirmed the molecular formula of the derivative, **2**.

3.1.4. *Metal complexes, [$ML_2$]*

Ciprofloxacin derivative, **2** was converted to its corresponding metal complexes **3**-**8** using Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Zn(II) salts. The ligand on interaction with metal salts in mole ratio, (ligand, L: metal= 2:1) yield complexes corresponding to the general proposed formula [$ML_2$]. The complexes were characterized based on physical constants, elemental analysis, FTIR, and UV-Vis spectra (Tables 1, 2 and 3). The complexes were colored, insoluble in water and common organic solvents. Besides, sharp different melting

point gave primary information about the formation of complexes. The low molar conductance values 12~17 $ohm^{-1}$ $cm^2$ $mol^{-1}$ of the complexes reveal their non-electrolytic in nature [24,25] and the elemental analysis results (% C, H, N, and M) of complexes were in very good agreement with the calculated ones, which established the proposed structural formula [$ML_2$].

Table 1. Physico-analytical data of the compounds **2-8.**

| Sample no. | Color | mp (±2 °C) | Yield % | Elemental analysis: Found (Calculated) % | | | |
|---|---|---|---|---|---|---|---|
| | | | | C | H | N | M |
| **2** | white | 225 | 73.60 | 60.38 (60.00) | 4.44 (4.41) | 11.51 (11.66) | - |
| **3** | cream | 289 | 69.30 | 56.81 (56.87) | 3.82 (3.98) | 11.01 (11.05) | 5.38 (5.42) |
| **4** | reddish | 243 | 76.14 | 56.43 (56.82) | 3.74 (3.97) | 11.01 (11.04) | 5.42 (5.50) |
| **5** | reddish | 248 | 80.76 | 56.78 (56.64) | 4.30 (3.96) | 11.08 (11.01) | 4.94 (5.79) |
| **6** | green | 255 | 73.80 | 56.58 (56.66) | 4.11 (3.96) | 11.08 (11.01) | 5.38 (6.77) |
| **7** | green | 262 | 78.85 | 56.48 (56.39) | 4.06 (3.94) | 11.18 (10.96) | 6.08 (6.22) |
| **8** | white | 267 | 79.43 | 56.34 (56.29) | 4.02 (3.94) | 11.05 (10.94) | 5.70 (6.38) |

Table 2. Selected IR absorption (KBr, ν $cm^{-1}$) of ligand and metal complexes.

| Group | Ligand | Metal complexes | | | | | |
|---|---|---|---|---|---|---|---|
| | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| ν O-H (COOH) | 3420 | -- | -- | -- | -- | -- | -- |
| ν C=O (COOH) | 1718 | -- | -- | -- | -- | -- | -- |
| νC-O (COOH) | 1338 | -- | -- | -- | -- | -- | -- |
| νasy COO- | -- | 1580 | 1572 | 1576 | 1574 | 1580 | 1576 |
| νsy COO- | -- | 1441 | 1438 | 1440 | 1441 | 1437 | 1440 |
| ν C=O Pyridone | 1627 | 1605 | 1609 | 1607 | 1608 | 1609 | 1612 |
| ν C-N | 1490 | 1484 | 1488 | 1486 | 1490 | 1490 | 1485 |
| ν C-F | 1267 | 1267 | 1267 | 1267 | 1267 | 1267 | 1267 |
| ν M-O | -- | 499 | 501 | 482 | 482 | 509 | 498 |

Table 3. Selected UV-Vis absorption and molar conductance.

| Sample no. | UV-Vis (DMSO, $\lambda_{max}$ nm) | | | ^ |
|---|---|---|---|---|
| | π- π* | d-d | n-π* | $ohm^{-1}$ $cm^2$ $mol^{-1}$ |
| 2 | 285 | - | 375 | -- |
| 3 | 264 | 596 | 374 | 12 |
| 4 | 258 | 553 | 364 | 17 |
| 5 | 218 | 692 | 407 | 14 |
| 6 | 220 | 698 | 404 | 14 |
| 7 | 215 | 682 | 401 | 17 |
| 8 | 215 | - | 403 | 12 |

There are four absorption bands in the IR spectrum of ligand at 3420, 1718, 1338 and 1627 $cm^{-1}$ assigned to νO-H of COOH, νC=O (COOH), νC-O (COOH) and νC=O (pyridone) stretching vibrations, respectively. On comparison of these IR frequencies, these bands completely vanished in the spectra of the metal complexes. Instead, the new strong absorption bands positioned at 1572–1580 and 1437-1441 $cm^{-1}$ indicating that νCOOH group emerged as two absorption bands νasyCOO and νsyCOO and its coordination with the metal atoms. Similarly, the band of pyridone moiety of ligand at 1627 $cm^{-1}$ disappeared and instead, a new band at 10–15 $cm^{-1}$ lower frequency (1606-1612 $cm^{-1}$) appeared in the complexes indicating the involvement of the carbonyl group in coordination. Likewise, the new peaks in the region at 480-515 $cm^{-1}$ can be assigned to stretching M-O in metal complexes. On the basis of these changes, we can propose that the ligand is acting as bidentate O-donor. The electronic spectra of the ligand showed two bands appeared at 285 nm and 375 nm, which assigned to π-π* and n-π* transition, respectively but it metal complexes showed two similar bands appeared at 215-286 nm and 374-407 nm, which can be assigned to π-π* and n-π* transition, respectively. The complexes of Mn (II), Fe(II) Co(II), Ni(II), and Cu(II) show new less intense bands in the region 553-698 nm, which can be assigned to d-d transition of metal ions except in Zn-complexes due to the occupied d-orbital in $Zn^{2+}$. The shifting of these bands in metal complexes together with color change authenticates complex formation. Taking into account the molar ratio of the reactants, as well as the obtained IR spectra for the synthesized complexes, we conclude that ligand, L coordinate with metal ions as bidentate O-donor ligand in a molar ratio of L: M = 2:1. One oxygen atom of the ligand carbonyl group and one oxygen atom from the deprotonated -COOH group are involved in chemical bonds with M(II) ions, forming the non-electrolytic tetrahedral complex $[ML_2]$. Similar research was performed by other scientists [26].

### 3.2. *Antibacterial test*

Zones of inhibition for Gram-positive and Gram-negative bacteria (Table 4 and 5) indicate that the benzoyl derivative, **2** and its metal complexes showed various degrees of activities compared to ciprofloxacin against the bacterial strains. The *p*-nitro-benzoyl derivative **2** (24.32±0.02 mm), metal complex, **3** (18.80±0.03 mm), **7** (18.60±0.01 mm) and **8** (26.42±0.02 mm) showed significantly enhanced activity compared to ciprofloxacin (18.20±0.05 mm) against *Staphylococcus aureus* but metal complex **4** (15.12±0.02 mm), **5** (14.52±0.02 mm) and **6** (13.10±0.05 mm) exhibited less activity compared to ciprofloxacin. The benzoyl derivative, **2** (17.50±0.05 mm) and Zn (II) metal complex, **8** (18.60±0.01 mm) showed significantly enhanced activities compared to ciprofloxacin (14.32±0.04 mm) but compounds **3** (14.82±0.02 mm), **4** (15.36±0.02 mm**), 5** (9.72±0.06 mm**), 6** (10.34±0.11 mm**)** and **7** (15.38±0.14 mm**)** exhibited less activity than parent (14.32±0.04 mm) against *Streptococci.* The metal compounds **3** (17.42±0.05 mm) and **8** (17.34±0.02 mm) were found to be enhanced activity compared to ciprofloxacin (16.36±0.01 mm) but derivatives **2**, **4**, **5**, **6** and **7** were found to be less or poor effective

against *Bacillus* spp compared to ciprofloxacin. The metal compound 3 (14.30±0.02 mm) and 5 (13.46±0.04 mm) were found to enhanced activity compared to ciprofloxacin (12.50±0.15 mm) but the rest of derivatives **2**, **4**, **6**, **7** and **8** were found to be less or no activity against Gram-negative bacteria *E. coli* compared to ciprofloxacin. Of the derivatives, metal compound **5** (24.82±0.11 mm) and 7 (25.24±0.08 mm) exhibited enhanced activity compared to ciprofloxacin (24.64±0.02 mm) whereas compound **8** (24.62±0.01 mm) showed similar activity and derivatives **2** (21.26±0.01 mm)**, 3** (18.44±0.05 mm)**, 4** (16.56±0.05 mm) and **6** (13.28±0.04 mm) showed poor activities compared to ciprofloxacin against *Klebsiella pneumoniae*. The metal complex derivatives, **3** (28.24±0.15 mm) and **8** (29.30±0.05 mm) showed enhanced activities compared to ciprofloxacin (28.22 ±0.04 mm) but the compounds **2 (**28.20±0.05 mm**), 4 (**23.26 ±0.02 mm**), 5 (**24.24±0.08 mm)**, 6 (**16.82±0.12 mm**)** & **7 (**14.42±0.04 mm**)** found to be less active compared to ciprofloxacin against *Pseudomonas* spp. Only the metal compound **7** (27.02±0.02 mm) was found to enhanced activity compared to ciprofloxacin (26.24±0.08 mm) but derivatives **2-6** and **8** showed less activity compared to ciprofloxacin against *Salmonella* spp. Only the metal compound **8** (27.68±0.08 mm) was found to enhanced activity compared to ciprofloxacin (26.24±0.08 mm) but derivatives **2-6** and **7** showed less activity compared to ciprofloxacin against *Salmonella typhi*. Only the metal compound **8** (28.38±0.02 mm) was found to enhanced activity compared to ciprofloxacin (26.24±0.08 mm) but derivatives **2-6** and **7** showed less activity compared to ciprofloxacin against *Shigella dysenteriae*. The derivative **7** (22.24±0.03 mm) showed enhanced activities compared to ciprofloxacin (21.72±0.04 mm) but compounds **2-6** and **8** were found to be less active compared to ciprofloxacin against *V. cholera*.

Table 4. Zone of inhibition (mm) of the compounds (100 µg/mL) against bacteria.

| Compound | Gram-positive bacteria | | | Gram-negative bacteria | |
|---|---|---|---|---|---|
| no. | a | b | c | d | e |
| 1 | 18.20±0.01 | 14.32±0.04 | 16.36±0.01 | 12.50 ±0.05 | 24.64±0.04 |
| 2 | 24.32±0.02 | 17.50±0.05 | 13.82±0.02 | 10.30±0.01 | 21.26±0.01 |
| 3 | 18.80±0.03 | 14.82±0.02 | 17.42±0.05 | 14.30±0.02 | 18.44±0.05 |
| 4 | 15.12±0.02 | 15.36±0.02 | 13.66±0.05 | 0 | 16.56±0.05 |
| 5 | 14.52±0.02 | 9.72±0.06 | 10.34±0.10 | 13.46±0.04 | 24.82±0.11 |
| 6 | 13.10±0.04 | 10.34±0.11 | 8.02±0.08 | 0 | 13.28±0.04 |
| 7 | 18.60±0.01 | 15.38±0.14 | 9.92±0.02 | 0 | 25.24±0.08 |
| 8 | 26.42±0.02 | 18.60±0.01 | 17.34±0.02 | 10.28±0.03 | 24.62±0.01 |

Gram-positive bacteria: a = *Staphylococcus aureus*, b = *Streptococci*, c = *Bacillus* spp and Gram-negative bacteria: d = *E. coli*, e = *Klebsiella pneumoniae*, f = *Pseudomonas* spp, g = *Salmonella* spp, h = *Salmonella typhi*, i = *Shigella dysenteriae*, j = *V. cholerae.*

Table 5. Zone of inhibition (mm) of the compounds (100 µg/mL) against bacteria.

| Compound | Gram-negative bacteria | | | | |
|---|---|---|---|---|---|
| no. | f | g | h | i | j |
| 1 | 28.22 ±0.04 | 26.24±0.08 | 26.24±0.06 | 26.24±0.08 | 21.72±0.04 |
| 2 | 28.20 ±0.05 | 15.22±0.06 | 24.30±0.03 | 24.48±0.05 | 15.62±0.05 |
| 3 | 28.24±0.15 | 19.28±0.25 | 17.62±0.04 | 17.38±0.04 | 11.48±0.04 |

| | | | | | |
|---|---|---|---|---|---|
| 4 | 23.26 ±0.02 | 13.66±0.15 | 14.32±0.06 | 21.88±0.03 | 10.62±0.02 |
| 5 | 24.24±0.08 | 17.72±0.18 | 12.48±0.15 | 22.24±0.07 | 10.68±0.08 |
| 6 | 16.82±0.12 | 18.38±0.05 | 13.61±0.25 | 21.36±0.06 | 11.66±0.05 |
| 7 | 14.42±0.04 | 27.02±0.02 | 26.40±0.12 | 26.72±0.02 | 22.24±0.03 |
| 8 | 29.30±0.05 | 25.88±0.12 | 27.68±0.08 | 28.38±0.02 | 20.38±0.01 |

### 3.3. *Antifungal activity*

Zones of inhibition for the fungi (Table 6) indicate that the derivatives, **2** (18.92±0.05 mm), **3** (18.10 ±0.15 mm), **4** (19.04 ±0.12 mm), **5** (18.14±0.06 mm), **6** (18.74 ±0.05 mm) **7** (19.62±0.01 mm) and **8** (25.04±0.03 mm) exhibited effective activities compared to ciprofloxacin (9.80 ±0.04 mm) against *Candida albicans* but less than that of miconazole nitrate (34.02±0.06 mm). Ciprofloxacin and its derivatives **2-8** exhibited poor activity against *Fusarium solani* and *Aspergillus fumigatus* compared to miconazole nitrate; however, among the derivatives compound, **8** found to be the most potent.

### 3.4. *Cytotoxicity*

The compounds **1-8** showed a varying degree of cytotoxic activities (Table 7). Most of the derivatives were found to have slightly more cytotoxic activities compared to ciprofloxacin. Among the compounds the lowest $LC_{50}$ is shown by derivatives **2 (**18.40 µg/mL), **5 (**18.20 µg/mL) and **8**, (18.04 µg/mL) confirmed the most potent cytotoxic agent compared to ciprofloxacin (36.50 µg/mL) but less than vincristine sulphate (0.78 µg/mL).

Table 6. Zone of inhibition (mm) of the compounds against various fungi.

| Compound no. | *Candida albicans* (µg/mL) | | | *Fusarium solani* (µg/mL) | | | *Aspergillus fumigatus* (µg/mL) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 20 | 40 | 60 | 20 | 40 | 60 | 20 | 40 | 60 |
| 1 | - | 8.04 ±0.04 | 9.80 ±0.04 | - | - | - | - | - | - |
| 2 | 15.18 ±0.01 | 17.02 ±0.08 | 18.92 ±0.05 | - | - | 8.08 ±0.02 | 8.10 ±0.15 | 9.82 ±0.12 | 10.68 ±0.02 |
| 3 | 13.46 ±0.02 | 15.84 ±0.14 | 18.10 ±0.15 | - | 8.12 ±0.04 | 9.22 ±0.02 | 8.04 ±0.01 | 8.96 ±0.03 | 10.02 ±0.02 |
| 4 | 12.54 ±0.11 | 15.22 ±0.02 | 19.04 ±0.12 | - | 8.71 ±0.08 | 9.64 ±0.34 | - | - | 8.62 ±0.04 |
| 5 | 11.34 ±0.06 | 14.44 ±0.15 | 18.14 ±0.06 | - | 9.46 ±0.16 | 10.84 ±0.04 | 8.02 ±0.02 | 9.30 ±0.06 | 11.20 ±0.05 |
| 6 | 11.08 ±0.10 | 14.12 ±0.04 | 18.74 ±0.05 | 8.20 ±0.07 | 9.28 ±0.02 | 10.02 ±0.01 | - | - | 8.22 ±0.06 |
| 7 | 10.42 ±0.18 | 16.32 ±0.06 | 19.62 ±0.01 | 8.60 ±0.02 | 10.21 ±0.01 | 10.72 ±0.04 | - | 8.06 ±0.04 | 10.14 ±0.12 |
| 8 | 18.20 ±0.02 | 24.24 ±0.04 | 25.04 ±0.03 | - | 8.04 ±0.06 | 10.44 ±0.15 | 8.40 ±0.02 | 10.80 ±0.05 | 12.92 ±0.10 |
| MN | 23.42 ±0.15 | 28.24 ±0.01 | 34.02 ±0.06 | 22.21 ±0.06 | 26.48 ±0.03 | 29.82 ±0.06 | 20.30 ±0.06 | 26.02 ±0.02 | 28.38 ±0.02 |

MN = Miconazole nitrate

Table 7. $LC_{50}$ of the compounds against brine shrimps.

| Compound no. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | VS |
|---|---|---|---|---|---|---|---|---|---|
| $LC_{50}$ (µg/mL) | 36.50 | 18.40 | 28.02 | 23.46 | 18.02 | 30.48 | 24.30 | 18.04 | 0.78 |

VS = Vincristine sulphate

## 4. Conclusion

In this paper, a new ligand and its six complexes have been successfully synthesized and characterized by various physico-chemical techniques. Based on the experimental data the ligand, *p*-nitrobenzoyl derivative of ciprofloxacin is a bidentate ligand. The molar conductance values expose that the ligand complexes were non-electrolyte in nature. The molar conductance values, elemental analysis, color, IR, and UV–Vis spectral observation suggested tetrahedral geometry of all metal complexes. The structural analogues of ciprofloxacin, **1** showed varying degree of antibacterial activity against the tested bacterial strains. Zones of inhibition of bacterial strains imply that *p*-nitro benzoyl derivative, **2** exhibited enhanced activity against *Staphylococcus aureus* & *Streptococci;* metal complex, **3** exhibited better activity against *Staphylococcus aureus*, *Bacillus* spp & *Pseudomonas* spp; **4** showed enhanced activity against only *Streptococci;* **5** showed better activity against *E. coli* & *Klebsiella pneumonia;* **7** exhibited enhanced activity against *Staphylococcus aureus*, *Streptococci*, *Klebsiella pneumoniae*, *Salmonella* spp, *Salmonella typhi*, *Shigella dysenteriae* & *V. cholera;* **8** exhibited better activity against *Staphylococcus aureus*, *Streptococci*, *Bacillus* spp *Pseudomonas* spp, *Salmonella typhi* & *Shigella dysenteriae* compared to ciprofloxacin. On the other hand, all the metal complexes, **3-8** possessed valuable antifungal properties against *Candida albicans* but poor activity against *Fusarium solani* and *Aspergillus fumigatus* compared with the parent, **1** did not demonstrate any activity. Most of the derivatives contained cytotoxic activity where derivatives **2**, **5** and **8** confirmed the most potent cytotoxic agent compared to ciprofloxacin. The comparison of the activities of different analogues of ciprofloxacin indicates that the amidic linkage of *p*-nitro benzoyl group at piperazine moiety and its transition metallic bond of the carboxylic group and the ketonic carbonyl function may be responsible for the change in the biological properties of the parent. The careful selection of the constituent for carboxylic group and the ketonic carbonyl function may result in more active biological activities based on ciprofloxacin.

## Acknowledgment

The authors express their thanks to Vaccine Research Laboratory, Gonoshasthaya Kendra, Savar, Dhaka for helpful suggestion to screen the antimicrobial effects of the compounds.

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