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ANTIBACTERIAL, ANTIFUNGAL AND CYTOTOXICITY STUDIES OF CIPROFLOXACIN-ACETYLATED AND ITS METAL COMPLEXES WITH Mn(II), Fe(II), Cu(II), Ni(II), Co(II) AND Zn(II) INORGANIC SALTS

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ABSTRACT

Some novel transition metal complexes of ciprofloxacin-acetylated derived from ciprofloxacin were synthesized and characterized on the basis of physical properties, elemental analysis, UV-VIS, IR, ESI-MS and NMR spectroscopy. All the compounds screened for their *in vitro* antibacterial and antifungal activities by agar well diffusion method. This acetylated derived, 2 and its metal complexes 3-8 were evaluated for their antibacterial activity against several bacterial strains and antifungal activity against *Candida albicans.* Cytotoxic activity also made against brine shrimp lethality assay. The acetylated derivative showed enhanced antibacterial activity compared to ciprofloxacin against most of the bacterial strains but the metal complexes possessed lower activities compared to acetylated derivative as well as ciprofloxacin. Among the metal complexes 3-8, Zn (II) derivative, 8 is more potent antibacterial. Interesting all of the derivatives has shown enhanced activities against *Candida albicans* compared to ciprofloxacin. Among which metal complexes 4, 5, 7 and 8 were found to be more active not only than ciprofloxacin but as well as for an antifungal drug, miconazole nitrate. Regarding cytotoxicity, most of the derivatives showed better cytotoxic agent than ciprofloxacin.

Keywords: Ciprofloxacin-acetylated, Metal complex, Antibacterial, Antifungal, Cytotoxicity.

INTRODUCTION

Ciprofloxacin hydrochloride belongs to the second-generation quinolone antibiotic which acts by inhibiting DNA gyrase, a type II topoisomerase and topoisomerase IV, enzymes necessary to separate bacterial DNA, thereby inhibiting cell division [1-3]. Ciprofloxacin has been approved for the treatment of urinary tract infections, prostatitis [4], shigellosis [5], gonorrhea, continuous ambulatory peritoneal dialysis infections [6], some diabetic foot infection [7], acute sinusitis, skin and skin structure infections, bone and joint infections, infection diarrhoea, also effected against Bacillus anthrax [8], typhoid fever etc. It has also been found to show anti-tumor activity against P388 leukemia [9]. Structure - Activity-Relationship, mechanism of action, resistance and clinical aspects of some fluoroquinolones antibacterial activity has been reported [10]. A series of benzoquinolizine-2-carboxylic acid arginine salt of nadifloxacin have been synthesized and were found to show activity against hospital infections of multi-drug-resistance with vancomycin-resistant *staphylococcus aureus* [11]. Effects of skeletal modification of ciprofloxacin on antibacterial, antifungal and cytotoxic activities have been observed and described that some of its derivatives having antifungal properties [12]. Ciprofloxacin have been incorporated to new series of Schiff base of 1, 2, 4-triazole via Mannich reaction and got comparable or superior antibacterial results than ciprofloxacin [13]. The pyrazolone derivatives with

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pyrazole ring extension have been synthesized from ciprofloxacin which showed more potential cytotoxicity than ciprofloxacin against brine shrimp neoplasm [14]. Five NH-derivatives of ciprofloxacin have been prepared by acetylation /benzoylation on ciprofloxacin. All of the derivatives showed enhanced activities against Gram-negatives bacteria compared to ciprofloxacin. Regarding the antifungal activity all of the compounds showed higher activity than ciprofloxacin and one of them showed enhanced activity than an antifungal drug miconazole nitrate [15].

Many metal complexes have powerful antimicrobial activities and are already in common day-to-day use in medicinal field such as silver bandages for treatment of burns, zinc antiseptic creams, bismuth drugs for the treatment of ulcers and metal clusters as anti-HIV drugs. Cu (II) complex of ciprofloxacin have been synthesized and found good antibacterial activity [16]. In Vitro antibacterial studies of ciprofloxacin-imines and their complexes with Cu (II), Ni (II), Co (II), and Zn (II) have been screened and obtained enhanced activities against Gram-positive and Gram-negative several bacterial strains [17].

The present work involves finding out the antibacterial, antifungal and cytotoxic activity of ciprofloxacin-acetylated derivative and its metal complexes. Our expectation is that these derivatives may be increased the biological activities such as antibacterial, antifungal and cytotoxic activities. We undertook the present study with the aim of substitution of the 2^0 amine of piperazine moiety of the parent molecule will be replaced with acetamido group to form derivative, **2** and subsequently converted to its metal complexes with Mn(II), Fe(II), Cu(II), Ni(II), Co(II) and Zn(II) inorganic salts in order to check the influence of newly introduced residue on the antibacterial, antifungal and cytotoxic properties of the ciprofloxacin, **1** (Scheme-1).

MATERIALS AND METHODS

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 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). 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 were examined by HPLC on a LC-20 AT liquid chromatograph equipped with UV detector SPD-20A at 278 nm and column oven CTO-10ASvp, using a mobile phase of acetonitrile and phosphoric acid (2.45g/L solution) in the ratio 13:87 and pH 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 40^0 C during analysis, with flow rate 1.5 mL/ min.

The compounds were purified by recrystallisation 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.

The UV-VIS spectra of the compounds were recorded on a Shimadzu UV-1601PC spectrometer using DMSO as solvent at the Gonoshasthaya Antibiotic Limited, Savar, Dhaka and λ_{max} are expressed in nm. The IR spectra of the compounds were recorded on Shimadzu FT-IR-8400s spectrometer using KBr pellet technique at the Gonoshasthaya Antibiotic Limited, Savar, Dhaka and Wazed Miah Science Research Centre, Jahangirnagar University, Savar, Dhaka and are expressed in cm^{-1}. ^{1}H-NMR and ^{13}C- NMR spectra were recorded on Bruker DRX-300 (300 MHz FT-NMR) & (75 MHz FT-NMR) using $CDCl_3$ solvent and TMS as internal standard at the Department of Nano Fusion Technology, Organic Optoelectronic Material Lab., Pusan National University, South Korea. Mass spectra were obtained using Shimadzu LC-MS (ESI) 2010A spectrophotometer and Elemental analysis (C, H, N) were obtained using a Carlo Erba NA-1500 analyzer at the Pusan National University, South Korea.

Regeneration of ciprofloxacin and synthesis of ciprofloxacin derivatives

A solution of ciprofloxacin hydrochloride (10 g, 27.19 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** (8.8 g, 98%).These precipitates were pure enough and used to as starting material for the acetylated reaction without purification. Ciprofloxacin was acetylated **2**, with acetic anhydride in acetic acid with 80% yield 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).

Experimental Protocol

Acetylation of Ciprofloxacin, 7-(4-N-acetyl-piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1, 4-dihydro-quinoline-3-carboxylic acid, **2**.

Ciprofloxacin (5.00 g, 15.09 mmol) was dissolved in acetic acid 40 mL and the solution was treated with acetic anhydride (1.55 mL). The reaction mixture was stirred at 60^0 C for one hour and allowed to cool for crystallization. The precipitate was collected by filtration, washed with 60% aqueous ethanol and dried under vacuum in a desiccator. The compound **2** was white crystalline solid; m.p. 255^0C (decomp.); yield 4.5 g, 80%. The purity of the compound was checked by TLC, R_f 0.89; mobile phase, acetonitrile: conc NH_3 solution: CH_3OH:

CH_2Cl_2(10: 20: 40: 40) and HPLC (system) purity was 99%.

UV-VIS: λ_{max} (DMSO), 275, 292, 396 nm; IR (KBr, ν cm^{-1}): 3402-2400(O-H str., H-bonded), 3093, 2917, 2853(C-H str., aromatic and aliphatic), 1720(C=O str., free -COOH), 1628(C=O str., keto conjugated), 1465 (C-N str.), 1392 (C-O str.), 1300 (C-F str.).

^{1}H-NMR ($CDCl_3$): δ 1.22(dd, 2H, *J*= 6.6, 4.2 Hz, H-8,8'), 1.41 (dd, 2H, *J* = 7.2, 6.6 Hz, H-7,7'), 1.69(s, 3H, H-9), 3.30(t, 4H, *J*= 4.2 Hz, H-1,1'), 3.37 (t, 4H, H-2,2'), 3.53-3.59(m, 1H, H-6), 7.37(d, 1H, J_{HF}= 6.6Hz, H-3), 8.02(d, 1H, J_{HF}= 12.6 Hz, H-4), 8.75(s, 1H, H-5), 11.22(s, 1H, H-10); ^{13}C-NMR ($CDCl_3$): δ 9.52(C-11, 11'), 22.56(C-15), 36.56(C-10), 47.31(C-13,13'), 51.44(C-12,12'), 97.35(C-8), 106.4(C-3), 109.46(C-5), 113.9(C-4a), 140.2(C-8a), 146.67(C-7), 148.84(C-2), 154.0(C-6), 168.09(C-9,-COOH), 170.5(C-14, amide), 178.3 (C-4,quinolinone C=O) Fig 1; Elemental Analysis: found % C 61.31, H 5.45, N 11.20; calculated C 61.12, H 5.40, N 11.21 for $C_{19}H_{20}FN_3O_4$; ESI-MS: 374.2095 (M+ H) for $C_{19}H_{21}FN_3O_4$.

Synthesis of Metal (II) Complexes of Ciprofloxacin Derivatives, 3-8

General synthesis procedure [18]: The acetylated derivative**, 2** (ligand) was dissolved in 5% aq. NaOH solution. Then metal salts were added (mole ratio, ligand : metals= 2:1) to the ligand solutions. The pH of the each reaction mixture was adjusted to 8. The solutions were stirred for 24 hours though a precipitate appeared after just an hour. Each of the reaction mass was filtered off, washed and dried under vacuum in a desiccator.

Mn (II) metal complex of ligand **2**, (metal complex **3**): Reagents: Ligand **2** = 0.50 g, 1.34 mmol; Mn SO_4, H_2O = 0.12 g, 0.71 mmol ; yield: 0.45 g, 84 %
Fe (II) metal complex of ligand **2**, (metal complex **4**): Reagents: Ligand **2** = 0.50 g, 1.34 mmol; Fe SO_4, 7 H_2O = 0.215 g, 0.67 mmol; yield: 0.42 g, 78 %
Co (II) metal complex of ligand **2**, (metal complex **5**): Reagents: Ligand **2** = 0.532 g, 1.42 mmol; Co SO_4, 7 H_2O = 0.20 g, 0.71 mmol; yield: 0.42 g, 73.45 %
Ni (II) metal complex of ligand **2**, (metal complex **6**): Reagents: Ligand **2** = 0.63 g, 1.68 mmol; $NiCl_2$, 6 H_2O = 0.20g, 0.84 mmol; yield: 0.54 g, 79.67%
Cu (II) metal complex of ligand **2**, (metal complex **7**): Reagents: Ligand **2** = 0.45 g, 1.20 mmol; $CuSO_4$, $5H_2O$ = 0.15g, 0.60 mmol; yield: 0.39 g, 80 %
Zn (II) metal complex of ligand **2**, (metal complex **8**): Reagents: Ligand 2 = 0.39 g, 1.04 mmol; $ZnSO_4$, $7H_2O$ = 0.15g, 0.52 mmol; yield: 0.38 g, 89.8 %

The metal complexes **3-8** were insoluble in water, ethanol, methanol, chloroform, acetone, ether, ethylene glycol, 2-propanol, carbon tetrachloride, cyclohexanone, dichloromethane and partially soluble in dimethyl sulfoxide but decomposed in diluted solutions of all strong acids. Physico-analytical data of the metal complexes are given in Table-1 and UV-VIS & IR data are given in Table-2.

Antibacterial Studies (*In vitro*)

Materials and methods: Agar well diffusion method [15] was employed to study, the antibacterial activity. Prepared the culture plate by 16 mL of Muller-Hinton agar for each Petridis and the diameter of each Petridis was 80 mm. In the agar well diffusion method wells were drugged in the media with the help of a sterile metallic borer. Two to eight hours old bacterial inoculums containing approximately 104~106 colony forming units (CFU/mL) were spread on the surface of agar with the help of a sterile cotton swab. Afterward 0.025 mL (100 µg) of (concentration 4 mg/mL of DMSO) solution of each sample was added in the designated wells. The plate was incubated immediately at 37^0 C for 20 h. Antibacterial activity was determined by measuring the diameter (mm) of zones showing extent of inhibition. The study was carried out in the department of Microbiology, Gono Bishwabidyalay, Savar, Dhaka-1344, Bangladesh.

Antibacterial (*in vitro)* results: The derivatives of ciprofloxacin 2-8 were screened for their antimicrobial affects against three Gram-positive organisms namely *Staphylococcus aureus, Streptococci* and *Bacillus spp*; and seven Gram-negative organisms e.g. *E. coli, Klebsiella pneumoniae, Pseudomonas spp, Salmonella spp, Salmonella typhi, Shigella dysenteriae* and *V. cholerae*. The results are summarized in Table-3.

Antifungal Studies (*In vitro*)

Materials and methods: Agar well diffusion method [15] was employed to study, the antifungal activity. Prepared the each culture plate by 16 mL of Muller-Hinton agar for each Petridis and the diameter of each Petridis was 80 mm. In the agar well diffusion method wells were drugged in the media with the help of a sterile metallic borer. Approximately 105 colony forming units (CFU/mL) fungal spore suspension were spread on the surface of agar with the help of a sterile cotton swab and then 0.010 mL (40 µg) of (concentration 4 mg/mL of DMSO) solution of each sample was added in the designated wells. The plate was incubated immediately at 37^0 C for 48 h. Antifungal activities were determined by measuring the diameter (mm) of zones showing extent inhibition. The antifungal activities were carried out in the department of Microbiology, Gono Bishwabidyalay, Savar, Dhaka-1344, Bangladesh.

Antifungal activity results (*in vitro*): All of the synthesized compounds 2-8 were screened for their antifungal affects against *Candida albicans* and compared with ciprofloxacin, 1 as well as an antifungal drug, miconazole nitrate. Miconazole nitrate was used as

standard drug. The results of (*in vitro*) antifungal activity are collected in Table-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 [19]. For determining cytotoxic activity 4.0 mg of each compound was dissolved in 10 mL of DMSO to get the initial concentration 400 µg / mL and diluted to 200, 100,50, 25, 12.5, 6.25, 3.125, 1.563, 0.781 µg / mL using DMSO with the help of micropipette. Measured amount of the vincristine sulphate was dissolved in DMSO to get an initial concentration of 20 µg / mL from which solution with decreasing concentration were made by serial dilutions using DMSO to get 10, 5, 2.5, 1.25, 0.625, 0.3125,0.15625,0.078125 and 0.0390 µg / mL. The test samples were then applied against *Artemia salina* in a 24 h *in vitro* assay. Vincristine sulphate was used as the positive control and DMSO as the negative control for the brine shrimp nauplii. The LC_{50} was determined from % of mortality vs log concentration graph. The bioassay was performed in triplicate. The cytotoxic activities were carried out in the department of Chemistry, Jahangirnagar University, Savar, Dhaka-1342. Cytotoxicity results (*in vitro*) for the compounds 1-8 are shown in tabular form in Table-5.

RESULTS AND DISCUSSION

Acetylation of Ciprofloxacin, 2

Ciprofloxacin was acetylated with acetic anhydride in acetic acid with 80% yield. The derivative, mp 255^0 C (decomp.) showed m/z peak at 374.2095 appropriate for (M+H), $C_{19}H_{21}FN_3O_4$. Derivative **2** was 99.0% pure as determined by HPLC. The elemental analysis (% C, H and N) gave satisfactory results in agreement with the molecular formula. The IR spectrum of **2** did not show any band at 1587 cm^{-1} for COO^- as observed in ciprofloxacin as acetylation of the secondary amine has destroyed the zwitter ion [20] character of ciprofloxacin. The moderately strong band at 2700-2250 cm^{-1} for NH_2^+ had also disappeared. The acetyl carbonyl in the form of an amide appeared at 1628 cm^{-1} which probably overlapped with the absorption for conjugated C=O group. That ciprofloxacin was converted to its acetyl derivative **2** was clearly shown by the ^{1}H- and ^{13}C-NMR spectrum. The methyl protons of the acetamido group appeared as a 3H singlet at δ 1.69 and a singlet at δ11.22 also appeared for free COOH which are not present in ciprofloxacin [21-22]. The other peaks were identical with that shown by ciprofloxacin. In the ^{13}C spectrum carbon of the acetamido group was seen at δ 170.5, a new one with respect to that of ciprofloxacin and a new peak δ 22.56 due to acetamido methyl carbon.

Metal (II) Complexes of Ciprofloxacin Derivatives, 3-8

In biological systems drugs are often observed to coordinate with metals and influence the activities of the drugs. In order to study the coordinating property of ciprofloxacin and its derivative, metal complexes of a number of them were prepared **3-8.** Mn (II) metal complex of ligand **2**, (metal complex **3**); Fe (II) metal complex of ligand **2**, (metal complex **4**); Co (II) metal complex of ligand **2**, (metal complex **5**); Ni (II) metal complex of ligand **2**, (metal complex **6**); Cu (II) metal complex of ligand **2**, (metal complex **7**) and Zn (II) metal complex of ligand **2**, (metal complex **8**).

The metal (II) complexes **3-8** were characterized on the basis of physical constants, % of C, H, N & M and UV-VIS, IR spectra. Ciprofloxacin derivative **2** was converted to their corresponding metal derivatives **3-8** using Mn (II), Fe (II), Co (II), Ni (II), Cu (II) and Zn (II) salts. The physico-analytical data of the metal complexes, **3-8** are shown in Table-1. However the elemental analysis results (% C, H, N and M) of those metal complexes gave satisfactory results which were established molecular formula. The IR and UV-VIS absorptions of the complexes are summarized in Table-2. All metal complexes to record the HPLC data and NMR spectra failed as the complexes were insoluble in common organic solvents. In the IR spectrum of all the metal complexes shifting of C=O absorption from the range 1718- 1722 cm^{-1} to 1624- 1629 cm^{-1} suggested that the group has coordinated with the metal ions. The expected shift of the carbonyl absorption to lower field was not either visible separately as it probably had overlapped with the amido carbonyl group. The formation of metal complexes is further confirmed by the IR absorptions in the region 480-515 cm^{-1} for M-O stretching. The UV-VIS spectra of the complexes, as expected showed the appearance of new bands in the visible range, 490-710 nm except in Zn-complexes probably due to the occupied d-orbital in Zn^{2+}.

Antibacterial Activity of Ciprofloxacin Derivatives

Inhibition zones for Gram-positive and Gram-negative bacteria (Table-3) indicate that the acetylated derivative **2** showed enhanced activities compared to ciprofloxacin against most of the bacterial strains. The inhibition zone (22 mm) for **2** against *Staphylococcus aureus* is higher than that of ciprofloxacin (18 mm). Similarly, metal compounds **3** (20 mm), **4** (18 mm), **5** (18 mm); **6** (19 mm), **7** (16 mm) and 8 (22 mm) also exhibits significant activity against *Staphylococcus aureus*. Derivative 2 (17.5 mm), metal complex **4** (17 mm) and 8 (16 mm) showed moderate enhanced activities against *Streptococci* compared to ciprofloxacin (14 mm). Derivative **2** (22 mm) showed enhanced activities against *Bacillus spp* compared to ciprofloxacin (16 mm). Only Zn (II) metal complex, **8** (16 mm) showed similar activity against *Bacillus spp* compared to ciprofloxacin (16 mm) whereas **3-7** possessed lower activities. Compound 2 showed significant enhanced activities and its metal

complexes 3-8 found to be less active against *Pseudomonas spp, Salmonella typhi* and *Shigella dysenteriae* compared to ciprofloxacin. On the other hand, both compound **2** and its metal complexes **3-8** were found to be poor activity against *E. coli, Klebsiella pneumoniae, Salmonella spp,* and *V. cholerae* compared to ciprofloxacin.

In summary, it is observed that derivative **2** showed enhanced activities compared to ciprofloxacin against most of the Gram-positive and Gram-negative strains. The metal complexes **3-8** exhibited poor activity compared to ciprofloxacin against most of the Gram-positive and Gram-negative strains. However metal complex, **8** exhibited better activity against most of the Gram-positive and Gram-negative strains compared to metal complexes, **3-7**.

Antifungal Activity of Ciprofloxacin Derivatives

Derivatives **2-8** were screened for their antifungal activity against *Candida albicans* which revealed that all the compounds showed enhanced activities than ciprofloxacin but less than that of miconazole nitrate whereas metal complexes **4**, **5, 7** and **8** were found to be more active not only than ciprofloxacin but also that of miconazole nitrate (Table-4).

Cytotoxicity of Ciprofloxacin Derivatives

The derivatives **2-8** showed a varying degree of cytotoxic activities. Of these compounds the lowest LC_{50} is shown by derivative **4,** LC_{50} 1.519 µg / mL meaning that it possesses the most potent cytotoxic agent. Most of the derivatives were found to have generally slightly more or similar cytotoxic activities compared to ciprofloxacin (Table-5).

Table 1. Physico-analytical data of (ligand) 2 and its metal complexes, 3-8

Prod. N0.	mp ^{0}C	Color	Yield %	Elemental analysis: Found (Calculated) %			
				C	H	N	M
2	255	white	80.0	61.31 (61.12)	5.45 (5.40)	11.20 (11.21)	---
3	287	cream	78.3	56.89 (57.07)	4.72 (4.79)	10.44 (10.53)	6.90 (6.87)
4	249	reddish	68.6	56.92 (57.01)	4.74 (4.78)	10.43 (10.50)	6.83 (6.98)
5	285	reddish	73.4	56.85 (56.79)	4.80 (4.77)	10.48 (10.46)	7.02 (7.33)
6	245	green	79.6	56.83 (56.81)	4.84 (4.77)	10.49 (10.47)	7.06 (7.31)
7	250	green	80.0	56.49 (56.47)	4.78 (4.74)	10.48 (10.40)	7.68 (7.86)
8	272	white	89.8	56.42 (56.34)	4.81 (4.73)	10.43 (10.37)	7.86 (8.07)
2	255	white	80.0	61.31 (61.12)	5.45 (5.40)	11.20 (11.21)	---

Table 2. UV-VIS and IR data of (ligand) 2 and its metal complexes, 3-8

Sample no	IR					UV-VIS		
	KBr, ν cm^{-1}					λ_{max} (DMSO) nm		
	C=O (COOH)	C=O (amide & keto)	C-N	C-O	O-M	π- π*	d-d	n-π*
2	1720	1627	1463	1340	-	275	-	392
3	-	1625	1482	1308	498	250	592	384
4	-	1624	1479	1298	511	254	533	394
5	-	1628	1466	1322	495	252	494	339
6	-	1624	1480	1304	496	251	557	392
7	-	1624	1479	1298	511	252	704	403
8	-	1626	1477	1338	487	251	-	392

Table 3. Antibacterial activates results (*In vitro*) of ciprofloxacin, 1 and its derivatives 2-8 against Gram-positive and Gram-negative bacteria

Sample number	Dose (µg)	Microbial species									
		a	b	c	d	e	f	g	h	i	j
1	100	18	14	16	12.5	31	34	38	32	34	22
2	100	22	17.5	22	8	21	45	24	35	34.5	21
3	100	20	12	12	0	18	26	16	19	24	12
4	100	18	17	10	0	17	32	25	16	25	11
5	100	18	10	08	0	17	26	17	12	22	10
6	100	19	09	08	0	16	26	18	13	21	11
7	100	16	10	09	0	14	27	13	16	24	14
8	100	22	18	16	0	27	32	24	27	29	22

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 4. Antifungal activity results (*in vitro*) against *Candida albicans* for 1, 2-8 and an antifungal agent miconazole nitrate

Sample notation	**Dose (µg)**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**mn**
Candida albicans	40	10	24	23.5	28.5	30	27	28	33	28

mn = Miconazole nitrate

Table 5. The brine shrimp lethality assay results of ciprofloxacin, 1 and its derivatives 2-8

Sample notation	**1**	**2**	**3**	**4**	**5**	**6**	7	8	VS
LC_{50} in µg / mL	1.632	1.544	1.544	1.519	1.525	1.568	1.562	1.700	0.539

VS = Vincristine sulphate (used as standard)

(Scheme-1)

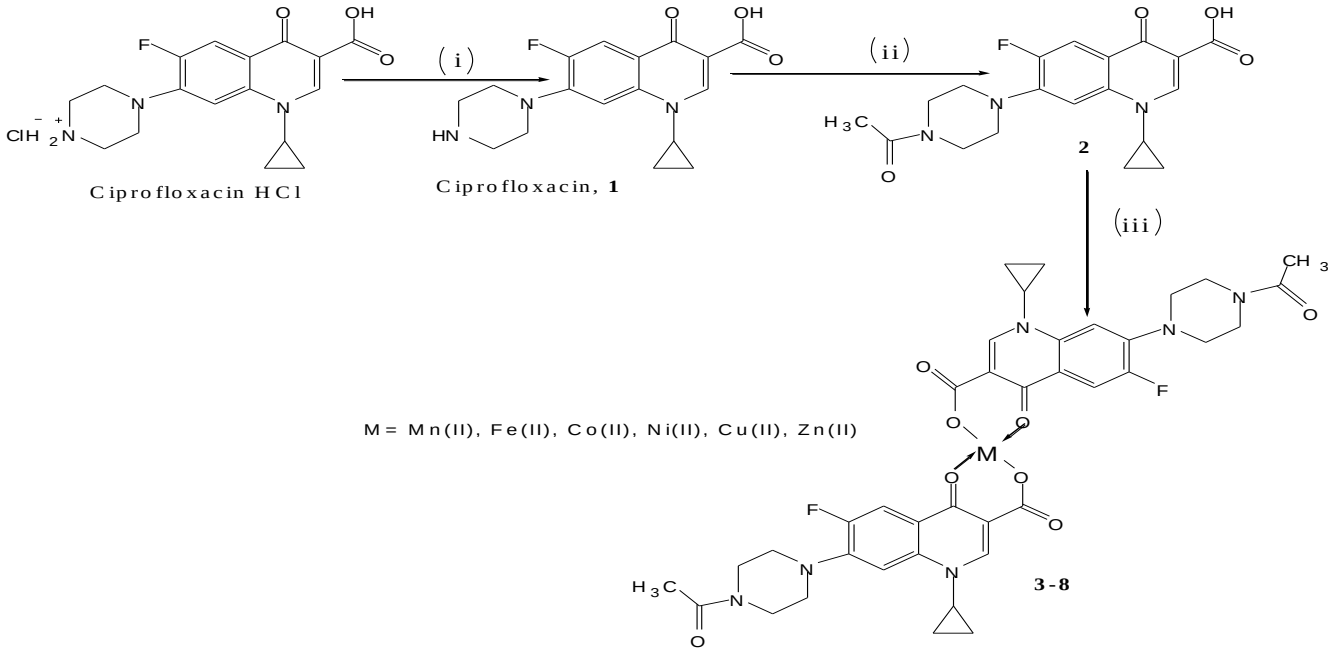
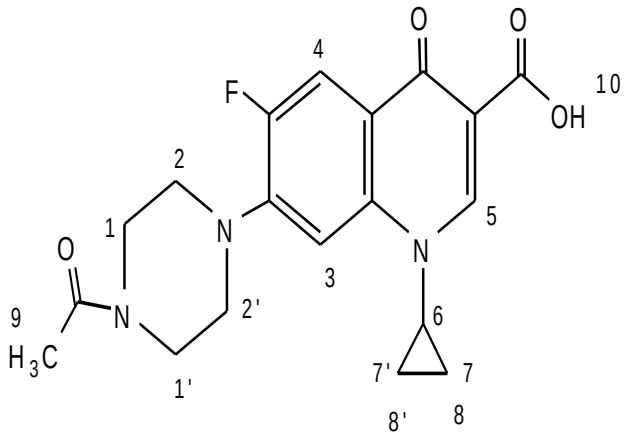
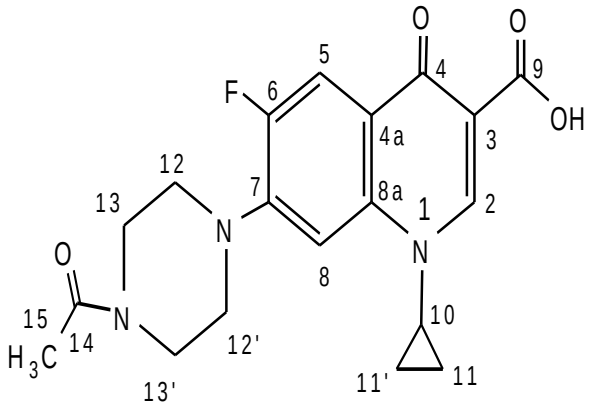


Fig 1. 2a for ^{1}H-NMR and 2b for ^{13}C-NMR



2a



2b

CONCLUSION

Comparison of the activities of different analogues of ciprofloxacin, **1** indicates that the amidic linkage at piperazine moiety may be responsible for the change in the antimicrobial properties of the present molecule. The carefully selection of the constituent for and or carboxylic group and the ketonic carbonyl function may result into more active antibiotics based on

ciprofloxacin. On the other hand, these preliminary studies of the metal complexes 3-8 provide us the idea that further modification on NH moiety of ciprofloxacin by suitable groups and their metal complexes may lead to more potent antifungal agent. It may be concluded that, antifungal activity of ciprofloxacin metal analogues may open new paths for designing antifungal agent based on ciprofloxacin skeleton.

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