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Synthesis and Antimicrobial Chattels of 1-Cyclopropyl-7-[(S,S)-2,8-Diazabicyclo [4.3.0]Non-8-Yl]-6-Fluoro-8-Methoxy-1,4-Dihydro-4-Oxo-3-Quinolinecarboxylic Acid Metal Complexes of Biological Interest

Najma Sultana[1], M. Saeed Arayne[2]* and Mehwish Akhtar[3]

[1]*Research Institute of Pharmaceutical Sciences, Faculty of Pharmacy, University of Karachi, Pakistan*

[2]*Department of Chemistry, University of Karachi, Pakistan*

[3]*Dow College of Pharmacy, Dow University of Health Sciences, Pakistan*

***Corresponding author**

Saeed Arayne, Department of Chemistry, University of Karachi, Pakistan, Tel: +922134664403; Email: msarayne@gmail.com

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- Essential and trace elements
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- ANOVA

Abstract

1-cyclopropyl-7-[(S,S)-2,8-diazabicyclo[4.3.0]non-8-yl]-6-fluoro-8-methoxy-1,4-dihydro-4-oxo -3-quinoline carboxylic acid (moxifloxacin) is a fluoroquinolone antibacterial agent. Metal complexes of moxifloxacin were synthesized with transition metal ions like Mg(II), Ca(II), Cr(III), Mn(II), Fe(III), Co(II), Ni(II), Cu(II), Zn(II) and Cd(II), usually present in the human body and heavy metals, as As(III), Ag(II), Cd(II) and lead (II). Their structures were elucidated physicochemically and spectroscopically using IR, NMR and elemental analysis. These investigations suggest that moxifloxacin interacts with the metal ions as a monoanionic, bidentate ligand and bound to the metal through the pyridone and one carboxylate oxygen atoms having general formula $[M^{I}(L)_2]^{+1}$, $[M^{II}(L)_2]^{+2}$, $[M^{III}(L)_2]^{+3}$ where L= moxifloxacin and M = Metal ion.

The complexes were screened against four Gram-positive and seven Gram-negative organisms and four fungi, which showed either comparable or increased antibacterial profile in comparison to the parent drug. The zones of inhibitions of these complexes and standard moxifloxacin were compared by post-hoc tests in ANOVA, which showed significant differences between individual zones of inhibitions. The biological data revealed that metal complexes of moxifloxacin showed good activity against *S. typhi*, *P. mirabilis*, *P. aeruginosa* and *B. subtilis*; moreover all complexes have excellent activity against *T. rubrum*, *F. solani*. The complexes showed comparable or increased antibacterial profile relative to the parent drug.

INTRODUCTION

Moxifloxacin (MOX) or 1-cyclopropyl-7-[(S,S)-2,8-diazabicyclo [4.3.0]Non-8-yl]-6-fluoro-8-methoxy-1,4-dihydro-4-oxo-3-quinoline carboxylic acid (Figure 1) is a synthetic third generation, 8-methoxyquinolone [1] broad spectrum antibacterial agent available for oral, intravenous administration and ophthalmic solution [2].

Many workers have reported the interactions of fluoroquinolones with metals of biological interest [3-13]. A study on the structure and activity of pipemidic acid, and interaction of its Cu(II) complexes on a DNA model, suggested that the intercalation of the quinolone complexed to a metal is an important step in the mechanism of action of these drugs [14]. Many drugs have modified pharmacological and toxicological properties in the form of metal complexes while the Cu(II) complexes of drugs have proved useful in several diseases such as gastric ulcers, rheumatoid arthritis, tuberculosis, and cancers [15,16-18]. Absorption of quinolone drugs is lowered when simultaneously administered with multivitamins containing minerals or antacids containing magnesium, aluminum, and others bivalent cations [19]. The reduction of metals below certain limit compromise many physiologically important functions [20].

Our research group earlier reported synthesis and characterization of metal complexes of levofloxacin [21,22],

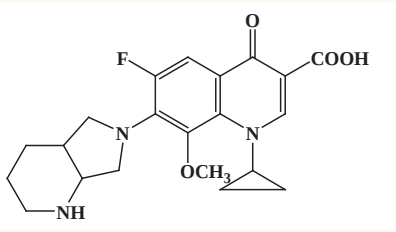

Figure 1 Moxifloxacin.

ciprofloxacin [23], lomefloxacin [24], sparfloxacin [25, 26], ofloxacin [27], enoxacin [28, 29], and gatifloxacin [30] and compared their antimicrobial activities with the parent drugs. Moreover, drug interactions of these quinolones, including moxifloxacin [31], were also reported.

Quinolones can form 1:1, 2:1, and 3:1 chelates depending on the particular metal ion, relative concentration of quinolone, and pH. It was found that neutral quinolones in the zwitterionic state are capable of forming simple complexes (bidentate chelating) [32,33]. The quinolones can also act as bridging ligands and thus are capable of forming polynuclear complexes [34].

These results encouraged us to study the coordination of MOX with transition metals of biological interest, and an attempt was made to investigate changes in antimicrobial and antifungal activities of resulting complexes. In the present study, we describe the synthesis and characterization of moxifloxacin metal complexes with elements of biological interest i.e., Mg^{2+}, Ca^{2+}, Cr^{3+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} and heavy metals As^{3+}, Ag^{+}, Cd^{2+} , and Pb^{3+}. The later are abundant as pollutants in many regions. These complexes were characterized by IR and NMR spectroscopy and elemental analysis. Prior to the synthesis, the metal ligand ratio in complex formation was established by conductometric titrations in methanol and by Job's plots [31]. Antibacterial and antifungal activities were determined by screening against a number of Gram positive and Gram negative bacteria and fungi. Most of these complexes were more potent as compared to the parent drug. The zones of inhibitions of synthesized complexes and standard (MOX) were compared by one-way ANOVA and post hoc test (Dunnett's test) which proved that activities of all complexes had significantly increased ($p<0.001$).

EXPERIMENTAL

Materials and methods

Moxifloxacin reference standard was gratis from Getz Pharma Pakistan (Pvt) Karachi and used without further purification. Essential and trace elements used were of analytical grade in the form of their hydrated salts as magnesium chloride ($MgCl_2.6H_2O$), calcium chloride ($CaCl_2.2H_2O$), chromium chloride ($CrCl_3.6H_2O$), manganese chloride ($MnCl_2.4H_2O$), ferric chloride ($FeCl_2.6H_2O$), cobalt chloride ($CoCl_2.6H_2O$), nickel chloride ($NiCl_2.6H_2O$), copper chloride ($CuCl_2.2H_2O$), and zinc chloride ($ZnCl_2$) and heavy metals, as arsenic trichloride ($AsCl_3$), silver chloride (AgCl), cadmium chloride monohydrate ($CdCl_2.H_2O$) and lead carbonate ($PbCO_3$) and were purchased from Merck Marker (Pvt) LTD Karachi. Methanol, acetone, carbon tetrachloride, dimethylsulfoxide, chloroform, and dichloromethane (Merck), were used without further purification. All the glasswares were washed with chromic acid followed by a thorough washing with de-ionized water which was freshly prepared daily in the laboratory.

Instrumentation

Conductometric titrations were performed on Vernier LabPro™ using Logger pro 3.2 software. For jobs plot, UV-Vis spectra of drug and metal solutions in variable ratios were recorded on a UV-Vis spectrophotometer (Shimadzu 1601coupled with a P IV–PC and loaded with UVPC version 3.9, software). Thin layer chromatography (TLC) was performed on HSF-254 TLC plate. Melting points were obtained manually by capillary method using a Gallenkamp™ apparatus. IR spectra were recorded on a Shimadzu prestige-21 200 VCE spectrometer in KBr pellets. ^{1}H-NMR spectra were obtained on a Bruker/XWIN-NMR spectrometer in CD_3OD using TMS as an internal standard. CHN analysis was carried out on an Elemental analyzer Carlo Erba 1106. Atomic absorption studies were carried out with a Perkin-Elmer AAnalyst 700 atomic absorption spectrometer using AAnalyst software.

Stoichiometric study

Conductometric Titrations: In conductometric titrations, 1 mM methanolic solution of MOX, and metal salts were prepared individually. Two mL of metal solution was added to a 20 mL drug solution at an intervals of two minutes, and conductance values were recorded until a state of chemical equilibrium was achieved. Figure 2a shows constantly increasing values; the conductance was corrected for dilution by means of the following equation.

$$\Omega_{corr} = \Omega_{obs} [(\nu_1 + \nu_2) / \nu_1]$$

Where Ω is electrolytic conductivity, ν_1 is initial volume, and ν_2 is added volume.

Job's Method: The Job's method of continuous variation of a 1 mM solution of a ligand (MOX) was employed, and metal salts were prepared in methanol individually. The ligand substrate ratio was varied keeping a constant volume (10 mL); solutions were kept at 37 °C for half an hour and then were analyzed on a UV/visible spectrophotometer at 290 nm [31]. The ligand substrate ratios were evaluated from the graph (Figure 2b) constructed between absorbance against molar concentration.

Synthesis of moxifloxacin metal complexes

Each metal salt (0.5 mM) and MOX(1 mM) solutions in distilled methanol were individually refluxed with frequent shaking for about 4 hours. The metal:ligand ratio was earlier determined by conductometric titrations and Job's method. The reaction was monitored by TLC (butanol:ammonia:methanol, 4:2:1). The reaction mixture was concentrated, filtered, and left for slow crystallization for two to three weeks. The crystals were filtrated, washed, recrystallized in methanol, dried and physical data were recorded (table 1). These complexes were characterized by IR and ^{1}H-NMR spectroscopic techniques and elemental analysis.

Antibacterial and antifungal activity

The synthesized metal complexes were screened for their antibacterial and antifungal activity. Antibacterial activity was

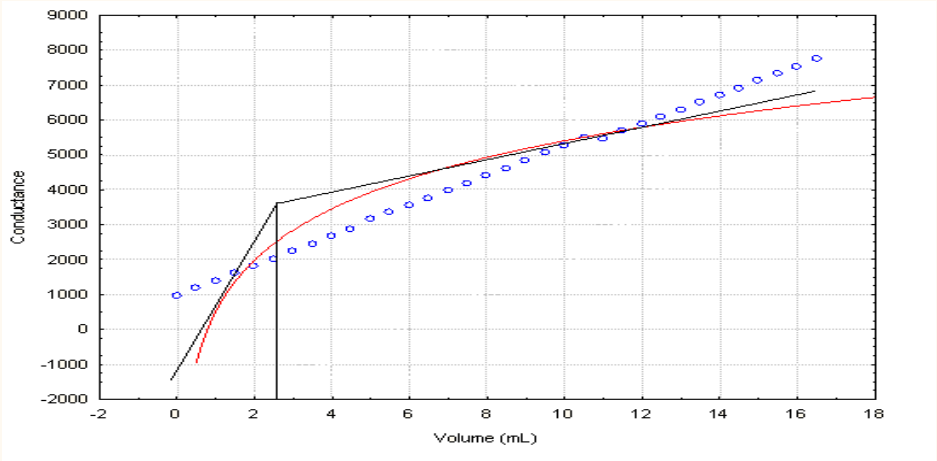

Figure 2a Representation of moxifloxacin metal complexes ratio via conductance.

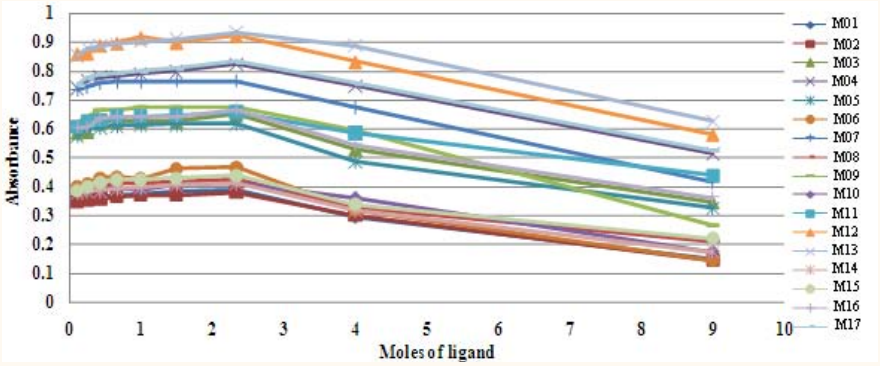

Figure 2b Representation of moxifloxacin metal complexes ratios via *job's* plot.

screened against Gram-positive organisms as Bacillus *subtilis*, *Micrococcus luteus*, *Staphylococcus aureus*, and *Streptococcus features* and Gram-negative organisms which included *Salmonella typhi*, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Escherichia coli, *Citrobacter species*, and *Shigella flexneri* by disc diffusion method [35]. The antifungal activity was investigated against *Candida albicans*, *Aspergillus parasiticus*, *Trichophyton rubrum*, *Aspergillus effuses*, *Fusarium solani*, and *Saccharomyces cerevisiae* by the agar diffusion disc variant method. Sabouraud dextrose agar was used.

The antibacterial discs (diameter 6 mm) were prepared in house in 5, 10, 20 and 40 µg mL^{-1} concentrations, dried at 37 °C and applied over each of the culture plates previously seeded with the 0.5 ml McFarland turbidity cultures of the test bacteria. DMSO paper discs were used as a negative control. These culture plates were then incubated at 37 °C for 18–24 h and then were allowed to stand for seven days for antifungal activity. The activity was determined by measuring the diameter of the inhibition zone (in mm). Growth inhibition was calculated with reference to the positive control i.e., moxifloxacin. For each compound, three replicate trials were conducted against each organism.

RESULTS AND DISCUSSION

An interaction study between moxifloxacin and transition metals is an important research area in bioinorganic chemistry. Many drugs are dependent on the coordination with metal ions [32], or/and the inhibition of the formation of metalloenzymes [14]. The proposed mechanism of the interaction is chelation between the 4-oxo and adjacent carboxyl group of quinolone and metal cations [5, 36-39]. Since these functional groups are required for antibacterial activity, it could be anticipated that all of the quinolones could be interacting with metal ions [22].

Synthesis of metal complexes

It was observed during the conductometric titration and continuous variation method that all the metals and moxifloxacin interacted in the ratio of 1:2 (Figure 2). Synthesis of solid drug: metal complexes was carried out according to a mole ratio as determined. Examination of solubility of these complexes shows that they are insoluble in benzene, chloroform, and dichloromethane, slightly soluble in water, soluble in methanol, DMF, and DMSO. Melting points of the complexes were taken at the time interval of 24 and 48 hours to check the stability. No appreciable changes in the melting points were observed which showed that all the complexes were stable at room temperature for two days. The physical characteristics of these complexes are given in Table 1. The structures of these complexes were elucidated on the basis of IR (Table 2), ^{1}H-NMR (Table 3) and elemental analyses (Table 4). The spectroscopic data coordinate well with the proposed formula and structures of the complexes.

Infrared spectroscopic studies

Identification of moxifloxacin metal complexes can be achieved by studying the most typical vibrations that are characteristic of coordination type of quinolones. Moxifloxacin itself act as unidentate, bidentate, or as bridging ligand. The IR spectra of transition metal complexes of MOX are compared with the parent drug molecule in Figure 3. Deacon and Phillips [40] determined that unidentate carboxylato complexes exhibit Δν values which are much larger than those of the ionic salts (Δν >200 cm^{-1}), bidentate or chelating carboxylate complexes, exhibit Δν significantly smaller than ionic values (Δν <100 cm^{-1}), and bridging complexes show Δν comparable to the ionic values (Δν ≈150 cm^{-1}) [41]. As discussed above, the proposed mechanism of the interaction between drug and metal cations was chelation between ketonic group (4-oxo) and carboxylic groups of quinolone [42]; thus we focused on these group vibrations in our study.

In the IR spectra of the complexes the absorption of the m(C=O) carb has disappeared. Two very strong characteristic bands are present in the range 1613–1695 cm^{-1} and 1315–1394 cm^{-1}, assigned as asymmetric, $\upsilon(CO_2)_{asym,}$ and symmetric, $\upsilon(CO_2)_{sym}$, stretching vibrations, respectively. The ionic carboxylates [43] show no carbonyl stretching at about 1700 cm^{-1}, but have two bands in the range of 1650–1510 cm^{-1} and 1460–1400 cm^{-1}that could be assigned as (O–C–O) asymmetric and symmetric stretching vibrations. In the spectrum of moxifloxacin the (O–C–O)a + (C=O)p band appears at 1600–1658 with maxima at 1638 cm^{-1}, (O–C–O)s band stretch at 1398 cm^{-1} , and C–O absorbs at 1282 cm^{-1}. The D values fall in the range 200–229 cm^{-1}, indicating a monodentate coordination mode of the carboxylato group of the ligand [36,44]. The band observed at 1,623 cm^{-1} is assigned to pure C=O stretching mode of ring carbonyl group. On complexation, the characteristic peak of (C=O)p + (C–O–C)a in all the metal complexes spectra shifted towards a lower frequency region, that is in the range 1,635-1695 cm^{-1}, and the symmetric vibrations occurred in the region of 1407–1370 cm^{-1} [45,46].

The IR spectra of all the compounds exhibited a broad, split band between 3315–3475 cm^{-1} , assigned to the O–H stretching vibrations of water molecules which also includes the N–H stretching vibration of the piperazinyl moiety. The overall changes of the IR spectra suggest that the neutral moxifloxacin in the zwitterionic state is capable of forming simple complexes. In these complexes, the metal ions are coordinated by four oxygen atoms from two moxifloxacin, which acts as a bidentate ligand, and are coordinated to metals via the pyridone and carboxylate oxygen [47]. Metal bonding with oxygen gave absorption at 453-995 cm^{-1} [48].

NMR spectroscopic studies

The proton NMR spectra of moxifloxacin complexes have been recorded in $CDCl_3$ (with one to two drops of CD_3OD) and compared to the spectrum of moxifloxacin. The aromatic protons that are H-2 and H-5 are very close to the coordination site of the ligand. Both H-2 and H-5 protons of MOX appeared at δ = 8.61

Table 1: Physical characteristic of metal complexes.

Code	MOX-metal complexes	Color	Yield (%)	M.P ˚C
MOX	1-cyclopropyl-6-fluoro-8-methoxy-7-(octahydro-1H-cyclopenta[b]pyridin-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate MOX	Yellow	58	206
M01	Bis(1-cyclopropyl-6-fluoro-8-methoxy-7-(octahydro-1H-cyclopenta[b]pyridin-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate)magnesium(II) chloride, $[Mg(MOX)_2]Cl_2$	Yellow	72	236
M02	Bis(1-cyclopropyl-6-fluoro-8-methoxy-7-(octahydro-1H-cyclopenta[b]pyridin-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate)calcium(II)chloride, $[Ca(MOX)_2]Cl_2$	Light green	71	223
M03	Bis(1-cyclopropyl-6-fluoro-8-methoxy-7-(octahydro-1H-cyclopenta[b]pyridin-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate)chromium(III)chloride, $[Cr(MOX)_2]Cl_3$	Yellow	68	216
M04	Bis(1-cyclopropyl-6-fluoro-8-methoxy-7-(octahydro-1H-cyclopenta[b]pyridin-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate) manganese(II)chloride, $[Mn(MOX)_2]Cl_2$	Brown	65	226
M05	Bis(1-cyclopropyl-6-fluoro-8-methoxy-7-(octahydro-1H-cyclopenta[b]pyridin-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate)ferrous(II)chloride, $[Fe(MOX)_2]Cl_2$	Green	71	220
M06	Bis(1-cyclopropyl-6-fluoro-8-methoxy-7-(octahydro-1H-cyclopenta[b]pyridin-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate)cobalt(II)chloride, $[Co(MOX)_2]Cl_2$	Light green	59	228
M07	Bis(1-cyclopropyl-6-fluoro-8-methoxy-7-(octahydro-1H-cyclopenta[b]pyridin-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate)nickel(II), $[Ni(MOX)_2]Cl_2$	Light green	62	108
M08	Bis(1-cyclopropyl-6-fluoro-8-methoxy-7-(octahydro-1H-cyclopenta[b]pyridin-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate)copper(II)chloride, $[Cu(MOX)_2]Cl_2$	Yellow	66	178
M09	Bis(1-cyclopropyl-6-fluoro-8-methoxy-7-(octahydro-1H-cyclopenta[b]pyridin-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate)zinc(II)chloride $[Zn(MOX)_2]Cl_2$	Yellow	67	218
M10	Bis(1-cyclopropyl-6-fluoro-8-methoxy-7-(octahydro-1H-cyclopenta[b]pyridin-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate) arsenic (III)chloride, $[As(MOX)_2]Cl_3$	Yellow	59	210
M11	Bis(1-cyclopropyl-6-fluoro-8-methoxy-7-(octahydro-1H-cyclopenta[b]pyridin-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate) silver(I)chloride, $[Ag(MOX)_2]Cl$	Yellow	81	206
M12	Bis(1-cyclopropyl-6-fluoro-8-methoxy-7-(octahydro-1H-cyclopenta[b]pyridin-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate) cadmium (II) chloride, $[Cd(MOX)_2]Cl_2$	Yellow	74	218
M13	Bis(1-cyclopropyl-6-fluoro-8-methoxy-7-(octahydro-1H-cyclopenta[b]pyridin-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate) lead (II), $[Pb(MOX)_2]CO_3$	Yellow	73	178

Table 2: Characteristic absorptions (in cm^{-1}) of IR spectra of the complexes.

Complexes	O-H stretching	υ (C=O)	Υ $(CO_2)_{as}$	υ $(CO_2)_s$	[a] Δ	υ (M=O)
MOXI	3475[c]	1613	1705[b]	-	-	804
M01	3392	1653	1582	1369	213	995
M02	3312	1653	1578	1369	209	673
M03	3352	1695	1586	1373	213	549
M04	3230	1653	1566	1354	212	533
M05	3475	1647	1599	1394	205	624
M06	3482	1653	1528	1320	208	453
M07	3238	1655	1532	1315	217	802
M08	3215	1653	1550	1319	231	682
M09	3244	1683	1566	1354	212	-

[a] Δ= υ $(CO2)_{asym}$ – υ $(CO2)_{sym}$, [b] υ (COOH), [c] NH (str

Table 3: ^1H NMR spectrum of complexes.

Complexes	H-2	H-5
MOXI	8.61	7.20
M01	8.64	7.61
M02	8.59	7.58
M03	8.62	7.63
M04	8.75	7.51
M05	8.73	7.24
M06	8.62	7.63
M07	8.65	7.67
M08	8.69	7.34
M09	8.82	7.85

Table 4: Elemental analysis % found (calcd.) of MOXI-M Complexes.

Metal complexes	C		H		N	
$[Mg(MOXI)_2(H_2O)_2]Cl_2.2H_2O$	52.33	(52.00)	5.69	(5.82)	8.68	(8.66)
$[Ca(MOXI)_2(H_2O)_2]Cl_2.2H_2O$	51.48	(51.17)	5.64	(5.73)	8.73	(8.52)
$[Cr(MOXI)_2(H_2O)]Cl_2$	52.48	(52.45)	5.52	(5.45)	8.82	(8.74)
$[Mn(MOXI)_2(H_2O)]Cl_2.H_2O$	52.34	(52.49)	5.61	(5.43)	8.52	(8.71)
$[Fe(MOXI)_2(H_2O)]Cl_2$	53.25	(53.23)	5.42	(5.32)	8.72	(8.87)
$[Co(MOXI)_2(H_2O)_2]Cl_2.H_2O$	51.26	(51.12)	5.75	(5.52)	8.35	(8.52)
$[Ni(MOXI)_2(H_2O)_2].Cl_2.2H_2O$	50.15	(50.22)	5.75	(5.62)	8.67	(8.84)
$[Cu(MOXI)_2(H_2O)].Cl_2.H_2O$	51.69	(51.83)	5.59	(5.38)	8.42	(8.63)
$[Zn(MOXI)_2(H_2O)_2].Cl_2.H_2O$	50.62	(50.79)	5.45	(5.48)	8.82	(8.46)

(s) and δ = 7.2 (d)ppm, respectively. Upon addition of ametal, these protons undergo the most significant changes. In the metal complexes the H-2 proton gives a new signal at δ = 8.7-8.8 ppm while H-5 proton appeared at δ = 7.6-7.85 ppm.

This shift is due to the complexation and difference in the configuration of complexes than ligand [49]. The signals for the aliphatic and piperazine protons are practically unchanged since they lie far from the binding site of the ligand [50]. Therefore, studies indicate that moxifloxacin acts as a bidentate ligand through the ring carbonyl oxygen atom and one of the oxygen atoms of the carboxylic group. The resonance of the carboxylic proton (COOH) is not detected in the spectra of the complexes which further suggest the coordination of moxifloxacin is through its carboxylate oxygen atoms [23]. The OH proton peak appears near 3.5 ppm, adjacent to the piperazine protons, due to the presence of lattice water [29].

Our studies suggest that moxifloxacin interacts with the metal centre through the 3-carboxylate and 4-oxygen atom and acts as a monoanionic bidentate ligand where the metal ion is coordinated to two bidentate moxifloxacin ligands and two aqua located in axial positions which completes the slightly distorted octahedral coordination of metal (Figure 4). Probably due to the

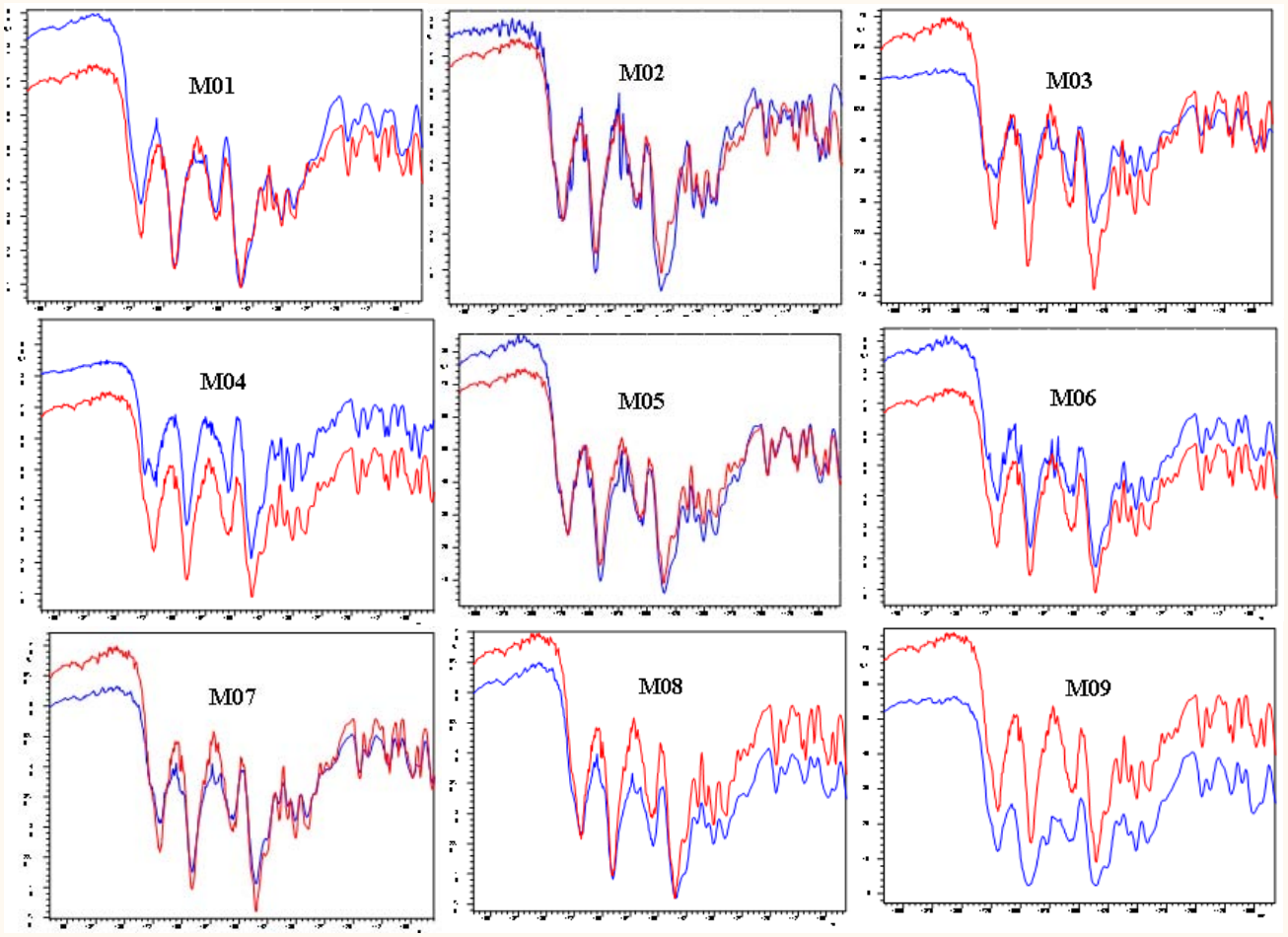

Figure 3 FTIR spectrum of moxifloxacin-metal complexes overlap with MOXI spectra (red).

steric crowding of the methyl group [51], the piperazine nitrogen does not interact with metal. Despite the crystalline nature of the products, we could not obtain crystals suitable for the determination of structures with X-ray crystallography [36, 52].

Antibacterial activity

Moxifloxacin is active against broad spectrum pathogens, encompassing Gram-negative and Gram-positive bacteria and also including antibiotic resistant *Streptococcus pneumonia.* It is useful in the treatment of respiratory tract infections, acute bacterial sinusitis, acute bacterial exacerbation of chronic bronchitis, and community acquired pneumonia. The results of the antibacterial studies against various microorganisms are given in Table 5a.

For antibacterial studies, disc susceptibility technique was employed [35]. The *in-vitro* antibacterial activity of all complexes was evaluated against Gram-negative Salmonella *typhi,* Klebsiella pneumoniae, Proteus mirabilis, *Pseudomonas aeruginosa,* Escherichia coli, *Citrobacter species,* and *Shigella flexneri* and Gram-positive organisms as Bacillus *subtilis*, *Micrococcus luteus, Staphylococcus aureus,* and *Streptococcus features* by disc diffusion method. Their zones of inhibition were compared with standard moxifloxacin. It is evident that the complexes possess inhibitory action against all microorganisms (Table 5a). All data were presented as zone of inhibition in diameter (mm) and screened at four different concentrations that were 5, 10, 20 and 40 µg (n = 3).

One way analysis of variance (ANOVA) was carried out to check any differences between the zones of inhibitions of all prepared complexes and the standard. Dunnett's test was applied, and differences were considered significant at $p \leq 0.05$. ANOVA showed significance difference in antibacterial activity between metals complexes and moxifloxacin (which was standard throughout the experiment) against *S. typhi, P. mirabilis, P. aeruginosa, E. coli, Citrobacter species,* and *S. flexneri.* Dunnett's test analyzed that all complexes were significantly increased ($p<0.05$) in antibacterial activity against S. *typhi, K. pneumonia, P. mirabilis* and *P. aeruginosa* excluding $Ni(MOX)_2$ which was insignificant in 10 μgmL^{-1} against S. *typhi*. Post hoc test considered that antibacterial activity of almost all complexes were significantly decreased ($p<0.001$) against *E. coli*, *Citrobacter species,* and *S. flexneri,* excluding $Ni(MOX)_2$, $Cu(MOX)_2$ and $Zn(MOX)_2$. $Ni(MOX)_2$ was insignificant in 5 and 10 μgmL^{-1} and decreased significantly at 20 and 40 μgmL^{-1} whereas $Cu(MOX)_2$ and $Zn(MOX)_2$ were increased significantly ($p<0.005$) against *E. coli* at all selected concentrations. Only $Cu(MOX)_2$ was significantly increased ($p<0.001$) in 5 μgmL^{-1}; $Mg(MOX)_2$, $Mn(MOX)_2$, $Fe(MOX)_2$ and $Cu(MOX)_2$ were significant increased in activities ($p<0.05$) in

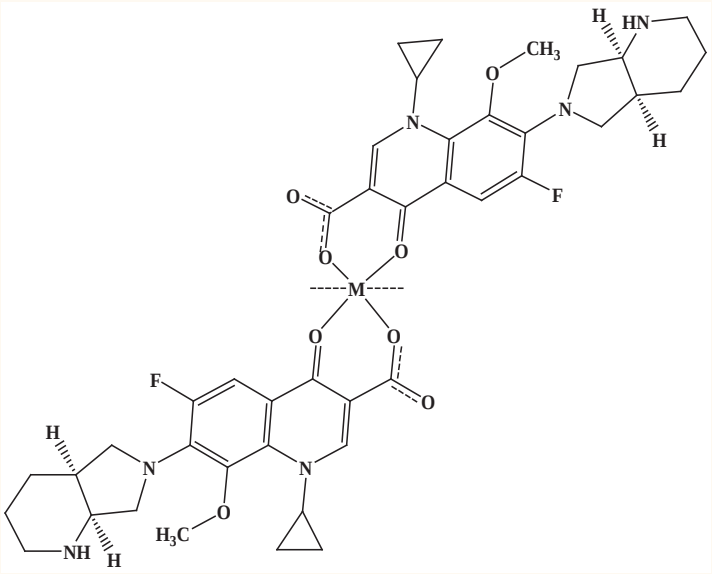

Figure 4 Predicted structure of MOXI-metal complexes.

Table 5a: Antibacterial activities of moxifloxacin and its metal complexes.

Complexes	B. subtilis	M. luteus	S. aureus	S. features	S. typhi	K. pneumoniae	Proteus mirabilis	P. aeruginosa	E. coli	Citrobacter species	S. flexneri
MOX	8.38	27.19	26.23	30.11	12.12	15.25	12.23	8.29	18.25	25.39	13.14
$Mg(MOX)_2$	14.14	13.6	14.38	13.27	13.55	13.27	15.33	15.45	13.28	14.2	14.72
$Ca(MOX)_2$	15.19	16.24	13.08	13.57	15.05	14.37	14.26	13.63	13.67	14.5	13.21
$Cr(MOX)_2$	13.91	11.21	11.53	13.59	14.25	13.34	13.13	13.48	15.48	13.67	13.7
$Mn(MOX)_2$	16.17	13.49	13.07	13.26	14.69	15.41	13.2	13.3	16.27	15.28	14.31
$Fe(MOX)_2$	14.26	15.26	15.38	14.26	19.28	16.57	15.19	15.23	15.54	18.3	14.64
$Co(MOX)_2$	15.65	15.24	15.21	13.32	16.18	13.56	14.54	15.67	12.63	14.31	13.23
$Ni(MOX)_2$	11.28	19.26	13.29	12.04	13.1	14.7	12.5	13.38	17.25	13.17	12.26
$Cu(MOX)_2$	14.17	13.2	13.54	12.27	13.7	14.03	14.72	13.55	20.22	13.19	14.28
$Zn(MOX)_2$	14.3	16.33	15.12	12.67	13.68	14.72	15.49	14.64	23.3	14.42	13.27
$As(MOX)_2$	16.29	10.31	13.07	14.21	13.21	25.25	14.03	14.13	25.18	15.23	15.31
$Ag(MOX)_2$	17.26	12.24	15.24	17.24	15.29	25.27	17.34	15.27	28.1	18.36	15.31
$Cd(MOX)_2$	16.59	12.24	12.33	14.21	14.46	25.24	14.23	13.37	24.3	15.2	16.18
$Pb(MOX)_2$	18.2	12.07	14.14	16.34	14.26	26.35	16.24	15.19	26.2	17.25	14.94
ANOVA	F-132.34	F-760.8	F-80.16	F-622.55	F-77.15	F-23.15	F-31.55	F-60.64	F-185.64	F-377.84	F-13.39

Mean (mm; n = 6); $p<0.001$

10 μgmL^{-1}; $Mg(MOX)_2$, $Mn(MOX)_2$, $Fe(MOX)_2$, $Cu(MOX)_2$ showed increase significantly ($p<0.005$) antibacterial activities in 20 μgmL^{-1}. Increased significant ($p<0.005$) activities of $Mn(MOX)_2$, $Fe(MOX)_2$, and $Cu(MOX)_2$ were confirmed in 40 μgmL^{-1} against *S. flexneri*. The synthesized complexes also produced significant differences against Gram-positive organisms. Dunnett's test confirmed antibacterial activities of all complexes were significantly increased ($p<0.001$) compared to standards.

The increased activity of metal chelates can be explained on the basis of the overtone concept and chelation theory. According to the overtone concept of cell permeability, the lipid membrane that surrounds the cell favors the passage of only lipid-soluble materials in which liposolubility is an important factor that controls the antimicrobial activity. Upon chelation, the polarity of the metal ion will be reduced due to overlap of ligand orbital and partial sharing of the positive charge of the metal ion with donor groups [53]. It is likely that the increased liposolubility of the ligand upon metal complexation may contribute to its facile transport into the bacterial cell which blocks the metal binding sites in enzymes of microorganisms [54]. These complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of the organism. The antimicrobial activity of the metal salts investigated revealed that they did not exhibit antimicrobial activity at the concentration range used to investigate the activity of the synthesized metal complexes [36,55].

Antifungal activity

Moxifloxacin is an antibacterial drug like other fluoroquinolones and not used as antifungal agent. MOX and its complexes were screened for their antifungal activity against *C. albicans, A. parasiticus, T. rubrum, A. effuses, F. solani,* and *S. cerevisiae* (Table 5b). Moxifloxacin and its complexes showed good activity against *C. albicans, F. solani*, very low activity against *T. rubrum* and no activity against rest of the fungi examined.

ANOVA showed significance differences between all prepared complexes against *C. albicans*. Dunnett's test analyzed that antifungal activities of all complexes were significantly decreased ($p<0.001$) except $Zn(MOX)_2$ and $Ni(MOX)_2$. $Ni(MOX)_2$ was insignificant in 5 and 10 μgmL^{-1} and $Zn(MOX)_2$ was insignificant in 5, 10, and 20 μgmL^{-1}. ANOVA showed significance differences between against *F. solani* and *T. rubrum*. Dunnett's test analyzed that antifungal activities of all complexes were significantly increased ($p<0.001$), but $Cu(MOX)_2$ influence was insignificant at 5 μgmL^{-1}. $Mg(MOX)_2$ was insignificant in 20 μgmL^{-1} and significantly decreased ($p<0.05$) at 40 $\mu gsmL^{-1}$. $Zn(MOX)_2$ was significantly decreased at 40 μgmL^{-1} ($p<0.05$) against *F. solani.*

Moxifloxacin showed low activity, and the complexes had excellent activity as compared to standard drug towards all the fungi (Table 5b). It is suggested that the antifungal activity of the complexes is due to either by killing the microbes or inhibiting their multiplication by blocking their active sites.

CONCLUSION

Synthesis and characterization of metal complexes of the third generation antibacterial agent moxifloxacin with transition metal ions of biological interest are reported. Physiochemical, spectroscopic, and elemental analysis studies revealed that moxifloxacin coordinated with metal ions to form stable complexes and acted as a bidentate ligand bound to metal through the pyridone oxygen and one carboxylate oxygen having a distorted octahedral geometry. This work also reports the evaluation of synergistic or antagonistic behavior of these complexes in comparison to the parent, through the differences in their biological activities against Gram-positive and Gram-negative organisms and fungi. Among the studied quinolones, moxifloxacin–metal complexes showed a diverse antimicrobial activity as compared to moxifloxacin which was attributed to the formation of metal drug chelates. All metal complexes were superior to moxifloxacin for antifungal activity as compared to the parent drug. One way analysis of variance and Dunnett's test were applied to differentiate the significance at $p \leq 0.05$. The biological data revealed that the metal complexes of MOX have good activity against *S. typhi, P. mirabilis*, P. aeruginosa, and B. *subtilis*. Excellent antifungal activities of all complexes had produce against *T. rubrum, and F. solani*. They were more potent antibacterial and antifungal agents as compared to the parent compound.

Table 5b: Antifungal activities of moxifloxacin and its metal complexes.

Complexes	S. flexneri	C. albicans	F. solani	T. rubrum
MOX	13.14	17.12	16.21	0
$Mg(MOX)_2$	14.72	14.19	16.28	10.21
$Ca(MOX)_2$	13.21	13.59	21.35	11.95
$Cr(MOX)_2$	13.7	13.04	22.2	12.52
$Mn(MOX)_2$	14.31	13.22	22.56	17.31
$Fe(MOX)_2$	14.64	13.29	22.27	14.72
$Co(MOX)_2$	13.23	13.21	19.14	12.29
$Ni(MOX)_2$	12.26	14.11	23.54	11.48
$Cu(MOX)_2$	14.28	13.16	18.28	16.28
$Zn(MOX)_2$	13.27	16.58	17.58	13.44
$As(MOX)_2$	15.31	13.15	20.17	17.29
$Ag(MOX)_2$	15.31	13.15	25.23	22.1
$Cd(MOX)_2$	16.18	15.29	22.22	18.17
$Pb(MOX)_2$	14.94	13.33	24.19	21.31
ANOVA	F-13.393	F-78.461	F-246.703	F-476.634

Mean (mm; n = 6); $p<0.001$

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