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# DNA-interaction and in vitro antimicrobial studies of some mixed-ligand complexes of cobalt(II) with fluoroquinolone antibacterial agent ciprofloxacin and some neutral bidentate ligands

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ABSTRACT

Six new mixed-ligand complexes of Co(II) with ciprofloxacin (Cip) and neutral bidentate ligands have been synthesized and characterized. Binding and cleavage of DNA with the complex were investigated using spectroscopic method, viscosity measurements and gel electrophoresis techniques. Antibacterial activity has been assayed against two Gram$^{(-ve)}$ and three Gram$^{(+ve)}$ microorganisms using the doubling dilution technique.



Derivatives of compounds composed of 3-carboxy-4-oxo-1,4-dihydroquinoline (i.e., 4-quinolones) are active against a wide range of gram-positive and gram-negative organisms.[1] The first member of the quinolone family kept forward for clinical practice was nalidixic acid; it is used for the treatment of urinary tract infections.[2] Major increase in the potency was obtained by addition of fluorine atom on position 6 of the quinoline ring, addition of piperazinyl group on position 7 to enhance permeability and potency.[3] Binding of DNA with the complexes have been studied by different methods to understand the selectivity and efficiency of DNA reorganization, cleavage by cobalt complexes, and to develop new effective useful DNA probes.

All the chemicals used in the experiment were of analytical grade and solvents were purified by standard methods.[4] Physical measurements were made as per S-1. The diamagnetic correction was made using Pascal's constant.[5] The Schiff bases $A^1$–$A^5$ were prepared by condensation of the amine and aldehyde/ketone in ethanol.[6] The complexes (I)–(VI) were prepared as per reported method.[7] Synthesis and physicochemical parameters of the ligands and synthesized complexes are as Supplementary data S-2. All the complexes were insoluble in common solvent while partially soluble in dimethylformamide and completely soluble in dimethylsulfoxide.

The $^1$H NMR spectra of ligands exhibiting peaks at about 6.65–7.91 ppm were assigned to the aromatic protons. The singlet peak which appeared at 8.6–8.7 ppm was assigned to the azomethine proton (–CH═N–). In the $^{13}$C NMR spectra, the peaks observed at about 115.0–136.4 and 113.5–144.5 ppm were assigned to aromatic and pyridine carbons, respectively. Peaks observed in range 123.5–140.0 and 160.5–180.5 ppm were assigned to C–N and C═N carbons, respectively.

The $\nu$(C═O) stretching vibration band appears at 1708 cm$^{-1}$ for ciprofloxacin, where as for complexes it appear at 1627–1633 cm$^{-1}$; this shift towards lower energy suggests that coordination occurs through the carbonyl oxygen of pyridine ring.[8] The sharp band in ciprofloxacin at 3520 cm$^{-1}$ due to hydrogen bonding;[9] which is attributed to ionic resonance structure and peak observed because of free hydroxyl stretching vibration. This band completely vanished in the spectra of the metal complexes indicating deprotonation of the carboxylic proton. The data were further supported by $\nu$(M–O)[10] band which appeared at 502–514 cm$^{-1}$. The strong absorption band obtained at 1624 cm$^{-1}$ and 1340 cm$^{-1}$ in ciprofloxacin were assigned to $\nu(COO)_{asy}$ and $\nu(COO)_{sym}$, respectively, while in the metal complexes these bands were observed about 1590 and 1375 cm$^{-1}$, respectively. The frequency separation ($\Delta\nu$) in the investigated complexes is greater than 200 cm$^{-1}$, suggesting a unidentate bonding nature for the carboxyl group.[11] In the investigated compound the $\nu$(C═N) band of 2,2′-bipyridylamine appeared at 1585 cm.$^{-1}$ This shifted to higher frequency at 1612 cm$^{-1}$ which points that N–N coordination of the chelating agent.[12] The $\nu$(C═N) peak for the Schiff bases $A^1$–$A^5$ was observed at 1601–1629 cm$^{-1}$ which on complexation were shifted to 1560–1605 cm$^{-1}$, which indicates the N–N coordination of the chelating agent.[13] This data was further supported by $\nu$(M–N)[14] which appeared at

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537–542 $cm^{-1}$. Detail IR Spectral data of complex is as Supplementary data S-3.

The magnetic moments of the complexes are in the range 4.7–5.1 BM show that all are paramagnetic and have three unpaired electrons indicating a high-spin octahedral configuration.[15] The Co(II) complexes exhibited well-resolved bands at 17471–17577 $cm^{-1}$ and a strong high-energy band at 20202–22122 $cm^{-1}$ were assigned to $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)(\nu_2)$, $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)(\nu_3)$ transitions, respectively, for a high-spin octahedral geometry.[16]

It was observed that all the complexes showed a loss in weight corresponding to three water molecules in the range 50–130 °C, indicating the presence of lattice water molecules. In the second step weight loss during 130–180 °C corresponding to two coordinated water molecules. For Co(II) complexes a loss in weight corresponding to a piperazine (pip) molecule was found between 180 and 250 °C, followed by liberation of Cip.(L) in the temperature range 250–500 °C. Finally, decomposition of $A^n$ occurred in the temperature range 520–800 °C, and the remaining weight was consistent with metal oxide.[17]

The ESI mass spectrum of the complex $[Co_2(Cip)_2(dcbd)_2(pip)(H_2O)_2]\cdot 3H_2O$ (I) shows the peak at $m/z = 1336$ corresponds to the molecular ion peak of complex (in absence of water of crystallization). For fragments at $m/z = 781.3$ and 763 there exist a peak at $m$, $m+2$ and $m+4$, which indicates presence of two Cl atoms in fragments. There exist several other fragments at $m/z = 710$, 669, 442 and 567 with peak at m and $m+2$ only, which indicates presence of single Cl atom[9] S-4.

The antimicrobial activity of all the complexes against all the five microorganisms is much higher than metal salt while in competition with the ciprofloxacin (S-5). The results of our study indicate that the compounds II, III and IV (MIC = 0.648, 0.223 and 0.754 μM, respectively) exhibit higher antimicrobial activity against *Staphylococcus aureus* compare to all tested drugs. Except VI (MIC = 1.672 μM) all the compounds posses higher antimicrobial activity against *Bacillus subtilis* compare to all tested drugs. In case of *Serratia merscences* compounds I (MIC = 0.717 μM) and IV (MIC = 1.131 μM) exhibit higher antimicrobial activity compare to all tested drug. In case of *Pseudomonas aeruginosa* compound II posses highest activity (MIC = 0.876 μM). In case of *Escherichia coli* compounds III (MIC = 0.876 μM) shows the best activity. Thus the synthesized complexes were found more potent against $gram^{(+ve)}$ and not much active against $gram^{(-ve)}$ species. The result indicates that the compound III is most potent against all the test species compares to other synthesized compounds. No doubt it is the most potent compare to all but weak in case of *S. merscences*. It was observed that all the complexes were more potent bactericides than the ligands. The inhibition activity seems to be governed in certain degree by the facility of coordination at the metal centre. This may support the argument that some type of bimolecular binding to the metal ions or intercalation or electrostatic interactions causing the inhibition of biological synthesis and preventing the organisms from reproducing. The strong antimicrobial activities of these complexes against tested organisms suggest further investigation on these complexes.[18]

Complex binding with DNA through intercalation usually results in hyperchromism and blue shift, because intercalative mode involving a strong stacking interaction between an aromatic chromophore and the base pairs of DNA. The electronic absorption spectra of complexes mainly consist of two resolved bands.[19] Change in absorbance at peak maximum shows moderate hypsochromism shift (~4 nm). For each complex, increasing concentration of DNA has been monitored for an evaluation of the intrinsic binding constant. Using absorption measurements for (I), a linear plot of $[DNA]/(\varepsilon_f/\varepsilon_a)$ versus [DNA] was obtained using Eq. 1 (S-6). Assuming all the molecules of complexes were bound with DNA, the experimental $K_b$ was obtained by substituting the absorbance into Beer's law. The $K_b$ value derived from the plot for all the complexes were in range of $1.0 \times 10^4 - 2.5 \times 10^4\ M^{-1}$. These spectral characteristics are consistent with a mode of interaction that involves a stacking interaction between the complex and the base pairs of DNA, which means that the titled complexes can intercalate into the double helix structure of DNA.

Hydrodynamic measurements that are sensitive to length change (i.e., viscosity and sedimentation) are regarded as the least ambiguous and the most critical tests of a binding model in solution in the absence of crystallographic structural data (S-7). A classical intercalation model demands that the DNA helix lengthens as base pairs are separated to accommodate the bound ligand, leading to the increase of sperm herring DNA viscosity. In contrast, a partial, non-classical intercalation of ligand could bend (or kink) the DNA helix, reduce its effective length and, concomitantly, its viscosity.[20] The effects of complexes on the viscosity of DNA are shown in Figure 1. For complexes the viscosity of DNA increases steadily with increasing concentration of the complex. The experimental results suggest that complexes bind to DNA through a classical intercalation mode and the order of intercalation mode of complexes to DNA are II > V > IV > VI > III > I. This difference of DNA-binding mode between complexes should be caused by their different ancillary ligands.

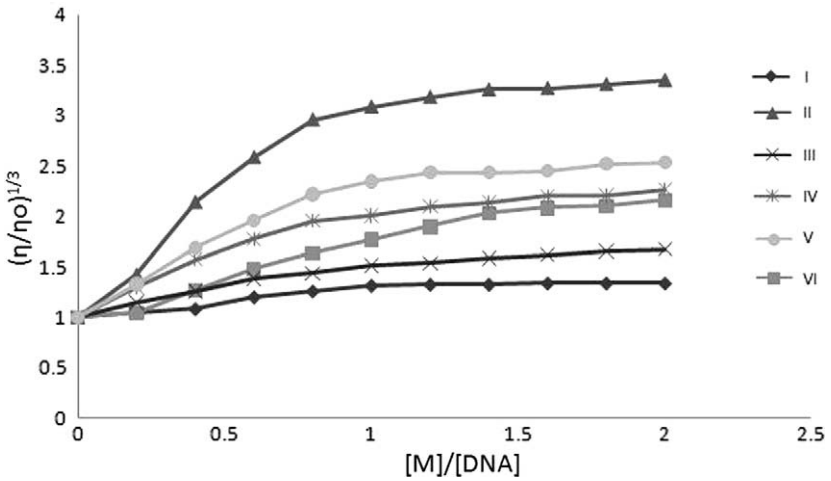


**Figure 1.** The effect of the complexes on the viscosity of DNA.

**Table 1**
Gel electrophoresis data of the compounds without $H_2O_2$

| Compound | Conc. of comp. (μM) | % SC | % NC | % OC | Ref. |
|---|---|---|---|---|---|
| DNA (control) | — | 87 | — | 13 | This work |
| DNA + $Co(NO_3)\cdot 6H_2O$ | 10 | 58 | — | 42 | This work |
| DNA + Cip | 10 | 40 | 16 | 44 | This work |
| DNA + I | 10 | 15 | 45 | 40 | This work |
| DNA + II | 10 | 28 | 42 | 30 | This work |
| DNA + III | 10 | 14 | 48 | 38 | This work |
| DNA + IV | 10 | 20 | 12 | 67 | This work |
| DNA + V | 10 | 20 | 29 | 51 | This work |
| DNA + VI | 10 | 21 | 62 | 17 | This work |
| DNA (control) | — | 85.8 | — | 14.2 | 24 |
| DNA + $[Co(en)_2Cl_2]^+$ | 500 | 78.1 | — | 21.9 | 24 |
| DNA + $[Co(bpy)_2Cl_2]^+$ | 500 | 77.4 | — | 22.6 | 24 |
| DNA (control) | | 93 | 7 | — | 25 |
| DNA + $[CuL_2]ClO_4$ | 33 | 54 | 46 | — | 25 |
| DNA + $[CuL(phe)]ClO_4$ | 33 | 29 | 71 | — | 25 |

**Table 2**
Gel electrophoresis data of the compounds with $H_2O_2$

| Compound | Conc. of comp. (μM) | Additional reagent | Conc. of reagent | % SC | % NC | % OC | Ref. |
|---|---|---|---|---|---|---|---|
| DNA (control) | — | | — | 87 | — | 13 | This work |
| DNA | — | $H_2O_2$ | 100 μM | — | 77 | 23 | This work |
| DNA + $Co(NO_3)_2\cdot 6H_2O$ | 10 | $H_2O_2$ | 100 μM | — | 69 | 31 | This work |
| DNA + Cip | 10 | $H_2O_2$ | 100 μM | — | 41 | 59 | This work |
| DNA + I | 10 | $H_2O_2$ | 100 μM | — | 75 | 25 | This work |
| DNA + II | 10 | $H_2O_2$ | 100 μM | 20 | 66 | 14 | This work |
| DNA + III | 10 | $H_2O_2$ | 100 μM | 16 | 73 | 11 | This work |
| DNA + IV | 10 | $H_2O_2$ | 100 μM | — | 86 | 14 | This work |
| DNA + V | 10 | $H_2O_2$ | 100 μM | 11 | 60 | 23 | This work |
| DNA + VI | 10 | $H_2O_2$ | 100 μM | 15 | 72 | 13 | This work |
| DNA (control) | — | — | — | 99 | 1 | — | 26 |
| DNA + $[CuL^2(phe)]ClO_4$ | 80 | $NaN_3$ | 100 μM | 91 | 9 | — | 26 |
| DNA + $[CuL^2(phe)]ClO_4$ | 80 | $D_2O$ | 14 μL | 1 | 99 | — | 26 |
| DNA (control) | — | — | — | 96 | 4 | — | 24 |
| DNA + $[Co(phe)_2Cl_2]^+$ | 500 | $NaN_3$ | 20 mM | 74.3 | 25.7 | — | 24 |
| DNA (control) | — | — | — | — | — | — | 27 |
| DNA + $[Cu(qnsa)_2(phe)]$ | 15 | $H_2O_2$ | 150 μM | — | — | — | 27 |

There has been considerable interest in DNA cleavage reactions that are activated by transition metal complex. The delivery of metal ion to the helix, in locally generating oxygen or hydroxide radicals, yields an efficient DNA cleavage reaction.[21,22] The cleavage of pUC19 DNA induced by the complexes under aerobic conditions in absence and presence of $H_2O_2$ was carried out using electrophoresis technique[23] (S-8). When circular plasmid DNA conducted by electrophoresis, the fastest migration will observed for the supercoiled form (SC). If one strand cleaved, the supercoiled will relax to produce a slower-moving open circular form (OC). If both strands cleaved, a nicked form (NC) will be generated that migrates in between. This clearly shows that the relative binding efficacy of the complexes to DNA is much higher than the binding efficacy of metal salt itself or ciprofloxacin. The different DNA-cleavage efficiency of the complexes was due to the different binding affinity of the complexes to DNA, which has been observed in other cases. One of the most interesting electrophoretic results of the complexes takes place when experiment done in presence of $H_2O_2$. The DNA + complex + $H_2O_2$ systems cleave the supercoiled DNA form (I) and convert into nicked form (III) to open circular form (II) more than complex alone. Gel electrophoresis data of the complexes with and without $H_2O_2$ along with some reported results are presented in Tables 1 and 2. Therefore, we concluded that the mixture of complex with $H_2O_2$ have been found to be efficient oxidant. Hence the proposed work seems to be worth for generating database to develop new effective useful DNA probes.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.03.078.

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