Synthesis, spectral characterization and DNA cleavage studies of Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II) complexes with benzofuran-2-carbohydrazide Schiff bases

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New Schiff bases have been synthesized from benzofuran-2-carbohydrazide and 1-naphthaldehyde [NMBC] or 6-methoxy-2-naphthaldehyde [MNMBC]. Complexes of the type MLX₂, where M = Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II), L = NMBC or MNMBC and X = Cl, have been synthesized and characterized on the basis of analytical data, IR, UV-vis, ¹H NMR, DART-MS and ESR spectral data and magnetic studies. All the complexes are soluble in DMF and DMSO. The measured molar conductance values indicate that the complexes are non-electrolytic in nature. The DNA cleavage activities of the Schiff bases and their Co(II), Ni(II) and Cu(II) complexes have been studied by agarose gel electrophoresis method. The Cu(II), Ni(II) and Co(II) complexes have also been studied for their plasmid (pBR322) DNA cleavage activity.

Keywords: Coordination chemistry, Benzofurans, Schiff bases, DNA cleavage, Copper, Nickel, Cobalt, Zinc, Cadmium, Mercury

Benzofuran compounds are abundant in nature, particularly among plants, and often possess useful pharmacological properties. Benzofuran compounds occur in nature in a variety of structural forms. The seed oil of plant “Egonoki” which contains a benzofuran derivative called “Egonal”, is an effective synergist for rotenone pyrethrum against house flies, mosquitoes, aphides and many other insects. Baker’s yeast contains a benzofuran derivative which acts as an antioxidant preventing hemorrhaging liver necrosis in rats and haemolysis of red cells in vitamin-E deficient rats. Some of the benzofuran derivatives such as 2-acetylbenzofuran and 2-nitrobenzofuran are well known biodynamic agents possessing various pharmacological properties. The compound, amiodarone hydrochloride, used as an ideal anti-arrhythmic drug contains a 2, 3-disubstituted benzofuran moiety.

In the past two decades, the properties of Schiff base metal complexes stimulated much interest for their contributions to single molecule-based magnetism, material science, catalysis of many reactions like carbonylation, hydroformylation, oxidation, reduction and epoxidation, their industrial applications and complexing ability towards some toxic metals. The high affinity for the chelation of the Schiff bases towards the transition metal ions is utilized in preparing their solid complexes. Schiff base complexes containing nitrogen and oxygen as donor atoms play an important role in biological systems and represent models for metalloproteins and metalloenzymes that catalyze the reduction of nitrogen and oxygen. Small bioactive ligands with low molecular weight, which also recognize and interact with DNA, are of significance as potential artificial gene regulators or cancer chemotherapeutic agents.

The aim of present work is to synthesize and characterize the Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II) metal complexes with newly synthesized Schiff bases derived from benzofuran-2-carbohydrazide and 1-naphthaldehyde/6-methoxy-2-naphthaldehyde. The DNA cleavage activities of Schiff bases and their Co(II), Ni(II) and Cu(II) complexes have been studied by agarose gel electrophoresis method. The Cu(II), Ni(II) and Co(II) complexes have also been investigated for their plasmid (pBR322) DNA cleavage activity.

Materials and Methods

All the chemicals used were of analytical reagent grade (AR), and of highest purity available. Benzofuran-2-carbohydrazide was synthesized...
according to the literature procedure. The metal and chloride contents were determined as per literature method. CHN analysis was carried out microanalytically on a Perkin-Elmer (model 240C) instrument at the Central Drug Research Institute (CDRI), Lucknow. The IR spectra of the Schiff bases and their Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II) complexes were recorded in KBr pellets in the region 4000-350 cm\(^{-1}\) on a Perkin-Elmer 783 FT-IR spectrophotometer. The electronic spectra of the Co(II), Ni(II) and Cu(II) complexes were recorded on an Elico-SL-164 double beam UV-visible spectrophotometer in the range 200-1100 nm in DMF (10\(^{-3}\) M) solution. The \(^1\)H NMR spectra were recorded in DMSO-\(d_6\) on a Bruker 300 MHz spectrophotometer using TMS as an internal standard. The ESR spectrum of the Cu(II) complex in polycrystalline state was recorded on a Varian-E-4X band EPR spectrophotometer using TCNE as ‘g’ marker (g=2.00277) at room temperature. DART-4X band EPR spectrophotometer using TCNE as ‘g’ marker (g=2.00277) at room temperature. DART-4X band EPR spectrophotometer using TCNE as ‘g’ marker (g=2.00277) at room temperature. DART-4X band EPR spectrophotometer using TCNE as ‘g’ marker (g=2.00277) at room temperature. DART-4X band EPR spectrophotometer using TCNE as ‘g’ marker (g=2.00277) at room temperature. DART-4X band EPR spectrophotometer using TCNE as ‘g’ marker (g=2.00277) at room temperature. DART-4X band EPR spectrophotometer using TCNE as ‘g’ marker (g=2.00277) at room temperature.

**General procedure for synthesis of Schiff bases and their metal complexes**

The Schiff bases, (E)-N-[((naphthalen-4-yl)methylene)benzofuran-2-carboxyhydrazide [NMBC] and (E)-N-((2-methoxynaphthalen-6-yl)methylene)benzofuran-2-carboxyhydrazide [MNMBC] were prepared as follows: A solution of benzofuran-2-carboxyhydrazide (1.76 g, 0.01 mol) in ethanol (25 mL) was added to 1-naphthaldehyde (1.56 g, 0.01 mol)/6-methoxy-2-naphthaldehyde (1.86 g, 0.01 mol) in ethanol (10 mL). The reaction mixture was refluxed on a water bath for about 4-5 h. The Schiff base was separated on partial removal of the solvent and cooling to the room temperature as light yellowish crystalline solid, which was filtered, washed with ethanol and recrystallised from ethanol.

\[ C_{32}H_{16}O_{3}N_2 \text{[NMBC]}: \text{Mol. wt} = 344, \text{m. pt.} = 221 ^\circ \text{C}, \text{Yield} = 68 \%. \]

\[ C_{37}H_{18}O_{3}N_2 \text{[MNMBC]}: \text{Mol. wt} = 344, \text{m. pt.} = 234 ^\circ \text{C}, \text{Yield} = 70 \%. \]

The metal complexes were synthesized by adding ethanolic solution (20 mL) of metal(II) chlorides (0.01 mol) to the Schiff base [NMBC]/[MNMBC] (3.14 g/3.44 g, 0.01 mol) in ethanol (30 mL). The reaction mixture was refluxed on a water bath for 6 h, and on partial removal of the solvent and cooling to the room temperature gave solid colored complexes which were filtered, washed thoroughly with alcohol and finally dried over fused CaCl\(_2\) in vacuum (Yield: 60-65 %).

**DNA cleavage**

DNA cleavage experiments were done according to the literature. Nutrient broth [peptone: 10 g/L; yeast extract: 3 g/L; NaCl: 10 g/L] was used for culturing of *Staphylococcus aureus*. The 50 mL media was prepared, autoclaved for 15 min at 121 °C under 15 lb pressure. The autoclaved media were inoculated with the seed culture and incubated at 37 °C for 24 h.

The fresh bacterial culture (1.5 mL) was centrifuged to obtain the pellet which was then dissolved in 0.5 mL of lysis buffer (100 mM tris pH 8.0, 50 mM EDTA, 50 mM lysozyme). To this, 0.5 mL of saturated phenol was added and incubated at 55 °C for 10 min. After centrifugation at 10,000 rpm for 10 min, to the supernatant, equal volume of chloroform:isoamyl alcohol (24:1) and 1/20 volume of 3 M sodium acetate (pH 4.8) was added. The solution was further centrifuged at 10,000 rpm for 10 min and to the supernatant, 3 volumes of chilled absolute alcohol was added. The precipitated DNA was separated by centrifugation and the pellet was dried and dissolved in tris buffer (10 mM tris pH 8.0) and stored in cold condition.

Cleavage products were analyzed by agarose gel electrophoresis method. Test samples (1 mg/mL) were prepared in DMSO. The samples (25 µg) were added to the isolated DNA of *S. aureus*, and incubated for 2 h at 37 °C. Then 20 µL of DNA sample (mixed with bromophenol blue dye at 1:1 ratio) was loaded carefully into the electrophoresis chamber wells along with standard DNA marker containing TAE buffer (4.84 g tris base, pH 8.0, 0.5 M EDTA/L) and finally loaded on agarose gel and passed the constant 50 V of electricity for around 30 min. Removed the gel and stained with 10 µg/mL ethidium bromide for 10-15 min and the bands observed under UV transilluminator and photographed to determine the extent of DNA cleavage. Then the results are compared with standard DNA marker.
Results and Discussion

Characterization of the complexes

The analytical data shows that all of the complexes have 1:1 stoichiometry (Table 1). The molar conductance values are too low to account for any dissociation of the complexes in DMF, indicating non-electrolytic nature of the complexes. All the complexes are light in colour, stable and non-hygrosopic in nature and possess high melting points. The complexes are insoluble in common organic solvents but soluble in DMF and DMSO.

Magnetic susceptibility measurements at room temperature exhibit paramagnetism for Co(II), Ni(II) and Cu(II) complexes (Table 1). The Co(II) complexes exhibit magnetic moments of 4.78 and 4.86 BM, suggesting octahedral geometry. The Ni(II) complexes show magnetic moment values of 2.85 and 2.96 BM, slightly higher than spin only value (2.83 BM), indicating an octahedral environment. The observed magnetic moments for the Cu(II) complexes are 1.65 and 1.67 BM, suggesting a distorted octahedral geometry.

The electronic spectra of the Co(II), Ni(II) and Cu(II) complexes were recorded for freshly prepared solution in DMF (10^{-3} M) at room temperature. The electronic spectra of Co(II) complexes show two bands at 16806, 20221 cm^{-1} and at 16013, 19379 cm^{-1} assignable to \(^4T_1g\) (F) → \(^4A_{2g}\) (F) (v_2) and \(^2T_{1g}\) (F) → \(^2T_{2g}\) (P) (v_3) transitions respectively in an octahedral environment. The \(v_1\) band could not be observed, however, \(v_1\) band was calculated using an equation suggested by Underhill and Billing. The Ni(II) complexes exhibit two bands at 15152, 25679 cm^{-1} and at 15803, 26210 cm^{-1} assignable to \(^3A_{2g}\) (F) → \(^3T_{1g}\) (F) (v_2) and \(^3A_{2g}\) (F) → \(^3T_{1g}\) (P) (v_3) transitions respectively in an octahedral environment. The lowest band \(v_1\) could not be observed due to limited range of the instrument used. However, it is calculated by using band fitting procedure. The Cu(II) complexes exhibit a single broad asymmetric band in the region 12428-16598 cm^{-1}. The broadness of the band indicates that the expected three transitions \(^2B_{1g}\) → \(^2A_{1g}\) (v_1), \(^2B_{1g}\) → \(^2B_{2g}\) (v_2) and \(^2B_{1g}\) → \(^2E_{g}\) (v_3) are similar in energy and give rise to only one broad band. The broadness of the band may

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<th>Comp.</th>
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be due to dynamic Jahn–Teller distortion. These data suggest a distorted octahedral geometry around Cu(II) ion. The octahedral geometry is further supported by the values of ligand field parameters such as Dq (897, 855 and 927, 976 cm\(^{-1}\)), B' (901, 867, and 869 and 849 cm\(^{-1}\)), \(\beta\) (0.928, 0.893 and 0.836, 0.816) and LFSE (15.377, 14.657 and 31.783, 33.463 kcal) respectively for Co(II) and Ni(II) complexes of the Schiff bases [NMBC] and [MNMBC].

The IR spectra of the free ligands were compared with the spectra of the metal complexes. The IR spectra of [NMBC] and [MNMBC] show strong bands at 3187 and 3114 cm\(^{-1}\) assigned to \(\nu(NH)\) stretch of the CONH group. The shifting of these bands to higher wave number in the complexes by 8-16 cm\(^{-1}\) indicates non-involvement of \('N' of CONH group in bonding. The strong bands observed at 1665 and 1704 cm\(^{-1}\) in the free ligands are assigned to \(\nu(C=O)\) stretch of carbonyl group. These bands shift to lower wave number in all the complexes by about 21-86 cm\(^{-1}\), indicating participation of the carbonyl oxygen in coordination. The bands at 1578 and 1611 cm\(^{-1}\) in the free ligands are assigned to \(\nu(C=N)\) stretch of the azomethine group. These bands shift to lower wave number in all the complexes indicating involvement of azomethine nitrogen in bonding. The medium intensity bands at 961 and 975 cm\(^{-1}\) are assigned to \(\nu(N-N)\) stretching vibration of hydrazine moiety. These bands in the complexes shift slightly to higher wave number confirming involvement of one of the nitrogen of N-N- in bonding with the metal ions.

Metal–ligand vibrations are generally observed in the far-IR region. The weak intensity non-ligand bands observed in the complexes, in the region 549-521 cm\(^{-1}\) and 476-448 cm\(^{-1}\) are assigned to \(\nu(M-O)\) and \(\nu(M-N)\) stretching vibrations, respectively. For polymeric complexes where both terminal and bridging metal–halogen linkages are present, \(\nu(M-Cl)\) stretch for terminal halide is observed at a higher wave number than that for bridging halide. In the present study the broad and weak intensity non-ligand bands is assigned to \(\nu(M-Cl)\) stretch for terminal in the region 394–452 cm\(^{-1}\) and \(\nu(M-Cl)\) stretch for bridging in the region 365 – 392 cm\(^{-1}\) in the case of Co(II), Ni(II) and Cu(II) complexes in support of their chloride bridged polymeric octahedral structures. Medium intensity bands in the region 375–402 cm\(^{-1}\) are assigned to \(\nu(M-Cl)\) stretch in Zn(II), Cd(II) and Hg(II) complexes.

\(^{1}\)H NMR spectra of [NMBC] and [MNMBC] and their Zn(II) and Cd(II) complexes were recorded in DMSO-\(d_6\). The signals at \(\delta\) (12.23, 12.16) (s, 1H) are assigned to amide proton (-CONH-) and the signals at \(\delta\) (8.83, 8.64) (s, 1H) to azomethine proton (-N=CH-) in both ligands. The signals due to (-CONH-) are shifted downfield in the spectra of Zn(II) at \(\delta\) (12.34, 12.28) (s, 1H) and Cd(II) at \(\delta\) (12.43, 12.31) (s, 1H) complexes, indicating coordination of oxygen of -CONH- with metal ions. The azomethine protons shift downfield in the region \(\delta\) (8.72 – 9.22) (s, 1H), supporting coordination of ‘N’ of the –N=CH- group with the metal ions. The aromatic protons at \(\delta\) (7.20 – 8.08) shift downfield in the complexes. Thus, \(^{1}\)H NMR spectral observations supplement the assigned geometry.

The DART-mass spectrum of the Schiff base (NMBC) showed a molecular ion peak at \(m/z\) 315, which is one mass unit more than that of the molecular weight of the Schiff base. The DART-mass spectrum of the Ni(II) complex of the Schiff base (NMBC) showed a molecular ion peak at \(m/z\) 444, which is the same as that of the molecular weight of the complex. The DART-mass spectrum of the Co(II) complex of the Schiff base (MNMBC) showed a molecular ion peak at \(m/z\) 345, which is one mass unit more than that of the molecular weight of the Schiff base. The DART-mass spectrum of the Co(II) complex of the Schiff base (MNMBC) showed a molecular ion peak at \(m/z\) 474 which is same as that of the molecular weight of the complex. Mass spectral studies support the proposed composition.

The ESR spectra of the Cu(II) complexes in polycrystalline state have been recorded at room temperature. The g\(_{||}\) and g\(_{\perp}\) values have been found to be 2.1999, 2.1985 and 2.0742, 2.0592 respectively. The g\(_{av}\) was calculated to be 2.1169 and 2.1067. The spectra have asymmetric bands with g\(_{||}\) > g\(_{\perp}\) > 2.00277 (TCNE), indicating that the unpaired electrons lie predominantly in the \(d_{x^2 - y^2}\) orbital with possibly mixing of \(d_{z^2}\) because of low symmetry. The axial symmetry parameter ‘G’ determined as \(G = (g_{||} - 2.00277)/(g_{\perp} - 2.00277) = 2.7598, 3.4685\) respectively, is found to be less than 4, suggesting considerable interaction in the solid state.

**DNA Cleavage**

The Schiff bases [MNMBC] and [NMBC] and their Co(II), Ni(II) and Cu(II) complexes were studied for their DNA cleavage activity by agarose gel electrophoresis method (Figs 1 and 2). The gel after
Fig. 1—DNA cleavage activity of Schiff bases, [MNMB C] and [NMBC] (B1 – B2).

Fig. 2—DNA cleavage activity of Co(II), Ni(II) and Cu(II) complexes of schiff bases, [MNMBC] and [NMBC] (P1 – P6).

Fig. 3—Plasmid (pBR322) DNA cleavage activity of Cu(II), Ni(II) and Co(II) complexes of schiff bases, [NMBC] and [MNMBC] (G1 – G6).

Fig. 4—Suggested structures of Co(II), Ni(II) and Cu(II) complexes of schiff bases, [NMBC] and [MNMBC].

Fig. 5—Suggested structures of Zn(II), Cd(II) and Hg(II) complexes of schiff bases, [NMBC] and [MNMBC].
electrophoresis clearly revealed that the intensity of all the treated DNA samples has diminished, possibly because of the cleavage of the DNA. The complete cleavage of DNA was observed by Ni(II) complex of the Schiff base [MNMBC] and partial cleavage of DNA was observed by Cu(II) complex of the Schiff base [MNMBC]. The difference was observed in the bands of the complexes (Lanes P1 – P6) compared to that of the control DNA of S. aureus. This shows that the control DNA alone does not show any apparent cleavage whereas the complexes show cleavage. However, the nature of reactive intermediates involved in the DNA cleavage by the complexes is not clear. These results indicate that the metal ions play an important role in the cleavage of isolated DNA. As the compound was observed to cleave the DNA, it can be concluded that the compound inhibits the growth of the pathogenic organism by cleaving the genome. The Cu(II), Ni(II) and Co(II) complexes were also studied for their plasmid (pBR322) DNA cleavage activity (Fig. 3). The difference was observed in the bands of the complexes (Lanes G1 – G6) compared to that of the control, pBR322 DNA.

The above study shows that the newly synthesized Schiff bases act as neutral bidentate ligand by coordinating through the oxygen of amide group and nitrogen of azomethine group. The DNA cleavage studies reveal that complete cleavage of DNA was observed by Ni(II) complex of (MNMBC) and partial cleavage of DNA was observed by Cu(II) complex of (MNMBC). Based on the analytical and spectral data we propose a chloride-bridged polymeric octahedral structures for Co(II), Ni(II) and Cu(II) complexes and tetrahedral structures for Zn(II), Cd(II) and Hg(II) complexes (Figs 4 and 5).

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References