Synthesis, characterization and DNA-Binding properties of the novel mononuclear Zn(II), Cd(II), and Mn(II) complexes with Pantoprazole

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Abstract: A novel mononuclear Mn(II), Zn(II) and Cd(II) complexes of pantoprazole (PA) was synthesized and characterized by elemental analysis, molar conductivity, magnetic susceptibility measurements, IR, UV-visible spectral studies, and thermal analysis. The electronic spectra along with magnetic data suggest octahedral geometry for Mn(II), Zn(II) and Cd(II) complexes. PA acts as an anionic bi-dentate ligand being coordinated by (S=O) oxygen and benzimidazolyl nitrogen atoms. The interaction of the complexes with calf thymus DNA (CT-DNA) was monitored by blue shift and hyperchromism in the UV-vis spectra. The observed intrinsic binding constants together with structural analysis of the complexes indicate the groove binding. The binding constants were determined at 303 K, 308 K and 313 K. A thermodynamic analysis showed that the reaction is spontaneous with ΔG being negative. The enthalpy ΔH and the entropy ΔS of reactions were all determined.

Keywords: Pantoprazole; Complexes; Binding constant; hyperchromism.

Introduction

Proton pump inhibitors are highly effective in the management of acid-related diseases, including duodenal ulcer (DU), gastric ulcer (GU), gastroesophageal reflux disease (GERD), erosive esophagitis, hypertensive syndromes like Zollinger -Ellison and Helicobacter pylori (H. pylori) infections 1-4. There are currently five different proton pump inhibitors (PPIs) available, including esomeprazole, lansoprazole, omeprazole, pantoprazole and rabeprazole 5-6. These agents are all substituted benzimidazoles that inhibit the final common pathway of gastric acid secretion 2,3. Proton pump inhibitors irreversibly inhibit the proton pump and acid production can only be restored through endogenous synthesis of new proton pumps 5,7-8. Metal complexes are gaining increasing importance in the design of respiratory, slow release and long acting drugs. Metal ions are therefore known to accelerate drug actions 11.

The efficacies of some therapeutic agents are known to increase upon coordination 12. Some metal complexes are known to exhibit remarkable antitumor, antifungal, antiviral and special biological activities 13,14. Therefore, complexation of chemotherapeutic agents has been found to be applicable useful in medicine and pharmacy 15.

Deoxyribonucleic acid (DNA) is the primary target molecule for most anticancer and antiviral therapies according to cell biology 16,17. Investigations of the interaction between small molecules and DNA are basic work in the design of new types of pharmaceutical molecules 18. Binding studies of small molecules with DNA are important in the design of new and more efficient drugs targeted to DNA 19. Several aromatic hydrocarbons and their derivatives have been shown to be carcinogenic, and in several instances, the carcinogenicity was attributed to their activity at the DNA level 20,49-50. Metal complexes, porphyrins, natural antibiotics, and a host of other planar heterocyclic cations have been investigated for their DNA binding affinity. Recently, the DNA sequence recognition by drugs as well as by small molecules that are conjugated to peptides or oligonucleotides has been of great interest. Binding studies with these various small molecules are valuable for the rational design of drugs as well as in understanding how

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proteins recognize and bind to specific DNA sequence \(^21,22\).

**Experimental Section**

**Materials**

All chemicals were of A.R. grade and were used without further purification; pure (how pure) Pantoprazole sodium sesquihydrate (PANa) was obtained from Smailx Laboratories Limited, India. Cadmium (II) chloride dihydrate (CdCl\(_2\)-2H\(_2\)O), zinc (II) chloride hexahydrate (ZnCl\(_2\)-6H\(_2\)O) and manganese (II) chloride hexahydrate (MnCl\(_2\)-6H\(_2\)O) were purchased from (Merck Co.). All solvents used are of analytical grade quality from Sigma-Aldrich.

**Methods**

All experiments involving the interaction of the complexes with DNA was carried out in tripus buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.0). In solution this DNA had a ratio of UV absorbance at 260 and 280 nm of about 1.90 indicating its purity \(^23\). DNA concentration per nucleotide was determined by absorbance at 260 nm using the molar absorption coefficient

\[ (6600 \text{ M}^{-1}\text{cm}^{-1}) \] \(^{24,25}\). All the experiments involving the binding of complexes with CT-DNA were carried out in a bidistilled water buffer with (5 mM) hydroxymethyl-aminoethanethanol (Tris, 5 mM) and sodium chloride (50 mM) and adjusted to pH 7.2. Absorption titration experiments were performed with fixed concentrations of the complexes (1 x 10\(^{-4}\) molL\(^{-1}\)) with varying concentration of DNA(0-60 \(\mu\)M). While measuring the absorption spectra, an equal amount of DNA was added to both the test solution and the reference solution to eliminate the absorbance of DNA itself. The values of the intrinsic binding constants \(K_b\) were calculated by regression analysis using the equation

\[ \frac{[DNA]}{\varepsilon_{a}-\varepsilon_f} = [DNA] + \frac{1}{K_b(\varepsilon_a-\varepsilon_f)} \] \(^{(1)}\)

where [DNA] is the concentration of CT-DNA in base pairs and \(\varepsilon_a\), \(\varepsilon_f\) and \(K_b\) are extinction coefficients of the apparent, free and bound metal complex, respectively and \(K_b\) is the equilibrium binding constant. In the plots of [DNA]/(\(\varepsilon_a - \varepsilon_f\)) versus [DNA], \(K_b\) is given by the ratio of slope to the intercept.

**Synthesis of the complexes**

To a colorless solution of PANa (431 mg, 1 mmol) in ethanol/ water (EtOH/ H\(_2\)O, 1:1) (10 ml), a solution of hydrated metal chloride (1 mmol) in ethanolic aqueous (H\(_2\)O/EtOH, 1:1) (10 ml) was added drop wise then the reaction mixture was stirred at room temperature for 1 hr. After that, the isolated solid was filtered off, washed with cold acetone and dried under vacuum at room temperature to yield M(II) pantoprazolate complexes.

**Instruments**

Elemental analyses for C, H, and N were performed with a Perkin-Elmer 2400 elemental analyzer. FTIR spectra were recorded on a BRUKER Tensor -37 FTIR spectrophotometer in the range 400-4000 cm\(^{-1}\) as KBr discs. For signal intensities the following abbreviations were used: br (broad), sh (sharp), w (weak), m (medium), s (strong), vs (very strong). The UV/vis absorption measurements were conducted at room temperature with concentration (10\(^{-4}\) M) on a Shimadzu UV-1601 spectrophotometer, in the range 200-800 nm, using quartz cuvettes (1 cm).

Molar conductivities of freshly prepared 1 x 10\(^{-3}\) molL\(^{-1}\) DMF solutions were measured using WPACM 35 conductivity meter. Finally the thermogravimetric analysis (TGA and DTG) were carried out in dynamic air atmosphere (30 mL/min) with a heating rate of 10°C/min using a Shimadzu TGA-50H thermal analyzer.

**Results and Discussion**

**Chemistry and synthesis**

The metal complexes were prepared by the reaction of an equimolar amount of MC\(_2\)-H\(_2\)O \(^{2-}\), \(Zn^{2+}\) and \(Cd^{2+}\) with PANa in ethanol/ water (1:1) media. The ease of synthesis and high yield in single step reaction from commercially inexpensive reagents make these extremely attractive in coordination compounds. These metal complexes are stable, soluble in DMF and DMSO and insoluble in all other organic solvents and in water. The elemental analysis and conductivity data of the free ligand and its complexes are presented in Table 1. All the metal complexes possess 1:1 (M: L) stoichiometry and the analytical data are in a good agreement with the proposed stoichiometry. The molar conductivity values for the complexes in DMF solvent (1.0 x 10\(^{5}\) molL\(^{-1}\)) which were in the range (0.016-0.022 mhos cm\(^{2}\)mol\(^{-1}\)) (Table 1) reveal their non-electrolytic nature \(^{26}\) and absence of Cl\(^-\) outside the coordination sphere which supported by negative AgNO\(_3\) test.

\[ \text{M}^{2+} + \text{L} \rightarrow \text{ML} \]
Table 1. Elemental analytical results and some properties of the PANa and complexes

<table>
<thead>
<tr>
<th>Compound</th>
<th>m.p (°C)</th>
<th>Y (%)</th>
<th>Elemental Analysis (%)</th>
<th>Calc. / (found)</th>
<th>Λη</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>H</td>
<td>N</td>
<td>S</td>
</tr>
<tr>
<td>PANa (C16H15F2N3NaO4 SNa)</td>
<td>198</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>[Cd(PA)Cl2(H2O)2] (C16H19Cl2CdF2N3O6S)</td>
<td>200</td>
<td>70</td>
<td>31.88</td>
<td>(31.76)</td>
<td>3.18</td>
</tr>
<tr>
<td>[Zn(PA)Cl2(H2O)2] (C16H19Cl2ZnF2N3O6S)</td>
<td>186</td>
<td>73</td>
<td>34.58</td>
<td>(34.35)</td>
<td>3.45</td>
</tr>
<tr>
<td>[Mn(PA)Cl2(H2O)2] (C16H19Cl2MnF2N3O6S)</td>
<td>194</td>
<td>72</td>
<td>35.24</td>
<td>(35.13)</td>
<td>3.51</td>
</tr>
</tbody>
</table>

**IR spectra and mode of chelation**

The study of the IR spectral data was quite informative in characterizing the metal-PA binding modes. The diagnostic IR spectral bands of the free ligand with those of its metal complexes were recorded in Table 2.

Table 2. IR spectral assignments of PANa and its bivalent transition metal complexes

<table>
<thead>
<tr>
<th>Compound</th>
<th>ν (O=)</th>
<th>ν (C=N)</th>
<th>ν (C-)</th>
<th>ν (S=O)</th>
<th>γ, δ</th>
<th>ν (M-)</th>
<th>ν (M-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PANa</td>
<td>3430</td>
<td>1590</td>
<td>1320</td>
<td>1120</td>
<td>1069</td>
<td>720</td>
<td>630</td>
</tr>
<tr>
<td></td>
<td>(m, br)</td>
<td>(s, sh)</td>
<td>(s, sh)</td>
<td>(s, sh)</td>
<td>(s, sh)</td>
<td>(w, br)</td>
<td>(w, br)</td>
</tr>
<tr>
<td>[Cd(PA)Cl2(H2O)2]</td>
<td>3432</td>
<td>1592</td>
<td>1304</td>
<td>1119</td>
<td>1040</td>
<td>718, 630</td>
<td>426</td>
</tr>
<tr>
<td></td>
<td>(m, br)</td>
<td>(s, sh)</td>
<td>(m, sh)</td>
<td>(s, sh)</td>
<td>(s, sh)</td>
<td>(w, br)</td>
<td>(w, br)</td>
</tr>
<tr>
<td>[Zn(PA)Cl2(H2O)2]</td>
<td>3417</td>
<td>1590</td>
<td>1303</td>
<td>1119</td>
<td>1039</td>
<td>719, 631</td>
<td>421</td>
</tr>
<tr>
<td></td>
<td>(m, br)</td>
<td>(s, sh)</td>
<td>(m, sh)</td>
<td>(s, sh)</td>
<td>(s, sh)</td>
<td>(w, br)</td>
<td>(w, br)</td>
</tr>
<tr>
<td>[Mn(PA)Cl2(H2)2]</td>
<td>3435</td>
<td>1592</td>
<td>1301</td>
<td>1120</td>
<td>1041</td>
<td>716, 629</td>
<td>419</td>
</tr>
<tr>
<td></td>
<td>(m, br)</td>
<td>(s, sh)</td>
<td>(s, sh)</td>
<td>(s, sh)</td>
<td>(s, sh)</td>
<td>(w, br)</td>
<td>(w, br)</td>
</tr>
</tbody>
</table>

sh, sharp; br, broad; s, strong; m, medium; w, week.

All complexes exhibit very similar infrared spectral features. When comparing the spectroscopic data of complexes with those of the free ligand, marked changes may be noticed in the ligand bands arising from various modes of donor groups involved in bonding to cadmium, zinc and manganese ions (Figure 1). The diagnostic IR spectral bands with their assignments of the free ligands and their metal complexes are shown in (Table 2). The IR stretching bands due to ν(C-N, imidazole) register a blue-shift of 10-11 cm⁻¹ in the complexes thus, indicating the coordination of the imidazole nitrogen to the ions [28], which is further confirmed by the appearance of a new band in the spectra of chelates at 443-445 cm⁻¹ assigned to a νM(II)-N vibration (M= Mn, Zn, Cd) [29]. Strong bands attributable to sulfonoyl oxygen ν(S=O) are evident; these all show a shift of 28-30 cm⁻¹ to lower energy in the complexes from the free ligand values indicative of coordination to Mn(II), Zn(II) and Cd(II) respectively [30]. The new IR band (456-475 cm⁻¹) is assigned to M-O vibrations [31] and supports the ligation of the sulfonoyl oxygen to metal ions. The bands ascribed to the vibration of the imidazole ν(C=N) and pyridine moiety remains unchanged in the complexes revealing that, the nitrogen atom of the (C=N) group does not take part in metal coordination [32]. Finally, very broad bands centered at ca. 3400 cm⁻¹ in all complexes can be assigned to the ν(OH) modes of lattice water in the corresponding framework [33-36].
Electronic spectra and magnetic susceptibility data

The formation of the metal complexes was also confirmed by UV/vis spectra. The absorption spectrum of PA (1x10⁻⁴ mol L⁻¹) in ethanol:DMF (1:9) (v/v) shows a maximum absorption band at 242 nm and around 295 nm due to the π-π* transition with high extinction coefficient 1.85x10⁴ mol cm⁻¹ L mol⁻¹, which can be assigned to the intra-pantoprazolate charge transfer (π→π*) transition, in the phenyl and imidazole parts of the PA ligand 37. In comparison to the ligand, the electronic spectra of the mononuclear complexes (1x10⁻⁴ mol L⁻¹) exhibit similar features, where all display blue shift which confirms the coordination of the ligand to the metal ions 38, which indicate that the benzimidazolyl nitrogen atom and the oxygen atom were involved in coordination with the metal ions. For Mn-complex, the absorption bands at 291 and 242 nm are assigned to ligand-centered π→π* transition 39. The Mn(II) complexes, exhibit new bands over 450 nm corresponding to the ⁶A₁g (F) → ⁴T₂g (G) transitions, as has been reported for Mn(II) octahedral complexes ⁴⁰. In the spectra of the complexes, the d-d transitions were not very well defined and were observed as shoulder bands. The geometry of Mn(II) complex is further confirmed ⁴²,⁴³ by the high μeff value (5.85 BM).

The diamagnetic Zn(II) and Cd(II) complexes show absorption bands at 292 nm and 242 nm. These bands are attributed to the charge transfer MLCT as the electronic configuration of these complexes confirmed the absence of any d-d transition ⁴⁴,⁴⁶. Effect of different solvents on UV-vis spectra for complexes is shown in Figure 2. The π-π* (295 nm) band shifted to lower wavelength as the polarity of solvent increase, following the order: ethanol ~methanol > DMF > acetonitrile > DMSO > water.
Figure 2. Electronic absorption spectra for Pantoprazole and their complexes at different solvent

**Thermal stability**

The TG-DTA analysis of manganese complex shows loss in weight corresponding to decomposition of water molecules in the temperature range 152 to 200 °C accompanied with the two endothermic peaks in first step decomposition. In that second step, there is continuous loss of the complex supported by an endothermic peak and the final product is metal oxide. The TG - DTA analysis of Cd-complex shows loss in weight in temperature range 160-220 °C may correspond the elimination of coordinated water molecules accompanied with an endothermic peak in the first step of decomposition. The decomposition continues up to 700°C and on further increasing the temperature no weight loss is observed which may be attributed to formation of stable metal oxide.

**Structure of the complexes**

The fact that these compounds were isolated as powders and not as single crystals means that no complete structure determination can be made. Accordingly, physicochemical and spectral as well as thermal analyses could help us to predict structure; the suggested structures of the Hydrated Mn(II), Zn(II) and Cd(II) pantoprazolate complexes can be represented below (Figure 4).

![Chemical structure image](image-url)

**Figure 4.** PANa coordination modes in metal complexes

**DNA-binding experiments**

DNA-binding studies are important for the rational design and construction of new and more efficient drugs targeted to DNA. Electronic absorption spectroscopy is one of the most useful techniques for DNA-binding studies of metal complexes. The spectral changes reflect the corresponding changes in DNA in its conformation and structures after the drug bound to DNA. Intercalative mode of binding usually results in hypochromism and bathochromism due to strong stacking interaction between an aromatic
chromophore and the base pairs of DNA. On the other hand, metal complexes which non-intercalatively or electrostatically bind with DNA may result in hyperchromism and hypochromism. The absorption spectra show clearly that the addition of DNA to the complexes lead to strong hyperchromism accompanied by the slight hypochromism to the [DNA]/[complex] (Figure 5). Obviously, these spectral characteristics suggest that all the complexes interact with DNA via electrostatically with the base pairs of DNA \(^{47-50}\)

![Graphs showing [DNA]/(ε_a - ε_b) versus [DNA] for Zn, Cd, and Mn complexes at different temperatures](image)

**Figure 5:** Plot of [DNA]/(ε_a - ε_b) versus [DNA]

The values of intrinsic DNA-binding constant, \(K_b\) of the complexes determined at different temperatures are listed in the Table 3.

**Table 3.** Binding constants and Thermodynamic parameters for the interaction of pantoprazole complexes with CT-DNA

<table>
<thead>
<tr>
<th>Temp.</th>
<th>Binding constant</th>
<th>R</th>
<th>(\Delta H) (kJ/mol)</th>
<th>(\Delta S) (J/mol.K)</th>
<th>(\Delta G) (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd-complex</td>
<td>298 K</td>
<td>2.88x10^3</td>
<td>0.9910</td>
<td>9.07x10^3</td>
<td>116.10</td>
</tr>
<tr>
<td>Cd-complex</td>
<td>308 K</td>
<td>3.50x10^3</td>
<td>0.9935</td>
<td>9.45x10^3</td>
<td>132.4</td>
</tr>
<tr>
<td>Cd-complex</td>
<td>318 K</td>
<td>3.68x10^3</td>
<td>0.9825</td>
<td>9.45x10^3</td>
<td>132.4</td>
</tr>
<tr>
<td>Mn-complex</td>
<td>298 K</td>
<td>1.86x10^5</td>
<td>0.9945</td>
<td>9.45x10^3</td>
<td>132.4</td>
</tr>
<tr>
<td>Mn-complex</td>
<td>308 K</td>
<td>1.97x10^5</td>
<td>0.9924</td>
<td>9.45x10^3</td>
<td>132.4</td>
</tr>
<tr>
<td>Mn-complex</td>
<td>318 K</td>
<td>2.39x10^5</td>
<td>0.9963</td>
<td>9.45x10^3</td>
<td>132.4</td>
</tr>
<tr>
<td>Zn-complex</td>
<td>298 K</td>
<td>2.5x10^4</td>
<td>0.9786</td>
<td>1.13x10^3</td>
<td>88.04</td>
</tr>
<tr>
<td>Zn-complex</td>
<td>308 K</td>
<td>2.56x10^4</td>
<td>0.9851</td>
<td>1.13x10^3</td>
<td>88.04</td>
</tr>
<tr>
<td>Zn-complex</td>
<td>318 K</td>
<td>2.58x10^4</td>
<td>0.9915</td>
<td>1.13x10^3</td>
<td>88.04</td>
</tr>
</tbody>
</table>

The thermodynamic parameters associated with temperature variation were analyzed in order to further characterize the acting forces between CT-DNA and complexes. The thermodynamic parameters, enthalpy change \((\Delta H)\) and entropy change \((\Delta S)\) of binding reaction, are the main evidence for confirming binding modes. From the thermodynamic standpoint, \(\Delta H > 0\) and \(\Delta S > 0\) implies a hydrophobic interaction; \(\Delta H < 0\) and \(\Delta S < 0\)
reflects the van der Waals force or hydrogen bond formation; and $\Delta H = 0$ and $\Delta S > 0$ suggesting an electrostatic force \cite{30b}. The temperature dependence of the binding constants was studied at three different temperatures (25, 35, and 45 °C). According to the following thermodynamic equations:

$$\ln K = -\frac{\Delta H}{RT} + \frac{\Delta S}{R}$$ (2)

$$\Delta G = \Delta H - T\Delta S = -RT\ln K$$ (3)

Where $K$ is the binding constant at constant temperature and $R$ is the gas constant, in which $\Delta H$ and $\Delta S$ of reaction could be determined from the linear relationship between $\ln K$ and the reciprocal absolute temperature. The free energy ($\Delta G$) could be calculated by Eq. (3). The results from the plots of $\ln K$ versus $1/T$ (Figure 6 which is based on the $K$ values of binding constant) are given in Table 3. The reaction of complexes with DNA is spontaneous as indicated from the negative value for $\Delta G$, both $\Delta H$ and $\Delta S$ are positive. This attributed to hydrophobic interactions being the leading contributor to the binding \cite{49, 51}. The binding constant increase with temperature suggesting that some covalent type interactions are at play in the binding.

Figure 6. Correlation between $\ln K$ versus $1/T$

Conclusion

Three new Mn(II), Zn(II) and Cd(II) complexes with pantoprazole sodium sesquihydrate (PANa) have been synthesized by mixing equimolar aqueous ethanol solution of PANa and MCl$_2$$\cdot$6H$_2$O (M = Mn$^{2+}$, Zn$^{2+}$ and Cd$^{2+}$). IR, UV-visible spectral studies, conductance measurements and thermogravimetric (TGA) and differential thermal analyses (DTG) have been used to characterize the isolated solid complexes.

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