Research Article

Synthesis, Crystal Structures, and DNA Binding Properties of Zinc(II) Complexes with 3-Pyridine Aldoxime

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The employment of 3-pyridine aldoxime, (3-py)CHNOH, in Zn II chemistry has afforded two novel compounds: \([\text{Zn(acac)}_2\{(3-py)\text{CHNOH}\}] \cdot \text{H}_2\text{O} (1 \cdot \text{H}_2\text{O})\) \([\text{where acac}^- \text{is the pentane-2,4-dionato(-1) ion}]\) and \([\text{Zn}_2(\text{O}_2\text{CMe})_4\{(3-py)\text{CHNOH}\}]/ (2)\). Complex 1 · H₂O crystallizes in the monoclinic space group \(P2_1/n\). The ZnII ion is five-coordinated, surrounded by four oxygen atoms of two acac⁻ moieties and by the pyridyl nitrogen atom of the (3-py)CHNOH ligand. Molecules of 1 interact with the water lattice molecules forming a 2D hydrogen-bonding network. Complex 2 crystallizes in the triclinic \(P\)-1 space group and displays a dinuclear paddle-wheel structure. Each ZnII exhibits a perfect square pyramidal geometry, with four carboxylate oxygen atoms at the basal plane and the pyridyl nitrogen of one monodentate (3-py)CHNOH ligand at the apex. DNA mobility shift assays were performed for the determination of the \emph{in vitro} effect of both complexes on the integrity and the electrophoretic mobility of pDNA.

1. Introduction

During the last decades, there has been considerable interest in the interaction of small molecules with DNA [1, 2]. DNA is generally the primary intracellular target of anticancer drugs, so such interactions can cause damage in cancer cells, block their division and consequently result in cell death [3]. Small synthetic binders can interact with DNA through the following three noncovalent modes: intercalation, groove binding, and external static electronic effects [4]. Transition metal complexes are a particularly interesting class of DNA-binders because of their cationic character, well-defined three-dimensional structure, aptitude to perform hydrolysis and redox reactions, as well as extensively developed substitution chemistry that allows easy modulations of their binding and reactive properties [5].

Among the various metal ions studied with nucleic acids and nucleobases, Zn II has occupied a special position [6], mainly due to the following reasons [7]: Zn II is a strong Lewis acid and exchanges ligands very rapidly; is of low toxicity; it has no redox chemistry, catalyzing only hydrolytic cleavage of DNA. For all the above mentioned reasons, the binding of Zn II complexes with DNA has attracted much attention [8, 9]. It has been reported that the binding properties of the complexes depend on several factors, such as the coordination geometry, the type of the donor-atoms and the planarity of ligands [10].

The ligand used in this work (Scheme 1) belongs to the family of pyridyl oximes. The coordination chemistry of these compounds has been extensively explored over the last two decades, mainly with paramagnetic 3d metal ions towards new molecular materials with interesting magnetic properties [11]. As a consequence, the diamagnetic character of the Zn II ion has led to a “gap” in the literature, concerning the area of the coordination chemistry of oximes. Recently, we have tried to fill this gap by the use of simple pyridyl oximes (the term “simple” means here ligands with only one pyridyl and one oxime group as donors) in Zn II coordination
chemistry. We reported the largest up to date Zn(II)/oxime cluster [12], as well as the first complexes of ZnII with 3- and 4-pyridine aldoxime [13].

In this study, our efforts were initiated by the synthesis and characterization of new ZnII/3-pyridine aldoxime complexes, while our next objective was to investigate the interaction of these compounds with plasmid DNA. The structural formula of the free ligand is illustrated in Scheme 1.

2. Experiments

2.1. Starting Materials and Physical Measurements. All manipulations were performed under aerobic conditions using reagents and solvents as received. Zinc acetylacetonate, zinc acetate, and 3-pyridinealdoxime were purchased from Aldrich Co. Elemental analyses (C, H, N) were performed by the University of Ioannina (Greece) Microanalytical Laboratory using an EA 1108 Carlo Erba analyzer. IR spectra (4000–450 cm\(^{-1}\)) were recorded on a Perkin-Elmer 16 PC FT-IR spectrometer with samples prepared as KBr pellets.

pDNA isolation was performed from a fully grown culture of Escherichia coli harboring the pBluescript plasmid. The Macherery-Nagel plasmid DNA isolation kit was used. All plastics and glassware used in the experiments were autoclaved for 30 min at 120°C and 130 Kpa.

2.2. Compound Preparation

2.2.1. Preparation of [Zn(acac)\(_2\)·(3-py)CHNOH]\(_2\)·H\(_2\)O (1·H\(_2\)O). Zn(acac)\(_2\)·H\(_2\)O (0.210 g, 0.80 mmol) was suspended in MeOH (10 cm\(^3\)) and then dissolved upon stirring by adding a solution of (3-py)CHNOH (0.195 g, 1.60 mmol) in the same solvent (10 cm\(^3\)). The resulting colourless solution was stirred at ambient temperature for 30 min and allowed to slowly evaporate at room temperature. Well-formed, X-ray quality colourless crystals of the product appeared within a period of four days. The crystals were collected by vacuum filtration, washed with cold MeOH (2 × 2 cm\(^3\)) and Et\(_2\)O (3 × 5 cm\(^3\)), and dried in air. The yield was ca. 65%. Found %: C, 47.60; H, 5.84; N, 6.73. Calc % for C\(_{18}\)H\(_{24}\)N\(_4\)O\(_{10}\)Zn: C, 47.36; H, 5.96; N, 6.90. IR data (KBr, cm\(^{-1}\)): 3468 (wb), 3196 (wb), 3084 (wb), 2998 (w), 2960 (w), 2914 (w), 2804 (w), 1956 (vw), 1654 (sh), 1586 (s), 1552 (vs), 1400 (s), 1340 (sh), 1316 (sh), 1268 (m), 1190 (m), 1124 (w), 1102 (vw), 1058 (vw), 1020 (w), 986 (m), 928 (w), 890 (vw), 818 (vw), 770 (w), 702 (w), 654 (w), 560 (w), 528 (vw), and 464 (vw).

2.2.2. Preparation of [Zn\(_2\)O\(_2\)CMe\(_4\)\(_4\)·(3-py)CHNOH\(_2\)]\(_2\) (2). Zn(O\(_2\)CMe)\(_2\)·H\(_2\)O (0.110 g, 0.50 mmol) was suspended in Me\(_2\)CO (10 cm\(^3\)) and then dissolved upon stirring by adding a solution of (3-py)CHNOH (0.122 g, 1.00 mmol) in the same solvent (10 cm\(^3\)). The resulting colourless solution was stirred at ambient temperature for 30 min and then layered with n-hexane (40 cm\(^3\)). Slow mixing gave well-formed, X-ray quality crystals of the product. The colourless crystals were collected by filtration, washed with cold Me\(_2\)CO (2 × 3 cm\(^3\)) and Et\(_2\)O (2 × 3 cm\(^3\)), and dried in air. The yield was ca. 53%. Found %: C, 39.10; H, 4.01; N, 9.23. Calc % for C\(_{20}\)H\(_{24}\)N\(_4\)O\(_{10}\)Zn\(_2\): C, 39.34; H, 3.94; N, 9.17. IR data (KBr, cm\(^{-1}\)): 3454 (wb), 3192 (m), 3084 (m), 2996 (m), 2962 (m), 2914 (m), 2808 (w), 1656 (sh), 1586 (vs), 1522 (s), 1400 (vs), 1340 (s), 1316 (s), 1268 (s), 1192 (w), 1124 (w), 1102 (vw), 1058 (vw), 1020 (m), 986 (s), 928 (m), 890 (w), 818 (m), 770 (m), 702 (m), 682 (w) 654 (w), 562 (w), and 418 (vw).

2.3. X-Ray Crystal Structure Determination. Crystals of 1·H\(_2\)O (0.22 × 0.34 × 0.45 mm) and 2 (0.12 × 0.14 × 0.24 mm) were mounted in capillary. Diffraction measurements for 1·H\(_2\)O were made on a Crystal Logic Dual Goniometer diffractometer using graphite monochromated Mo radiation, and for 2 on a P2\(_1\) Nicolet diffractometer upgraded by Crystal Logic using graphite monochromated Cu radiation. Unit cell dimensions were determined and refined by using the angular settings of 25 automatically centered reflections in the ranges of 11 < 2\(θ\) < 23° (for 1·H\(_2\)O) and 22 < 2\(θ\) < 54° (for 2) and they appear in Table 1. Intensity data were recorded using a \(θ\)-2\(θ\) scan. Three standard reflections monitored every 97 reflections showed less than 3% variation and no decay. Lorentz, polarization and psi-scan absorption (only for 1·H\(_2\)O) corrections were applied using Crystal Logic software. The structures were solved by direct methods using SHELXS-97 [14] and refined by full-matrix least-squares techniques on F\(^2\) with SHELXL-97 [15]. Further experimental crystallographic details for 1·H\(_2\)O: \(θ\)\(_{max}\) = 50°; reflections collected/unique/used, 3449/3284 [\(R_{int}\) = 0.0179]/3284; 269 parameters refined; (\(Δ\sigma\))\(_{max}\) = 0.001; (\(Δ\rho\))\(_{max}/(\Delta\rho)_{min}\) = 0.453/−0.457 e\(Å\)^{-1}; R1/wR2 (for all data), 0.0371/0.834. Further experimental crystallographic details for 2: \(θ\)\(_{max}\) = 118°; reflections collected/unique/used, 1964/1820 [\(R_{int}\) = 0.0538]/1820; 167 parameters refined; (\(Δ\sigma\))\(_{max}\) = 0.000; (\(Δ\rho\))\(_{max}/(\Delta\rho)_{min}\) = 0.559/−0.553 e\(Å\)^{-1}; R1/wR2 (for all data), 0.0524/0.1205. Hydrogen atoms were either located by difference maps and were refined isotropically or were introduced at calculated positions as riding on bonded atoms. All nonhydrogen atoms were refined anisotropically. CCDC codes 789600 and 789661 contain the supplementary crystallographic data for this paper. This data can be obtained free of charge at http://www.ccdc.cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; Fax: ++44-1223-356 033; E-mail: deposit@ccdc.cam.ac.uk].

2.4. Effect on pDNA. 0.1 μg of pBluescript were incubated at 25°C in the presence of various concentrations of the
complexes under study. After 1 h at 25°C the reaction was terminated by the addition of loading buffer consisting of 0.25% bromophenol blue, 0.25% xylene cyanol FF and 30% (v/v) glycerol in water. The products resulting from DNA-compound interactions were separated by electrophoresis on agarose gels (1% w/v), which contained 1% sodium acetate, 2 mM Na$_2$EDTA, at 5 V/cm. Agarose gel electrophoresis was performed in a horizontal gel apparatus of in MeOH, which affered colourless parallelepiped crystals of 2 upon layering of the reaction solution with n-hexane. The chemically balanced equation for the synthesis of 2 is:

\[
2 \text{Zn(O}_2\text{CMe)}_2 \cdot 2\text{H}_2\text{O} + 2(3\text{-py})\text{CHNOH} \rightarrow \text{Me}_2\text{CO} \left[ \text{Zn}_2(\text{O}_2\text{CMe})_4 \left\{ (3\text{-py})\text{CHNOH} \right\}_2 \right] + 4\text{H}_2\text{O}
\]

(2)

As a next step, we tried to modify the structural identity of 1·H$_2$O by using excess of Zn(acac)$_2$·H$_2$O. A probable result would be the isolation of a paddle wheel structure with four bidentate bridging acac$^-$ ligands and two monodentate (3-py)CHNOH ligands, that is, a structure analogous to that of compound 2. Unfortunately, our efforts did not yield fruits; all the reactions lead to the isolation of solids corresponding to compound 1·H$_2$O, emphasizing the reduced (compared to carboxylates) bridging capability of acac$^-$. 

### 3. Results and Discussion

#### 3.1. Synthetic Comments. 
Our previous investigation on the reaction between Zn(O$_2$CPh)$_2$ and (3-py)CHNOH led to a trinuclear benzoate cluster [13]. In a subsequent step, we expanded our research to similar or different types of bidentate ligands, such as MeCO$_2$ and acac$^-$, respectively.

Our initial efforts involved the reaction of Zn(acac)$_2$·H$_2$O with one equivalent of (3-py)CHNOH in MeOH, which afforded colourless parallelepiped crystals of 1·H$_2$O upon slow evaporation of the reaction solution. Its formation can be represented by the equation (1)

\[
\text{Zn(acac)}_2 \cdot \text{H}_2\text{O} + (3\text{-py})\text{CHNOH} \rightarrow \text{MeOH} \left[ \text{Zn(acac)}_2 \left\{ (3\text{-py})\text{CHNOH} \right\} \right] \cdot \text{H}_2\text{O}
\]

(1)

Treatment of Zn(O$_2$CMe)$_2$·2H$_2$O with two equivalents of (3-py)CHNOH in Me$_2$CO afforded colourless parallelepiped crystals of 2 upon layering of the reaction solution with n-hexane. The chemically balanced equation for the synthesis of 2 is:

\[
2 \text{Zn(O}_2\text{CMe)}_2 \cdot 2\text{H}_2\text{O} + 2(3\text{-py})\text{CHNOH} \rightarrow \text{Me}_2\text{CO} \left[ \text{Zn}_2(\text{O}_2\text{CMe})_4 \left\{ (3\text{-py})\text{CHNOH} \right\}_2 \right] + 4\text{H}_2\text{O}
\]

(2)

As a next step, we tried to modify the structural identity of 1·H$_2$O by using excess of Zn(acac)$_2$·H$_2$O. A probable result would be the isolation of a paddle wheel structure with four bidentate bridging acac$^-$ ligands and two monodentate (3-py)CHNOH ligands, that is, a structure analogous to that of compound 2. Unfortunately, our efforts did not yield fruits; all the reactions lead to the isolation of solids corresponding to compound 1·H$_2$O, emphasizing the reduced (compared to carboxylates) bridging capability of acac$^-$. 

#### 3.2. Description of Structures. 
Aspects of the molecular and crystal structures of complexes 1·H$_2$O and 2 are shown in Figures 1–4. Selected interatomic distances and angles are listed in Tables 2 and 3, while important hydrogen bonding interactions are presented in Table 4.

Complex 1·H$_2$O crystallizes in the monoclinic space group $P2_1/n$. Its crystal structure consists of mononuclear [Zn(acac)$_2${(3-py)CHNOH}] molecules and H$_2$O molecules

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1·H$_2$O</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula</td>
<td>C$<em>{16}$H$</em>{12}$N$_2$O$_3$Zn</td>
<td>C$<em>{20}$H$</em>{24}$N$<em>4$O$</em>{10}$Zn$_2$</td>
</tr>
<tr>
<td>$F_w$</td>
<td>403.73</td>
<td>611.17</td>
</tr>
<tr>
<td>Space group</td>
<td>$P2_1/n$</td>
<td>$P-1$</td>
</tr>
<tr>
<td>$a$ (Å)</td>
<td>10.531(4)</td>
<td>7.934(5)</td>
</tr>
<tr>
<td>$b$ (Å)</td>
<td>15.779(5)</td>
<td>10.153(6)</td>
</tr>
<tr>
<td>$c$ (Å)</td>
<td>11.602(4)</td>
<td>8.392(5)</td>
</tr>
<tr>
<td>$α$ (°)</td>
<td>90</td>
<td>70.05(2)</td>
</tr>
<tr>
<td>$β$ (°)</td>
<td>101.17(1)</td>
<td>87.34(2)</td>
</tr>
<tr>
<td>$γ$ (°)</td>
<td>90</td>
<td>89.07(3)</td>
</tr>
<tr>
<td>$V$ (Å$^3$)</td>
<td>1891.4(11)</td>
<td>634.8(7)</td>
</tr>
<tr>
<td>$Z$</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>$T$ (°C)</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Radiation</td>
<td>Mo $Kα$</td>
<td>Cu $Kα$</td>
</tr>
<tr>
<td>$ρ_{calc}$ (g cm$^{-3}$)</td>
<td>1.418</td>
<td>1.599</td>
</tr>
<tr>
<td>$μ$ (mm$^{-1}$)</td>
<td>1.331</td>
<td>2.856</td>
</tr>
<tr>
<td>Reflections with $I &gt; 2σ(I)$</td>
<td>2865</td>
<td>1585</td>
</tr>
<tr>
<td>$R_1$</td>
<td>0.0302</td>
<td>0.0438</td>
</tr>
<tr>
<td>$wR_2$</td>
<td>0.0785</td>
<td>0.1125</td>
</tr>
</tbody>
</table>

$^a w = 1/[σ^2(F_0^2) + (αP^2)^2 + bP]$ and $P = (max F_0^2, 0) + 2F_2^2/3$ 
$R_1 = Σ[|F_o| - |F_e|] / Σ|F_o|$ and $wR_2 = (Σ[w(F_o^2 - F_e^2)^2] / Σ[w(F_e^2)^2])^{1/2}$. 

---

### Table 1: Crystallographic data for complexes 1·H$_2$O and 2.
being present in the lattice. The metal center is five-coordinated surrounded by two acetylacetonate (acac\(^-\)) and one (3-py)CHNOH ligand. Each of the acac\(^-\) moiety acts in a bidentate chelating way, while the (3-py)CHNOH behaves as a monodentate ligand via the nitrogen atom of the pyridine ring. The coordination geometry of the Zn\(^{II}\) ion is heavily distorted and thus it can be either described as distorted square pyramidal or as distorted trigonal bipyramidal. Analysis of the shape-determining angles using the approach of Reedijk and coworkers \[16\] yields a trigonality index, \(\tau\), value of 0.53 (\(\tau = 0\) and 1 for perfect sp and tbp geometries, respectively). By adopting the square pyramidal geometry, the basal plane is occupied by four acetylacetonate oxygen atoms, while the apical position is taken by the pyridyl nitrogen atom of the oxime ligand. Adopting the trigonal bipyramidal description, the axial positions are occupied by O(2) and O(4) and the equatorial ones by O(3), O(5), and N(1).

In the crystal lattice of 1·H\(_2\)O, the molecules of 1 interact with the water lattice molecules through hydrogen bonds, forming a 2D network (Figure 2, Table 4).

Complex 2 is a new member of Zn(II) carboxylate complexes with a paddle wheel structure \[17–20\]. The Zn\(^{II}\) ions are bridged by four \(\text{syn}, \text{syn}^{-}\eta^{1}:\eta^{1}:\eta^{1}:\mu^{2}\text{MeCO}_{2}\) ligands and each one has a perfect square pyramidal coordination geometry (\(\tau = 0.01\), with the apex provided by the pyridyl nitrogen atom of a monodentate (3-py)CHNOH ligand. The Zn···Zn distance is 2.923(2) Å, while each Zn\(^{II}\) ion lies 0.386 Å out of its least-squares basal plane towards the pyridyl nitrogen atom. The mean Zn–O(carboxylate) bond length is approximately 2.044 Å which is typical and unremarkable \[21\]. There is a crystallographically imposed inversion center in the midpoint of the Zn···Zn distance.

Table 2: Selected interatomic distances (Å) and angles (°) for complex 1·H\(_2\)O.

<table>
<thead>
<tr>
<th></th>
<th>Zn-O(2)</th>
<th>Zn-O(3)</th>
<th>Zn-O(5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O(2)</td>
<td>2.055(2)</td>
<td>1.988(2)</td>
<td>2.000(2)</td>
</tr>
<tr>
<td>O(3)</td>
<td>2.029(2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N(1)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3.3. IR Spectroscopy. The IR spectra of 1·H₂O and 2 exhibit weak bands at 3468 and 3454 cm⁻¹, respectively, assignable to the ν(OH) vibration of the coordinated pyridyl oxime ligands [22]. The broadness and relatively low frequency of these bands are both indicative of hydrogen bonding. The medium intensity bands at 1636 and 1124 cm⁻¹ in the spectrum of the free ligand (3-py)CHNOH are assigned to ν(C=O) modes of the acetate ligands, respectively [26]; the lower frequency band (1552 cm⁻¹) is attributed to ν(C=N)oxime, respectively [23]. In the spectra of the complexes, these bands are observed at approximately the same wavenumbers, confirming the nonparticipation of the oxime group in coordination. The in-plane deformation band of the pyridyl ring of free (3-py)CHNOH (638 cm⁻¹) shifts upwards (654 cm⁻¹) in the spectra of 1·H₂O and 2, confirming the crystallography established involvement of the ring-N atom in coordination [24].

The presence of chelating acac⁻ ligands in complex 1·H₂O is reflected by the presence of two strong intensity bands at 1500–1600 cm⁻¹. The higher frequency band (1586 cm⁻¹) is attributed to ν(C=O) coupled with ν(C–C), while the lower frequency band (1552 cm⁻¹) is attributed to ν(C=C) coupled with ν(C−O) [25].

The strong intensity bands at 1522 and 1400 cm⁻¹ in the spectrum of 2 are assigned to the νas(CO₂) and νs(CO₂) modes of the acetate ligands, respectively [26]; the former may also involve a pyridyl stretching character. The difference Δ, where Δ = νas(CO₂) – νs(CO₂), is 122 cm⁻¹, less than that for NaO₂CMe (164 cm⁻¹), as expected for bidentate bridging ligation [26].

3.4. Effect on pDNA. DNA mobility shift assays were carried out to investigate the ability of complexes 1·H₂O and 2, as well as that of the (3-py)CHNOH free ligand to interact with plasmid DNA. The initial amount of pDNA was incubated with increasing concentrations of the tested compounds. When circular pDNA is subjected to electrophoresis, relaxed form (form I) will be generated and migrate between the supercoils will relax to generate a slower-moving open form (form II). If both strands are cleaved, a relatively fast migration will be observed for the supercoiled linear form (form III). If scission occurs on one strand (nicking), a less than that for NaO₂CMe (164 cm⁻¹), as expected for bidentate bridging ligation [26].

Figure 5 shows the gel electrophoretic separations of pDNA after incubation with 1·H₂O, 2 and (3-py)CHNOH.
at various concentrations. Both complexes can break the double strand of pDNA and convert it to the relaxed form (II) and in a less extent to its linear form (III), at a concentration of 5 mM (Figure 5, lanes 1 and 2). At lower concentrations, the complexes display a minor effect on the integrity and electrophoretic mobility of pDNA, whereas the latter remains mostly in the supercoiled form (I). The (3-py)CHNOH ligand (Figure 5, lanes 3 and 6) does not display any interaction.

4. Conclusions

Two new complexes of Zn\textsuperscript{II}, with 3-pyridinealdoxime as ligand, have been synthesized and characterized by single-crystal X-ray crystallography, elemental analyses, and IR spectroscopy. In both structures, (3-py)CHNOH acts as a monodentate ligand via the pyridyl nitrogen, while the oxime group does not participate in coordination. This coordination mode is the only one observed in complexes of (3-py)CHNOH with any metal up to date. Complexes \textbf{1}⋅H\textsubscript{2}O and \textbf{2} are the second and the third structurally characterized Zn(II) complexes of (3-py)CHNOH.

The two complexes affect both the integrity and electrophoretic mobility of pDNA. At the highest tested concentration, \textbf{1}⋅H\textsubscript{2}O and \textbf{2} are able to totally convert the supercoiled form of pDNA to the relaxed form and in less extent to its linear form. Other types of DNA-binding experiments are currently in progress in order to determine the way of interaction with pDNA. In the future, synthetic efforts with different types of anionic ligands (e.g., NO\textsubscript{3}, SO\textsubscript{4}\textsuperscript{2−}) can lead to a variety of (3-py)CHNOH complexes with potentially interesting DNA-binding properties.

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References


