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Nickel–quinolones interaction. Part 2 – Interaction of nickel(II) with the antibacterial drug oxolinic acid

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ABSTRACT

The mononuclear nickel(II) complexes with the first-generation quinolone antibacterial agent oxolinic acid in the presence or absence of nitrogen-donor heterocyclic ligands (2,2′-bipyridine, 1,10-phenanthroline or pyridine) have been synthesized and characterized. The experimental data suggest that oxolinic acid acts as deprotonated bidentate ligand coordinated to Ni(II) ion through the ketone and carboxylato oxygens. The crystal structure of (2,2′-bipyridine)bis(oxolinato) nickel(II), **2** has been determined by X-ray crystallography. The cyclic voltammograms of the complexes recorded in dmso solution and in 1/2 dmso/buffer (containing 150 mM NaCl and 15 mM trisodium citrate at pH 7.0) solution have shown that in the presence of calf-thymus DNA (CT DNA) they can bind to CT DNA by the intercalative binding mode. UV study of the interaction of the complexes with CT DNA has shown that the complexes bind to CT DNA and bis(aqua)bis(oxolinato) nickel(II) exhibits the highest binding constant to CT DNA. Competitive study with ethidium bromide (EB) has shown that the complexes can displace the DNA-bound EB indicating that they bind to DNA in strong competition with EB. The complexes exhibit good binding propensity to human or bovine serum albumin protein having relatively high binding constant values.

1. Introduction

Oxolinic acid, Hoxo (Fig. 1), is a first-generation quinolone antimicrobial drug [1,2] and is used for the treatment of urinary tract infections [2–5]. Quinolones (or quinolonecarboxylic acids) are a group of antibacterial agents that effectively inhibit DNA replication and are commonly used as treatment for many infections [2,6,7]. Complexes of oxolinic acid structurally characterized till now are limited to three; a Cu(II) complex, Cu(oxo)(bipy)Cl·MeOH [8], and two Zn(II) ones, Zn(oxo)(bipy)Cl·MeOH and $Zn(oxo)_2$-(phen)·2MeOH [9], where bipy = 2,2′-bipyridine and phen = 1,10-phenanthroline.

Since the characterization of urease as a nickel enzyme in 1975 [10], the role of nickel in bioinorganic chemistry has been rapidly expanding [11,12]. Additionally, nickel complexes of biological interest have been reported with the most structurally characterized acting as antiepileptic [13], anticonvulsant [14] agents or vitamins [15] or showing antibacterial [16–19], anticancer/antiproliferative [20–26], antifungal [16,27,28] and antimicrobial [29] activity. The interaction of Ni(II) complexes with DNA has been mainly dependent on the structure of the ligand exhibiting intercalative behavior [30–32] and/or DNA cleavage ability [33,34].

We have initiated the study of nickel(II) complexes with quinolone antimicrobial agents [35]. In this context, we report the synthesis, the structural characterization, the electrochemical and the biological properties of the neutral mononuclear nickel(II) complexes with the first-generation quinolone antibacterial drug oxolinic acid in the absence ($Ni(oxo)_2(H_2O)_2$, **1**) or presence of a nitrogen-donor heterocyclic ligand such as bipy, phen or pyridine (=py) ($Ni(oxo)_2(bipy)\cdot 6H_2O$, **2** $Ni(oxo)_2(phen)$, **3** and $Ni(oxo)_2(py)_2$, **4**). The crystal structure of complex $Ni(oxo)_2(bipy)$, **2,** has been determined by X-ray crystallography. The binding properties of the complexes with calf-thymus DNA (CT DNA) have been investigated with UV spectroscopy and cyclic voltammetry. Competitive binding studies with ethidium bromide (EB) have been performed in order to investigate the existence of a potential intercalation of the complexes to CT DNA. The affinity of Hoxo and complexes **1**–**4** for bovine (BSA) and human serum albumin (HSA) has been investigated with fluorescence spectroscopy.

2. Experimental

2.1. Materials – instrumentation – physical measurements

Oxolinic acid, CT DNA, BSA, HSA and EB were purchased from Sigma, NaCl and all solvents were purchased from Merck, trisodium citrate was purchased from Riedel-de Haen and $NiCl_2\cdot 6H_2O$,

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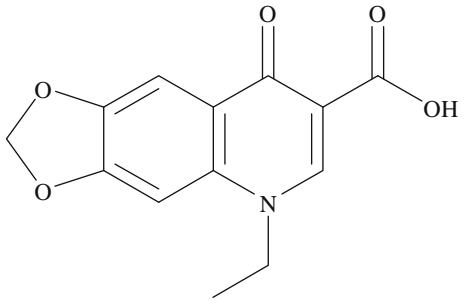

Fig. 1. Oxolinic acid (Hoxo = 5,8-dihydro-5-ethyl-8-oxo-1,3-dioxolo[4,5-g]quinoline-7-carboxylic acid).

bipy, phen, py and KOH were purchased from Aldrich Co. All the chemicals and solvents were reagent grade and were used as purchased. Tetraethylammonium perchlorate (TEAP) was purchased from Carlo Erba and, prior to its use, it was recrystallized twice from ethanol and dried under vacuum.

DNA stock solution was prepared by dilution of CT DNA to buffer (containing 15 mM trisodium citrate and 150 mM NaCl at pH 7.0) followed by exhaustive stirring for three days, and kept at 4 °C for no longer than a week. The stock solution of CT DNA gave a ratio of UV absorbance at 260 and 280 nm (A_{260}/A_{280}) of 1.89, indicating that the DNA was sufficiently free of protein contamination [36]. The DNA concentration was determined by the UV absorbance at 260 nm after 1:20 dilution using $\varepsilon = 6600\ M^{-1}\ cm^{-1}$ [37].

Infrared (IR) spectra (400–4000 cm^{-1}) were recorded on a Nicolet FT-IR 6700 spectrometer with samples prepared as KBr disk. UV–visible (UV–vis) spectra were recorded as Nujol mulls and in solution at concentrations in the range 10^{-5}–10^{-3} M on a Hitachi U-2001 dual beam spectrophotometer. Room temperature magnetic measurements were carried out by the Faraday method using mercury tetrathiocyanatocobaltate(II) as a calibrant. C, H and N elemental analysis were performed on a Perkin–Elmer 240B elemental analyzer. Molecular conductivity measurements were carried out with a Crison Basic 30 conductometer. Fluorescence spectra were recorded in solution on a Hitachi F-7000 fluorescence spectrophotometer.

Cyclic voltammetry (CV) studies were performed on an Eco chemie Autolab Electrochemical analyzer. Cyclic voltammetric experiments were carried out in a 30 mL three-electrode electrolytic cell. The working electrode was platinum disk, a separate Pt single-sheet electrode was used as the counter electrode and a Ag/AgCl electrode saturated with KCl was used as the reference electrode. The cyclic voltammograms of the complexes were recorded in 0.4 mM dmso solutions and in 0.4 mM 1/2 dmso/buffer solutions at $\nu = 100\ mV\ s^{-1}$, where TEAP and the buffer solution were the supporting electrolytes, respectively. Oxygen was removed by purging the solutions with pure nitrogen which had been previously saturated with solvent vapors. All electrochemical measurements were performed at 25.0 ± 0.2 °C.

2.2. Synthesis of the complexes

2.2.1. $Ni(oxo)_2(H_2O)_2$, **1**

A methanolic solution (15 mL) of oxolinic acid (0.4 mmol, 105 mg) and KOH (0.4 mmol, 22 mg), was added to a methanolic solution (10 mL) of $NiCl_2{\cdot}6H_2O$ (0.2 mmol, 48 mg) and the reaction mixture was refluxed for 1 h. The reaction solution was filtered and left for slow evaporation. After a few days a light blue microcrystalline product was deposited, collected with filtration, washed with methanol and dried. Yield: 100 mg, 80%. *Anal.* Calcd. for $Ni(oxo)_2(H_2O)_2$ ($C_{26}H_{24}N_2NiO_{12}$) (MW = 615.19): C 50.76, H 3.93, N 4.55; found C 50.89, H 4.12, N 4.45. IR: ν_{max}/cm^{-1} $\nu(O–H)_w$, 3395 (m(medium)); $\nu(C{=}O)_{ket}$, 1636 (vs(very strong)); $\nu(CO_2)_{asym}$, 1590 (vs); $\nu(CO_2)_{sym}$, 1400 (vs); $\Delta = \nu_{asym}(CO_2) - \nu_{sym}(CO_2)$: 190 cm^{-1} (KBr disk); UV–vis: λ/nm ($\varepsilon/M^{-1}\ cm^{-1}$) as Nujol mull: 755, 620, 474 (sh(shoulder)), 415 (sh), 324, 281; in dmso: 760 (9), 635 (15), 469 (sh) (20), 420 (sh) (110), 329 (1900), 315 (1800), 284 (1600); 10Dq = 13,158 cm^{-1}, $B = 1106\ cm^{-1}$. $\mu_{eff} = 2.96$ BM. The complex is soluble in dmso and dmf and is non-electrolyte.

2.2.2. $Ni(oxo)_2(bipy){\cdot}5.27H_2O$, **2**$\cdot 5.27H_2O$

Oxolinic acid (0.4 mmol, 105 mg) was dissolved in methanol (15 mL) followed by the addition of KOH (0.4 mmol, 22 mg). The resultant solution was added slowly, and simultaneously with a methanolic solution of bipy (0.2 mmol, 31 mg), to a methanolic solution (10 mL) of $NiCl_2{\cdot}6H_2O$ (0.2 mmol, 48 mg) and stirred for 30 min. The solution was left for slow evaporation. Blue–green crystals of $Ni(oxo)_2(bipy){\cdot}5.27H_2O$, **2**$\cdot 5.27H_2O$ suitable for X-ray structure determination, were deposited after a few days. Yield: 110 mg, 65%. *Anal.* Calcd. for $Ni(oxo)_2(bipy){\cdot}5.27H_2O$ ($C_{36}H_{38.54}N_4NiO_{15.27}$) (MW = 830.29): C 52.08, H 4.68, N 7.07; found C 51.91, H 4.61, N 6.73. IR: ν_{max}/cm^{-1}; $\nu(C{=}O)_{ket}$: 1642 (vs); $\nu_{asym}(CO_2)$: 1590 (vs); $\nu_{sym}(CO_2)$: 1390 (vs); $\Delta = \nu_{asym}(CO_2) - \nu_{sym}(CO_2)$: 200 cm^{-1} (KBr disk); UV–vis: λ/nm ($\varepsilon/M^{-1}\ cm^{-1}$) as Nujol mull: 775, 604, 470 (sh), 405 (sh), 331, 285 (sh); in dmso: 765 (5), 612 (10), 467 (sh) (30), 402 (sh) (150), 336 (1530), 305 (3600), 290 (3400); 10Dq = 13, 072 cm^{-1}, $B = 1145\ cm^{-1}$. $\mu_{eff} = 2.79$ BM. The complex is soluble in dmso, dmf, ethanol and CH_3CN and is non-electrolyte.

2.2.3. $Ni(oxo)_2(phen)$, **3**

Complex **3** was prepared by the addition of a methanolic solution (15 mL) of oxolinic acid (0.4 mmol, 105 mg) and KOH (0.4 mmol, 22 mg), and of a methanolic solution of phen (0.2 mmol, 36 mg) to a methanolic solution (10 mL) of $NiCl_2{\cdot}6H_2O$ (0.2 mmol, 48 mg). The dark green microcrystalline product was collected after a few days. Yield: 105 mg, 70%. *Anal.* Calcd. for $Ni(oxo)_2(phen)$ ($C_{38}H_{28}N_4NiO_{10}$) (MW = 759.37): C 60.15, H 3.72, N 7.38; found C 59.85, H 3.93, N 7.53. IR: ν_{max}/cm^{-1} $\nu(C{=}O)_{ket}$ 1639 (vs); $\nu_{asym}(CO_2)$: 1589 (vs); $\nu_{sym}(CO_2)$: 1385 (vs); $\Delta = \nu_{asym}(CO_2) - \nu_{sym}(CO_2)$: 204 cm^{-1} (KBr disk); UV–vis: λ/nm ($\varepsilon/M^{-1}\ cm^{-1}$) as Nujol mull: 775, 612, 470 (sh), 412 (sh), 328, 307 (sh), 287; in dmso: 760 (sh) (8), 612 (20), 465 (sh) (28), 420 (sh) (95), 329 (1620), 312 (1400), 291 (sh) (1800); 10Dq = 13,158 cm^{-1}, $B = 1143\ cm^{-1}$. $\mu_{eff} = 2.81$ BM. The complex is soluble in dmso and ethanol and is non-electrolyte.

2.2.4. $Ni(oxo)_2(py)_2$, **4**

Complex **4** was prepared by the addition of a methanolic solution (15 mL) of oxolinic acid (0.4 mmol, 105 mg) and KOH (0.4 mmol, 22 mg) to a methanolic solution (10 mL) of $NiCl_2{\cdot}6H_2O$ (0.2 mmol, 48 mg) followed by the addition of 2 mL of pyridine. The light blue microcrystalline product was collected after a few days. Yield: 104 mg, 70%. *Anal.* Calcd. for $Ni(oxo)_2(py)_2$ ($C_{36}H_{30}N_4NiO_{10}$) (MW = 737.37): C 58.64, H 4.10, N 7.60; found C 58.90, H 4.18, N 7.83. IR: ν_{max}/cm^{-1} $\nu(C{=}O)_{ket}$ 1635(vs); $\nu_{asym}(CO_2)$: 1595 (vs); $\nu_{sym}(CO_2)$: 1390 (vs); $\Delta = \nu_{asym}(CO_2) - \nu_{sym}(CO_2)$: 205 (KBr disk); UV–vis: λ/nm ($\varepsilon/M^{-1}\ cm^{-1}$) as Nujol mull: 755, 611, 483 (sh), 408 (sh), 327, 285; in dmso: 762 (sh) (5), 618 (10), 465 (sh) (25), 412 (sh) (160), 329 (1800), 311 (1500), 292 (sh) (2200); 10Dq = 13,123 cm^{-1}, $B = 1151\ cm^{-1}$. $\mu_{eff} = 2.98$ BM. The complex is soluble in dmso and is non-electrolyte.

2.3. X-ray crystal structure determination

Single-crystal X-ray diffraction data of **2**$\cdot 5.27H_2O$ were collected at room temperature with a Nonius Kappa CCD diffractometer with graphite monochromated Mo-Kα radiation ($\lambda = 0.71,\ 073$ Å). The