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Nickel–quinolones interaction. Part 4 — Structure and biological evaluation of nickel(II)–enrofloxacin complexes compared to zinc(II) analogues

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ABSTRACT

The nickel(II) complexes with the second-generation quinolone antibacterial agent enrofloxacin in the presence or absence of the nitrogen-donor heterocyclic ligands 1,10-phenanthroline, 2,2′-bipyridine or pyridine have been synthesized and characterized. Enrofloxacin acts as bidentate ligand coordinated to Ni(II) ion through the ketone oxygen and a carboxylato oxygen. The crystal structure of (1,10-phenanthroline)bis(enrofloxacinato)nickel(II) has been determined by X-ray crystallography. UV study of the interaction of the complexes with calf-thymus DNA (CT DNA) has shown that they bind to CT DNA and bis(pyridine)bis(enrofloxacinato)nickel(II) exhibits the highest binding constant to CT DNA. The cyclic voltammograms of the complexes have shown that in the presence of CT DNA the complexes can bind to CT DNA by the intercalative binding mode which has also been verified by DNA solution viscosity measurements. 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. The biological properties of the complexes have been evaluated in comparison to the corresponding Zn(II) enrofloxacinato complexes as well as Ni(II) complexes with the first-generation quinolone oxolinic acid.

1. Introduction

Quinolones (quinolonecarboxylic acids or 4-quinolones) are antibacterial drugs and are commonly used as treatment for many infections [1–5] since they can effectively inhibit DNA replication. Enrofloxacin (=Herx, Fig. 1) is a typical second-generation quinolone antimicrobial drug presenting a broad spectrum of activity against a wide range of Gram-negative and Gram-positive bacteria, including those resistant to β-lactam antibiotics and sulfonamides [4,5]. Enrofloxacin is the first fluoroquinolone developed for veterinary application and is usually used for the treatment of some urinary tract, respiratory tract and skin infectious diseases in pets and livestock [6–9].

In many cases, the metal complexes of drugs are more active than their parent compounds [10–12]. From this point of view, diverse metal complexes of quinolones have been synthesized in an attempt to investigate the physicochemical properties and to evaluate their biological properties (antibacterial activity and DNA-binding properties) in comparison to the free quinolones [4]. A thorough survey of the literature has revealed that two Zn(II) [13,14] and two out of three structurally characterized Cu(II) enrofloxacin complexes [15,16] have been reported by our lab.

Nickel is an element of expanding biological interest [17]. Therefore, diverse nickel complexes have been reported to act as antiepileptic [18], anticonvulsant [19] agents or vitamins [20], while other Ni(II) complexes have shown antibacterial [21,22], antifungal [22,23], antimicrobial [24] and anticancer/antiproliferative [25–27] activities. On the basis that DNA is one of the principal targets of drugs [28,29], the interaction of Ni(II) complexes with diverse ligands with DNA has been studied showing that they can intercalate to the DNA bases [29–34] and/or cleave DNA [35,36]. To the best of our knowledge, Ni(II) complexes with the first-generation quinolones oxolinic acid [32] and flumequine [33] as well as with the third-generation quinolone sparfloxacin [30,31] have been structurally characterized and their biological properties have been investigated.

Our studies have been focused on the coordination chemistry and the biological activity of carboxylate-containing antimicrobial [13–15,37–41] or anti-inflammatory [12,42–45] agents with metal ions in an attempt to examine their mode of binding and possible biological relevance. Taking into consideration the reported biological role and activity of nickel and its complexes, the significance of the quinolones in medicine and the fact that metal complexes with drugs may exhibit more pronounced biological properties in comparison to

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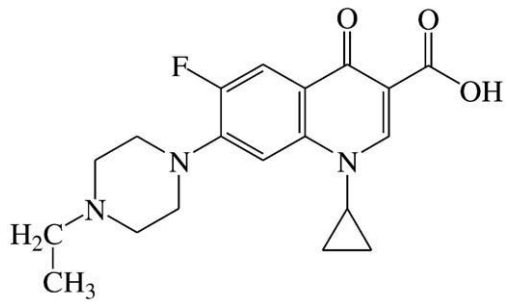

Fig. 1. Enrofloxacin (Herx = 1-cyclopropyl-7-(4-ethyl-piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid).

the free drugs, we have initiated the study of nickel(II) complexes with quinolone antimicrobial agents [30–33]. In this context, we report the synthesis, the structural characterization, the electrochemical and the biological properties of the nickel(II) complexes with the second-generation quinolone antibacterial drug enrofloxacin. The interaction of Ni(II) with enrofloxacin results in the complex $[Ni(erx)_2(H_2O)_2]$, **1,** while in the presence of a nitrogen-donor heterocyclic ligand such as 1,10-phenanthroline (phen), 2,2′-bipyridine (bipy) and pyridine (py), the neutral complexes $[Ni(erx)_2(phen)]$, **2**, $[Ni(erx)_2(bipy)]$, **3** and $[Ni(erx)_2(py)_2]$, **4** have been obtained, respectively. The crystal structure of complex $2{\cdot}2MeOH{\cdot}1.7H_2O$ has been determined by X-ray crystallography. The study of the biological properties of the complexes has been focused on (i) the binding properties of the complexes with CT DNA investigated by UV spectroscopy, viscometry measurements and cyclic voltammetry (ii) competitive binding studies with ethidium bromide (EB) performed by fluorescence spectroscopy in order to investigate the existence of a potential intercalation of the complexes to CT DNA and (iii) the affinity for bovine (BSA) and human serum albumin (HSA), proteins involved in the transport of metal ions and metal–drug complexes through the blood stream, investigated by fluorescence spectroscopy and the results have been evaluated in comparison to the Zn(II) analogues [13,14]. Additionally, a comparison of the biological behavior between Ni(II)-first-generation quinolone oxolinic acid complexes, previously reported [32], and the present compounds is being attempted.

2. Experimental

2.1. Materials–instrumentation–physical measurements

Enrofloxacin, 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 $NiCl_2{\cdot}6H_2O$, 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 [46]. The DNA concentration was determined by the UV absorbance at 260 nm after 1:20 dilution using $\varepsilon = 6600\ M^{-1}\ cm^{-1}$ [47].

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 on a magnetic susceptibility balance of Sherwood Scientific (Cambridge, UK) by the Faraday method using mercury tetrathiocyanatocobaltate(II) as a calibrant. C, H and N elemental analysis was performed on a Perkin-Elmer 240B elemental analyzer. Molar conductivity measurements of 1 mM DMSO solutions of the complexes were carried out with a Crison Basic 30 conductometer. Fluorescence spectra were recorded in solution on a Hitachi F-7000 fluorescence spectrophotometer. Viscosity experiments were carried out using an ALPHA L Fungilab rotational viscometer equipped with an 18 mL LCP spindle.

Cyclic voltammetry studies were performed on an Eco chemie Autolab Electrochemical analyzer. Cyclic voltammetry 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. $[Ni(erx)_2(H_2O)_2]$, 1

A methanolic solution (15 mL) of enrofloxacin (0.4 mmol, 144 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 stirred for 1 h and the solution was left for slow evaporation. After a few days a green microcrystalline product was deposited and collected with filtration. Yield: 120 mg, 75%. *Anal.* Calcd. for $[Ni(erx)_2(H_2O)_2]$ ($C_{38}H_{46}F_2N_6NiO_8$) (MW = 811.53): C 56.24, H 5.71, N 10.36; found C 55.89, H 5.98, N 10.45. IR: ν_{max}/cm^{-1} ν(O–H)$_w$, 3410 (m (medium)); $\nu(C{=}O)_{ket}$, 1629 (vs(very strong)); $\nu_{asym}(CO_2)$, 1616 (vs); $\nu_{sym}(CO_2)$, 1381 (vs); $\Delta = \nu_{asym}(CO_2) - \nu_{sym}(CO_2)$: 235 cm^{-1} (KBr disk); UV–vis: λ/nm (ε/$M^{-1}\ cm^{-1}$) as nujol mull: 745, 645, 463 (sh(shoulder)), 415 (sh), 322, 283; in DMSO: 755 (10), 650 (10), 467 (sh) (20), 420 (sh) (130), 330 (2450), 290 (13,200); 10Dq = 13,245 cm^{-1}, B = 1104 cm^{-1}. μ_{eff} = 2.93 BM. The complex is soluble in DMSO, DMF, acetonitrile, dichloromethane and ethanol and is non-electrolyte (Λ_M = 13 mho $cm^2\ mol^{-1}$).

2.2.2. $[Ni(erx)_2(phen)]{\cdot}2MeOH{\cdot}1.7H_2O$, $2{\cdot}2MeOH{\cdot}1.7H_2O$

Complex **2** was prepared by the addition of a methanolic solution (15 mL) of enrofloxacin (0.4 mmol, 144 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). After stirring for 30 min, the resultant solution was left for slow evaporation. Dark green crystals of $[Ni(erx)_2(phen)]{\cdot}2MeOH{\cdot}1.7H_2O$, $2{\cdot}2MeOH{\cdot}1.7H_2O$, suitable for X-ray structure determination, were collected after a few days. Yield: 150 mg, 70%. *Anal.* Calcd. for $[Ni(erx)_2(phen)]{\cdot}2MeOH{\cdot}1.7H_2O$ ($C_{52}H_{61.4}F_2N_8NiO_{9.7}$) (MW = 1050.40): C 59.46, H 5.89, N 10.69; found C 59.27, H 6.08, N 10.53. IR: ν_{max}/cm^{-1} $\nu(C{=}O)_{ket}$ 1631 (vs); $\nu_{asym}(CO_2)$: 1617 (vs); $\nu_{sym}(CO_2)$: 1376 (vs); $\Delta = \nu_{asym}(CO_2) - \nu_{sym}(CO_2)$: 241 cm^{-1} (KBr disk); UV–vis: λ/nm (ε/$M^{-1}\ cm^{-1}$) as nujol mull: 762, 650, 458 (sh), 398 (sh), 330, 298; in DMSO: 765 (sh) (15), 648 (15), 460 (sh) (18), 395 (sh) (190), 330 (2200), 298 (22,300); 10Dq = 13,072 cm^{-1}, B = 1211 cm^{-1}. μ_{eff} = 2.85 BM. The complex is soluble in DMSO, DMF, acetonitrile, ethanol and dichloromethane and is non-electrolyte (Λ_M = 10 mho $cm^2\ mol^{-1}$).

2.2.3. $[Ni(erx)_2(bipy)]$, 3

Enrofloxacin (0.4 mmol, 144 mg) was dissolved in methanol (15 mL) followed by the addition of KOH (0.4 mmol, 22 mg). This solution was added slowly, and simultaneously with a methanolic