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Copper(II) complexes with antimicrobial drug flumequine: Structure and biological evaluation

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ABSTRACT

The copper(II) complexes with the first-generation quinolone antibacterial agent flumequine(Hflmq) in the presence or absence of the nitrogen donor heterocyclic ligands 2,2′-bipyridylamine(bipyam), 2,2′-bipyridine(bipy), 1,10-phenanthroline(phen) or pyridine(py) have been synthesized and characterized. Flumequine acts as bidentate ligand coordinated to Cu(II) atom through the pyridone oxygen and a carboxylato oxygen. The crystal structures of the complexes [Cu(flmq)(bipyam)Cl], [Cu(flmq)(bipy)Cl] and [Cu(flmq)(phen)Cl] have been determined by X-ray crystallography revealing a distorted square pyramidal geometry for Cu(II) atom. The interaction of the complexes with bovine or human serum albumin proteins has been studied by fluorescence spectroscopy revealing their good binding propensity to the proteins with relatively high binding constant values. UV study of the interaction of the complexes with calf-thymus DNA (CT DNA) has shown that they bind to CT DNA and $[Cu(flmq)_2(py)_2]$ 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.

1. Introduction

Quinolonecarboxylic acids (quinolones) are a group of antibacterial agents that effectively inhibit DNA replication and are commonly used as treatment for many infections [1,2]. The targets of quinolones are both gyrases (type II topoisomerases) and topoisomerase IV (enzymes that participate in the DNA replication); therefore, quinolones may inhibit effectively DNA replication [1,3]. Many quinolones showing a broad spectrum of activity are classified in generations based on their activity [3]. In brief, the quinolones are used for the treatment of urinary tract infections, soft tissue infections, respiratory infections, bone-joint infections, typhoid fever, sexually transmitted diseases, prostatitis, community acquired pneumonia, acute bronchitis and sinusitis [4,5].

Flumequine, Hflmq (Scheme 1), is a synthetic first-generation quinolone structurally related to nalidixic and oxolinic acids or ofloxacin [5–7]. Hflmq is chiral and a racemic mixture is used as a ligand. Flumequine is highly effective in treating urinary tract infections, while activity against some Gram-positive and Gram-negative microorganisms has also been reported [8,9]. Metal complexes of flumequine with Ni(II) have been structurally characterized recently by our group [10].

Copper, one of the most interesting biometals [11,12] due to its biological role and its potential synergetic activity with drugs [13], has been the subject of a large number of research studies [14]. Copper(II) complexes with diverse drugs have shown numerous biological activities such as antitumor [15,16], antioxidant [17,18], antibacterial [19–21] and antifungal [22]. In many cases, mixed-ligand copper(II) complexes with drugs and N-donor ligands have shown better activity than free drug; the Cu(II) with ligands the antibacterial quinolone drug N-propyl-norfloxacin [15,16], the non-steroidal antiinflammatory drugs mefenamic acid, naproxen and diclofenac [17,18] or phenoxyalkanoic herbicides [23] and the N-donors 2,2′-bipyridine (bipy), 1,10-phenanthroline (phen), 2,2′-bipyridylamine (bipyam) or pyridine (py) are some representative examples of complexes with enhanced biological activity.

The interaction of copper(II) with diverse quinolone ligands has been thoroughly studied; the structures and study of (i) binary copper(II) complexes of ciprofloxacin [24–26], cinoxacin [27,28] and sparfloxacin [29], (ii) mixed-ligands neutral mononuclear copper(II) complexes with cinoxacin [30,31], ciprofloxacin [21,32], enrofloxacin [33], nalidixic acid [34], ofloxacin [35], oxolinic acid [36], pipemidic acid [37], N-propyl-norfloxacin [15,16] and sparfloxacin [38,39] as well as (iii) the ionic copper(II) complexes of protonated norfloxacin [40,41] and ciprofloxacin [42] have been reported in the literature.

Our recent studies have been focused on the study of divalent Co(II), Ni(II), Cu(II) or Zn(II) complexes with antimicrobial [10,43–50] or antiinflammatory drugs [17,18,51–53] in the absence or presence

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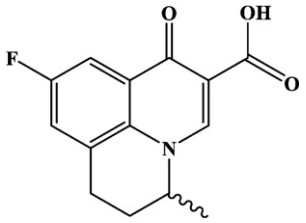

Scheme 1. The racemic mixture of flumequine (=Hflmq).

of N-donor(s) heterocyclic ligands. Taking into consideration the significance of the quinolones in medicine [1–4] and the fact that metal complexes with drugs may exhibit more pronounced biological properties in comparison to the free drugs, we have initiated the interaction of Cu(II) with quinolones [15,16,29,33,36–38]. In this context, we report the synthesis, the characterization, the electrochemical and the biological properties of a series of copper(II) complexes with flumequine, ($[Cu(flmq)_2(H_2O)]$ (=**1**), $[Cu(flmq)(bipyam)Cl]\cdot H_2O$ (=**2**·H_2O), $[Cu(flmq)(bipy)Cl]\cdot 2H_2O$ (=**3**·$2H_2O$), $[Cu(flmq)(phen)Cl]\cdot 5H_2O$ (=**4**·$5H_2O$) and $[Cu(flmq)_2(py)_2]$ (=**5**) while the crystal structures of complexes **2**, **3** and **4** have been determined by X-ray crystallography. The study of the biological properties of the complexes has been focused on (i) 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, (ii) the binding properties of the complexes with CT DNA investigated by UV spectroscopy, viscosity measurements and cyclic voltammetry and (iii) 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.

2. Experimental

2.1. Materials–instrumentation–physical measurements

Flumequine (a racemic mixture), $CuCl_2\cdot 2H_2O$, bipy, phen, bipyam, py, KOH, NaCl, trisodium citrate, tetraethylammonium perchlorate (TEAP), CT DNA, BSA, HSA and EB were purchased from Sigma-Aldrich Co and all solvents were purchased from Merck. All the chemicals and solvents were reagent grade and were used as purchased. TEAP was recrystallized twice from ethanol, prior to its use, 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 3 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}) in the range of 1.8–1.9, indicating that the DNA was sufficiently free of protein contamination. The DNA concentration was determined by the UV absorbance at 260 nm after 1:20 dilution using $\varepsilon = 6600\ M^{-1}\ cm^{-1}$ [49–52].

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 DMSO solution at concentrations in the range 10^{-5}–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 were 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. $[Cu(flmq)_2(H_2O)]$, 1

Flumequine (0.4 mmol, 105 mg) and KOH (0.4 mmol, 22 mg) dissolved in 15 mL of methanol, were added to a methanolic solution (10 mL) of $CuCl_2\cdot 2H_2O$ (0.2 mmol, 34 mg) and the reaction mixture was refluxed for 1 h. A light blue microcrystalline product of $[Cu(flmq)_2(H_2O)]$, **1** (Yield: 95 mg, 80%) was collected after a few days. *Anal.* Calcd for $[Cu(flmq)_2(H_2O)]$ **1** ($C_{28}H_{24}F_2N_2O_7Cu$) (MW = 602.05): C 55.86, H 4.02, N 4.65; found C 56.15, H 4.13, N 4.82. IR: ν_{max}/cm^{-1} $\nu(O-H)_w$, 3402 (m(medium)); $\nu(C{=}O)_{pyridone}$, 1636 (vs(very strong)); $\nu_{asym}(CO_2)$, 1586 (vs); $\nu_{sym}(CO_2)$, 1396 (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: 845 (sh(shoulder)), 665, 415 (sh), 339(sh), 330, 310; in DMSO: 840(sh) (15). 650 (25), 400 (sh) (210), 341 (5800), 328 (6800), 308 (sh) (7800). μ_{eff} = 1.86 BM. The complex is soluble in DMSO (Λ_M = 4 mho $cm^2\ mol^{-1}$, in 1 mM DMSO solution).

2.2.2. $[Cu(flmq)(bipyam)Cl]\cdot H_2O$, $2\cdot H_2O$

Flumequine (0.4 mmol, 105 mg) was dissolved in CH_3OH (15 mL) and KOH (0.4 mmol, 22 mg) was added. After 30 min of stirring, $CuCl_2\cdot 2H_2O$ (68 mg, 0.4 mmol) in CH_3OH (10 mL) and bipyam (0.4 mmol, 68 mg) in CH_3OH (10 mL) were added slowly and simultaneously. The reaction mixture was stirred for 1 h. The blue solution was reduced in volume under pressure and left for slow evaporation. Blue-green crystals of $[Cu(flmq)(bipyam)Cl]\cdot H_2O$, **2**·$H_2O$, (Yield: 140 mg, 65%) suitable for X-ray structure determination, were deposited after a fortnight. *Anal.* Calcd. for $[Cu(flmq)(bipyam)Cl]\cdot H_2O$ ($C_{24}H_{22}ClN_4FO_4Cu$) (MW = 548.45): C 52.56, H 4.04, N 10.22; found C 52.32, H 4.09, N 10.29. IR: ν_{max}/cm^{-1} $\nu(C{=}O)_{pyridone}$, 1632 (vs); $\nu_{asym}(CO_2)$, 1601 (vs); $\nu_{sym}(CO_2)$, 1367 (vs); Δ = 234 cm^{-1} (KBr disk); UV–vis: λ/nm ($\varepsilon/M^{-1}\ cm^{-1}$) as nujol mull: 840(sh) 655, 411 (sh), 342, 327, 310; in DMSO: 835 (sh) (20), 665 (85), 406 (190), 340 (11850), 326 (16500), 315(sh) (17500). μ_{eff} = 1.79 BM. The complex is soluble in DMSO (Λ_M = 8 mho $cm^2\ mol^{-1}$, in 1 mM DMSO solution) and DMF.

In a similar way, complexes (**3**) and (**4**) were prepared with the use of the corresponding N-donor heterocyclic ligand.

2.2.3. $[Cu(flmq)(bipy)Cl]\cdot 2H_2O$, $3\cdot 2H_2O$

Bipy (0.4 mmol, 62 mg) dissolved in methanol (5 mL) was used instead. Blue-green crystals of $[Cu(flmq)(bipy)Cl]\cdot 2H_2O$, **3**·$2H_2O$, (Yield: 150 mg, 70%) suitable for X-ray structure determination, were deposited after 5 days. *Anal.* Calcd. for $[Cu(flmq)(bipy)Cl]\cdot 2H_2O$ ($C_{24}H_{23}ClFN_3O_5Cu$) (MW = 551.44): C 52.27, H 4.20, N 7.62; found C 51.94, H 4.14, N 7.74. IR: ν_{max}/cm^{-1} $\nu(C{=}O)_{pyridone}$, 1629 (vs); $\nu_{asym}(CO_2)$, 1602 (vs); $\nu_{sym}(CO_2)$, 1385 (vs); Δ = 217 cm^{-1} (KBr disk); UV–vis: λ/nm ($\varepsilon/M^{-1}\ cm^{-1}$) as nujol mull: 840 (sh), 653, 409 (sh), 344, 327, 313; in DMSO: 835 (sh) (20), 650 (75), 410 (sh) (150), 341 (12400), 328 (15900), 313 (16400). μ_{eff} = 1.84 BM. The complex is soluble in DMSO (Λ_M = 7 mho $cm^2\ mol^{-1}$, in 1 mM DMSO solution).