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Nickel(II) complexes of flufenamic acid: Characterization, structure and interaction with DNA and albumins

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ABSTRACT

When $NiCl_2{\cdot}6H_2O$ reacts with the non-steroidal anti-inflammatory drug flufenamic acid (Hfluf), complex $[Ni(fluf\text{-}O)_2(MeOH)_4]$, **1** was isolated. When the same reaction takes places in the presence of a N,N′-donor heterocyclic ligand 2,2′-bipyridylamine (bipyam), 2,2′-bipyridine (bipy) and 1,10-phenanthroline (phen), the complexes $[Ni(fluf\text{-}O,O')_2(bipyam)]$, **2**, $[Ni(fluf\text{-}O)_2(phen)(MeOH)_2]$, **3** and $[Ni(fluf\text{-}O)_2(bipy)(MeOH)_2]$, **4** were isolated, respectively. Complexes **1–4** were characterized by physicochemical and spectroscopic techniques and the crystal structure of complex **2** was determined by X-ray crystallography. The interaction of the complexes with serum albumins was investigated by fluorescence emission spectroscopy and the corresponding binding constants were calculated. UV–Vis spectroscopy, viscosity measurements and fluorescence emission spectroscopy for the competitive studies of the complexes with ethidium bromide were the techniques used in order to investigate the interaction of the complexes with calf-thymus DNA; intercalation was revealed as the most possible mode of DNA-binding.

1. Introduction

Nickel was considered an element without important biological role until its establishment in the active center of the enzyme urease in 1975 [1,2]. Thereafter, the biological significance of nickel increased due to its presence in the active center of more enzymes [3,4]. Nickel may be considered an occupational hazard due to acute toxicity and carcinogenicity caused by chronic exposure [5] and is recognized as an allergen being the cause of contact allergy cases (i.e. contact dermatitis) [6,7]. Nevertheless, nickel compounds have gained increasing interest in bioinorganic chemistry not only the Ni-complexes bearing vitamins [8], antitumor antibiotics [9], anticonvulsant [10] or antiepileptic agents [11] as ligands but also those showing antifungal [12], antileishmanial [13], antimicrobial [14], antioxidant [15,16] or antiproliferative activity [17] and the ones acting as DNA-intercalating [18] or DNA-cleaving agents [19]. Considering the nickel complexes with non-steroidal anti-inflammatory drugs (NSAIDs) as ligands, a series of Ni(II)-diclofenac (one structure) [20] and Ni-mefenamato (four structures) [21] complexes were reported in the literature.

Flufenamic acid (Hfluf, Fig. 1(A)) is a NSAID as a member of the anthranilic acid derivatives (fenamates) [22]. Hfluf is a cyclooxygenase inhibitor preventing formation of prostaglandins, similarly to other fenamates (e.g. mefenamic acid and tolfenamic acid) in clinical use [23]. Hfluf has analgesic, anti-inflammatory and antipyretic effects and is used in musculoskeletal and joint disorders being administered orally and topically [24]; due to its gastrointestinal side-effects it is not widely used for humans [25]. It is also used as a cation-regulator [26] either by activating potassium [27] and neuronal sodium channels [24] or by inhibiting nonselective cation channels [28]. Among reports concerning the metal-NSAID complexes, few copper(II) [29–31], cobalt [32] and zinc [33] complexes were characterized and biologically evaluated.

As a continuation of our research in regard to metal-NSAID complexes [20–22,31–38], we present herein the interaction of Ni(II) with Hfluf in the absence or presence of the nitrogen-donor ligands 2,2′-bipyridylamine (bipyam), 1,10-phenanthroline (phen) or 2,2′-bipyridine (bipy) (Fig. 1). The resultant complexes $[Ni(fluf\text{-}O)_2(MeOH)_4]$, **1**, $[Ni(fluf\text{-}O,O')_2(bipyam)]$, **2**, $[Ni(fluf\text{-}O)_2(phen)(MeOH)_2]$, **3** and $[Ni(fluf\text{-}O)_2(bipy)(MeOH)_2]$, **4** were characterized by physicochemical (elemental analysis, molecular conductivity

Abbreviations: bipy, 2,2′-bipyridine; bipyam, 2,2′-bipyridylamine; BSA, bovine serum albumin; CT, calf-thymus; DMF, dimethylformamide; EB, ethidium bromide, 3,8-diamino-5-ethyl-6-phenyl-phenanthridinium bromide; $fluf^-$, anion of flufenamic acid; Hfluf, flufenamic acid; HSA, human serum albumin; K, SA-binding constant; K_b, DNA-binding constant; k_q, quenching constant; K_{SV}, Stern–Volmer constant; m, medium; NSAID, non-steroidal anti-inflammatory drug; *r*, [compound]/[DNA] ratio; *r*′, [DNA]/[compound] ratio; s, strong; SA, serum albumin; sh, shoulder; vs, very strong; $\Delta\nu(CO_2)$, $\nu_{asym}(CO_2) - \nu_{asym}(CO_2)$.

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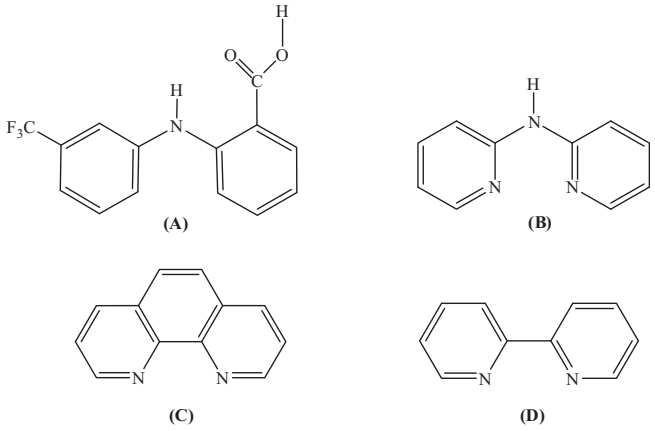

Fig. 1. The syntax formula of (A) flufenamic acid, (B) bipyam, (C) phen and (D) bipy.

and room-temperature magnetic measurements) and spectroscopic (IR and UV–Vis) techniques; the crystal structure of [Ni(fluf-O,O′)$_2$(bipyam)], **2** was determined by X-ray crystallography. Complexes **1**–**4** were also evaluated in regard to the binding mode and affinity to calf-thymus (CT) DNA by UV spectroscopy, viscosity measurements and via their ability to displace ethidium bromide (EB) from the EB-DNA compound by fluorescence spectroscopy and their binding to bovine (BSA) and human serum albumin (HSA) studied by fluorescence emission spectroscopy.

2. Experimental

2.1. Materials – instrumentation – physical measurements

Flufenamic acid, $NiCl_2{\cdot}6H_2O$, bipy, bipyam, phen, KOH, trisodium citrate, NaCl, CT DNA, BSA, HSA and EB were purchased from Sigma–Aldrich and all solvents were purchased from Merck. All the chemicals and solvents were reagent grade and were used as purchased. 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 1.86–1.88, indicating that the DNA was sufficiently free of protein contamination [39]. The DNA concentration was determined by the UV absorbance at 260 nm after 1:20 dilution using $\varepsilon = 6600\ M^{-1}\ cm^{-1}$ [40].

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). C, H and N elemental analysis were performed on a Perkin–Elmer 240B elemental analyzer. Molar conductivity measurements of 1 mM DMSO solution 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.

2.2. Synthesis of the complexes

2.2.1. Synthesis of [Ni(fluf-O)$_2$(MeOH)$_4$], **1**

A methanolic solution (15 mL) containing Hfluf (0.5 mmol, 141 mg) and KOH (0.5 mmol, 28 mg) was stirred for 1 h. The solution was added to a methanolic solution (5 mL) of $NiCl_2{\cdot}6H_2O$ (0.25 mmol, 59 mg) and the reaction mixture was stirred for 30 min. The reaction solution was filtered and left to slowly evaporate. Pale-blue microcrystalline product of [Ni(fluf)$_2$(MeOH)$_4$], **1** (yield: 120 mg, 65%) was collected after twenty days. *Anal.* Calc. for $C_{32}H_{34}F_6N_2NiO_6$ (MW = 747.33) C, 51.43; H, 4.87; N, 3.75. Found: C, 51.34; H, 4.73; N, 3.88%. IR (KBr disk): ν_{max}, cm^{-1}; $\nu_{asym}(CO_2)$, 1590(very strong (vs)); $\nu_{sym}(CO_2)$, 1382(strong (s)); $\Delta\nu(CO_2) = \nu_{asym}(CO_2) - \nu_{asym}(CO_2) = 208\ cm^{-1}$. UV–Vis: as Nujol mull, λ/nm: 995(shoulder (sh)), 675, 420(sh), 326, 295; in DMSO, λ/nm ($\varepsilon/M^{-1}\ cm^{-1}$): 990(10), 720(15), 410(sh)(50), 322(3800), 297(6800); 10Dq = 10,100 cm^{-1}, B = 586 cm^{-1}, 10Dq/B = 17.2. μ_{eff} at room temperature = 3.10 BM. Soluble in methanol, DMF and DMSO ($\Lambda_M = 5\ S\ cm^2\ mol^{-1}$, in 1 mM DMSO solution).

2.2.2. Synthesis of complex [Ni(fluf-O,O′)$_2$(bipyam)], **2**

Potassium hydroxide (0.5 mmol, 28 mg) was added to a methanolic solution of flufenamic acid (0.5 mmol, 141 mg) and the solution was stirred for 1 h. The solution was added simultaneously with a methanolic solution of bipyam (0.25 mmol, 43 mg) to a methanolic solution of $NiCl_26H_2O$ (0.25 mmol, 59 mg) under stirring for 30 min. Light-blue crystals of [Ni(fluf)$_2$(bipyam)], **2**, suitable for X-ray structure determination were collected after a month. Yield: 120 mg, 60%. *Anal.* Calc. for [Ni(fluf)$_2$(bipyam)] ($C_{38}H_{27}F_6N_5NiO_4$) (MW = 792.38): C, 57.60; H, 3.69; N, 8.84. Found: C, 57.71; H, 3.62; N, 8.55%. IR (KBr disk): ν_{max}/cm^{-1}; $\nu_{asym}(CO_2)$: 1583(vs); $\nu_{sym}(CO_2)$: 1408(s); $\Delta\nu(CO_2) = 175\ cm^{-1}$; $\rho(C{-}H)_{bipyam}$: 770(m (medium)). UV–Vis: as Nujol mull, λ/nm: 995, 648, 405(sh), 335, 301; in DMSO, λ/nm ($\varepsilon/M^{-1}\ cm^{-1}$): 1015 (10), 645 (20), 409(sh) (110), 332(sh) (6200), 301 (12,700); 10Dq = 9852 cm^{-1}, B = 693 cm^{-1}, 10Dq/B = 14.2. μ_{eff} at room temperature = 3.22 BM. The complex is soluble in DMF and DMSO ($\Lambda_M = 10\ S\ cm^2\ mol^{-1}$, in 1 mM DMSO).

2.2.3. Synthesis of complex [Ni(fluf-O)$_2$(phen)(MeOH)$_2$], **3**

The complex was prepared in a similar way to **2** with the use of phen (0.25 mmol, 45 mg) instead of bipyam. Pale-blue microcrystalline product of complex **3** (yield: 150 mg, 70%) was isolated after a few days. *Anal.* Calc. for $C_{42}H_{34}F_6N_4NiO_6$ (MW = 863.46): C, 58.42; H, 3.97; N, 6.49. Found: C, 58.28; H, 4.09; N, 6.57%. IR (KBr disk): ν_{max}, cm^{-1}: $\nu_{asym}(CO_2)$: 1588(vs); $\nu_{sym}(CO_2)$: 1384(s); $\Delta\nu(CO_2) = 204\ cm^{-1}$; $\rho(C{-}H)_{phen}$: 726(m). UV–Vis: as Nujol mull, λ/nm: 980 (sh), 645, 405(sh), 335, 297; in DMSO, λ/nm ($\varepsilon/M^{-1}\ cm^{-1}$): 1020 (10), 660 (45), 406(sh) (90), 331(sh) (9900), 297 (15800); 10Dq = 9804 cm^{-1}, B = 691 cm^{-1}, 10Dq/B = 14.2. μ_{eff} at room temperature = 3.40 BM. The complex is soluble in methanol, DMSO, DMF and acetone ($\Lambda_M = 8\ S\ cm^2\ mol^{-1}$, in 1 mM DMSO).

2.2.4. Synthesis of complex [Ni(fluf-O)$_2$(bipy)(MeOH)$_2$], **4**

The complex was prepared in a similar way to **2** with the use of bipy (0.25 mmol, 39 mg) instead of bipyam. Pale-blue microcrystalline product of complex **4** (yield: 145 mg, 70%) was isolated after three weeks. *Anal.* Calc. for $C_{40}H_{34}F_6N_4NiO_6$ (MW = 839.44): C, 57.23; H, 4.83; N, 6.67. Found: C, 57.09; H, 4.71; N, 6.65%. IR (KBr disk): ν_{max}, cm^{-1}: $\nu_{asym}(CO_2)$: 1586(vs); $\nu_{sym}(CO_2)$: 1387(s); $\Delta\nu(CO_2) = 199\ cm^{-1}$; $\rho(C{-}H)_{bipy}$: 764(m); UV–Vis: as Nujol mull, λ/nm: 975, 640, 415(sh), 325, 299; in DMSO, λ/nm ($\varepsilon/M^{-1}\ cm^{-1}$): 1010(10), 650(45), 405(65), 320(5500), 300(9600); 10Dq = 9901 cm^{-1}, B = 691 cm^{-1}, 10Dq/B = 14.3. μ_{eff} at room temperature = 3.35 BM. The complex is soluble in methanol, DMSO, DMF and acetone ($\Lambda_M = 10\ S\ cm^2\ mol^{-1}$, in 1 mM DMSO).

2.3. X-ray structure determination

Single-crystals of complex **2** were obtained from the reaction mixture after slow evaporation. For the structure determination, single-crystals of the compound were mounted on a Bruker Kappa