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# Biological evaluation of cobalt(II) complexes with non-steroidal anti-inflammatory drug naproxen

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ABSTRACT

Cobalt(II) complexes with the non-steroidal anti-inflammatory drug naproxen in the presence or absence of nitrogen-donor heterocyclic ligands (pyridine, 2,2′-bipyridine or 1,10-phenanthroline) have been synthesized and characterized with physicochemical and spectroscopic techniques. The deprotonated naproxen acts as monodentate ligand coordinated to Co(II) ion through a carboxylato oxygen. The crystal structure of [bis(aqua)bis(naproxenato)bis(pyridine)cobalt(II)], **2** has been determined by X-ray crystallography. The EPR spectrum of complex **2** in frozen solution reveals that it retains its structure. UV study of the interaction of the complexes with calf-thymus DNA (CT DNA) has shown that the complexes can bind to CT DNA and [(2,2′-bipyridine)bis(methanol)bis(naproxenato)cobalt(II)] exhibits the highest binding constant to CT DNA. The cyclic voltammograms of the complexes recorded in DMSO solution and in the presence of CT DNA in 1/2 DMSO/buffer (containing 150 mM NaCl and 15 mM trisodium citrate at pH 7.0) solution have shown that they 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. Naproxen and its cobalt(II) complexes exhibit good binding propensity to human or bovine serum albumin proteins having relatively high binding constant values. The antioxidant activity of the compounds has been evaluated indicating their high scavenging activity against hydroxyl free radicals and superoxide radicals.


## 1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are used as analgesic, anti-inflammatory and antipyretic agents and are among the most frequently used medicinal drugs [1]. NSAIDs have exhibited anti-tumorigenic activity by reducing the number and size of carcinogen-induced colon tumors and a synergistic role on the activity of certain antitumor drugs, although the mechanism has not been completely clarified [2,3]. They can act by inhibiting the cyclooxygenase(COX)-mediated production of prostaglandins or via COX-independent mechanisms by modulating cell proliferation and cell death in cultured colon cancer cells lacking COX [4,5]. The direct interaction of NSAIDs and their metal complexes with DNA is of interest since their anticancer as well as the anti-inflammatory activity may be explained [6,7]. It has been also reported that the metal complexes of some NSAIDs are more active than their parent compounds [8,9].

Phenylalkanoic acids, anthranilic acids, oxicams, salicylate derivatives, sulfonamides and furanones are the main chemical classes of NSAIDs [10]. Naproxen (=Hnap) (Scheme 1) belongs to the NSAID group of phenylalkanoic acids [10]. It exhibits favorable anti-inflammatory, analgesic and antipyretic properties [11] and is used in painful and inflammation conditions like rheumatoid arthritis, spondilytis, and osteoarthritis [12]. In the literature, the crystal structures of four copper(II) [13–16] and one cadmium(II) [17] complexes of naproxen have been reported.

The biological role of cobalt is mainly focused on its presence in the active center of vitamin B12, which regulates indirectly the synthesis of DNA. Additionally, cobalt is involved in the co-enzyme of vitamin B12 used as a supplement of the vitamin [18] and at least eight cobalt-dependent proteins have been reported [19]. Since the first reported studies into the biological activity of Co complexes [20] in 1952, diverse structurally characterized cobalt complexes showing antitumor-antiproliferative [21,22], antimicrobial [23,24], antifungal [25,26], antiviral [27,28] and antioxidant [29] activities have been reported. To the best of our knowledge, the only cobalt(II) complexes with NSAIDs as ligands are two mefenamato ones recently reported by our lab [29], a meloxicam [30] and a ketoprofenato one [31].

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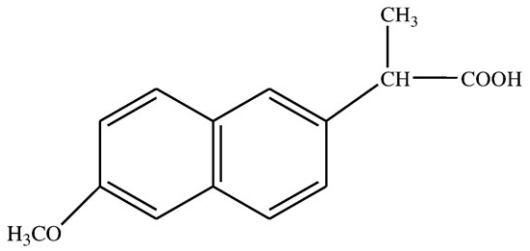

**Scheme 1.** Naproxen (=Hnap).

Our recent studies have been focused on the co-ordination chemistry of carboxylate-containing antimicrobial [32–36] or anti-inflammatory [15,29,37,38] agents with metal ions in an attempt to examine their mode of binding and possible biological relevance. In addition, we have reported studies on the interaction of these metal complexes with biomolecules (nucleic acids and serum albumin proteins) and their potential biological (antimicrobial, anticancer, antioxidant) activity. Taking into consideration the biological role and activity of cobalt and its complexes as well as the significance of the NSAIDs in medicine, we have initiated the investigation of the interaction of cobalt(II) with NSAIDs as ligands [29]. In this context, we report the synthesis, the structural characterization, the electrochemical and the biological properties of the neutral mononuclear cobalt(II) complexes with the NSAID naproxen in the absence ($[Co(nap)_2(MeOH)_4]$ **1**) or presence of a nitrogen-donor heterocyclic ligand such as pyridine (=py), 1,10-phenanthroline (=phen) or 2,2′-bipyridine (=bipy) ($[Co(nap)_2(py)_2(H_2O)_2]$ **2**, $[Co(nap)_2(phen)(MeOH)_2]$ **3** and $[Co(nap)_2(bipy)(MeOH)_2]$ **4**). The crystal structure of **2** has been determined by X-ray crystallography. The investigation of the biological properties of the Hnap and its complexes has been focused on (i) the binding properties to calf-thymus (CT) DNA performed with UV spectroscopy, DNA solution viscosity measurements, cyclic voltammetry and competitive binding studies with ethidium bromide (EB), (ii) the affinity for bovine (BSA) and human serum albumin (HSA) proteins involved in the transport of metal ions and metal complexes with drugs through the blood stream, performed with fluorescence spectroscopy and (iii) the antioxidant capacity, since the use of NSAIDs in medicine as anagelsics and anti-inflammatories may be related to free radicals scavenging and their metal complexes have exhibited synergistic activity. Additionally, the antioxidant capacity of the recently reported Cu(II) naproxenato complexes [15] has been evaluated.

## 2. Experimental

### 2.1. Materials–instrumentation–physical measurements

All the chemicals ($CoCl_2\cdot 6H_2O$, naproxen, bipy, phen, bipyam, py, KOH, CT DNA, BSA, HSA, EB, NaCl, trisodium citrate, nordihydroguairetic acid (NDGA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS) and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox)) were purchased from Sigma-Aldrich Co and all solvents were purchased from Merck. All the chemicals and solvents were of reagent grade and were used as purchased without any further purification. 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 1.87, 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 $\varepsilon = 6600\ M^{-1}\ cm^{-1}$ [15,29].

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 analyses were performed on a Perkin-Elmer 240B elemental analyzer. Molar conductivity measurements were carried out with a Crison Basic 30 conductometer. Fluorescence spectra were recorded in solution on a Hitachi F-7000 fluorescence spectrophotometer. Solid state and solution EPR measurements were taken in the temperature range 4–300 K on a Bruker 200D-SRC X-Band spectrometer, equipped with an Oxford ESR 9 Cryostat, operating at 9.412 GHz, 10 db.

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. $[Co(nap)_2(MeOH)_4]$, 1

A methanolic solution (15 mL) containing naproxen (0.4 mmol, 92 mg) and KOH (0.4 mmol, 22 mg) was stirred for 1 h. The solution was added to a methanolic solution (10 mL) of $CoCl_2\cdot 6H_2O$ (0.2 mmol, 48 mg) and the reaction mixture was stirred for 1 h. The reaction solution was filtered and left for slow evaporation. Rose-colored microcrystalline product of $[Co(nap)_2(MeOH)_4]$, **1**, (73 mg, yield: 61%) was collected after a few days. *Anal.* Calcd. for $C_{32}H_{40}CoO_{10}$ (MW = 645.61) C, 59.53; H, 6.56%; found: C, 59.35; H, 6.59%. IR: $\nu_{max}$, $cm^{-1}$; $\nu_{asym}(CO_2)$, 1604(vs (very strong)); $\nu_{sym}(CO_2)$, 1398 (vs); $\Delta = \nu_{asym}(CO_2) - \nu_{sym}(CO_2)$: 206 $cm^{-1}$ (KBr disk); UV–vis: λ, nm (ε, $M^{-1}\ cm^{-1}$) as nujol mull: 720, 565, 440, 396, 335, 318; in DMSO: 735 (25), 562 (90), 445 (50), 389 (150), 333 (4500), 320 (3800). $\mu_{eff} = 4.55$ BM. The complex is soluble in DMSO and DMF and is non-electrolyte ($\Lambda_M = 5$ mho $cm^2\ mol^{-1}$).

#### 2.2.2. $[Co(nap)_2(py)_2(H_2O)_2]$, 2

The complex was prepared by the addition of a methanolic solution (15 mL) of Hnap (0.4 mmol, 92 mg) and KOH (0.4 mmol, 22 mg), after 30 min of stirring, to a methanolic solution (10 mL) of $CoCl_2\cdot 6H_2O$ (0.2 mmol, 48 mg) followed by the addition of 2 mL of pyridine. Pink-colored crystals of $[Co(nap)_2(py)_2(H_2O)_2]$, **2** (109 mg, yield: 60%) suitable for X-ray structure determination, were deposited after a few days. *Anal.* Calcd. for $C_{38}H_{40}CoN_2O_8$ (MW = 711.65) C, 64.13; H, 5.67; N, 3.94%; found C, 63.93; H, 5.45; N, 3.92%. IR: $\nu_{max}$, $cm^{-1}$; $\nu_{asym}(CO_2)$: 1605 (vs); $\nu_{sym}(CO_2)$: 1389 (vs); $\Delta = \nu_{asym}(CO_2) - \nu_{sym}(CO_2)$: 216 $cm^{-1}$ (KBr disk); UV–vis: λ, nm (ε, $M^{-1}\ cm^{-1}$) as nujol mull: 730, 565, 445(sh (shoulder)), 385, 335, 318; in DMSO: 740 (25), 572 (40), 448 (45), 390 (230), 333 (2100), 320 (1650). $\mu_{eff} = 4.34$ BM. The complex is soluble in DMSO, DMF and acetonitrile and is non-electrolyte ($\Lambda_M = 6$ mho $cm^2\ mol^{-1}$).