Journal of Inorganic Biochemistry 123 (2013) 53–65

Contents lists available at SciVerse ScienceDirect

Journal of Inorganic Biochemistry

journal homepage: www.elsevier.com/locate/jinorgbio

# Copper(II) interacting with the non-steroidal antiinflammatory drug flufenamic acid: Structure, antioxidant activity and binding to DNA and albumins

Charikleia Tolia [a], Athanassios N. Papadopoulos [b], Catherine P. Raptopoulou [c], Vassilis Psycharis [c], Claudio Garino [d], Luca Salassa [e], George Psomas [a,*]

[a] Department of General and Inorganic Chemistry, Faculty of Chemistry, Aristotle University of Thessaloniki, GR-54124 Thessaloniki, Greece
[b] Department of Nutrition and Dietetics, Faculty of Food Technology and Nutrition, Alexandrion Technological Educational Institution, Sindos, Thessaloniki, Greece
[c] Institute of Advanced Materials, Physicochemical Processes, Nanotechnology & Microsystems, Department of Materials Science, NCSR "Demokritos", GR-15310 Aghia Paraskevi Attikis, Greece
[d] Department of Chemistry and NIS Centre of Excellence, University of Turin, Via P. Giuria 7, 10125 Turin, Italy
[e] CIC biomaGUNE, Paseo Miramón 182, 20009 Donostia–San Sebastián, Spain

ARTICLE INFO

*Article history:*
Received 28 December 2012
Received in revised form 8 February 2013
Accepted 20 February 2013
Available online 28 February 2013

*Keywords:*
Copper complexes
Non-steroidal antiinflammatory drugs
Flufenamic acid
Interaction with DNA
Interaction with serum albumins
Antioxidant activity

ABSTRACT

Copper(II) complexes with the non-steroidal antiinflammatory drug flufenamic acid (Hfluf) in the presence of N,N-dimethylformamide (DMF) or nitrogen donor heterocyclic ligands (2,2′-bipyridylamine (bipyam), 1,10-phenanthroline (phen), 2,2′-bipyridine (bipy) or pyridine (py)) have been synthesized and characterized. The crystal structures of $[Cu_2(fluf)_4(DMF)_2]$, **1**, and [Cu(fluf)(bipyam)Cl], **2**, have been determined by X-ray crystallography. Density functional theory (DFT) (CAM-B3LYP/LANL2DZ/6-31G**) was employed to determine the structure of complex **2** and its analogues (complexes [Cu(fluf)(phen)Cl], **3**, [Cu(fluf)(bipy)Cl], **4** and $[Cu(fluf)_2(py)_2]$, **5**). Time-dependent DFT calculations of doublet–doublet transitions show that the lowest-energy band in the absorption spectrum of **2**–**5** has a mixed d–d/LMCT character. UV study of the interaction of the complexes with calf-thymus DNA (CT DNA) has shown that the complexes can bind to CT DNA with [Cu(fluf)(bipy)Cl] exhibiting the highest binding constant to CT DNA. The complexes can bind to CT DNA via intercalation as concluded by studying the cyclic voltammograms of the complexes in the presence of CT DNA solution and by DNA solution viscosity measurements. Competitive studies with ethidium bromide (EB) have shown that the complexes can displace the DNA-bound EB suggesting strong competition with EB. Flufenamic acid and its Cu(II) complexes exhibit good binding affinity to human or bovine serum albumin protein with high binding constant values. All compounds have been tested for their antioxidant and free radical scavenging activity as well as for their *in vitro* inhibitory activity against soybean lipoxygenase showing significant activity with [Cu(fluf)(phen)Cl] being the most active.



## 1. Introduction

Copper is one of the most important biometals not only because of its role in proteins but also due to its potential synergetic activity with drugs [1,2]. Copper(II) complexes with drugs as ligands exhibiting increased activity in comparison to free drugs have been the subject of many research studies [2–4] and a plethora of copper(II) complexes with diverse ligands showing potential antitumor [5,6], antioxidant [7,8], antibacterial [9–11] and antifungal [12] activity may be found in the literature.

Non-steroidal antiinflammatory drugs (NSAIDs) are the most frequently administrated analgesic, antiinflammatory and antipyretic drugs with known side-effects [13]. In general, the action of NSAIDs is the inhibition of the cyclo-oxygenase (COX)-mediated production of prostaglandins [14]. NSAIDs can also induce the apoptosis of a series of cancer cell lines including colon, breast, prostate, human myeloid leukaemia and stomach cell lines [15] and have a synergistic role in the activity of certain antitumor drugs [16]; these antitumorigenic properties of the NSAIDs may be attributed to COX-independent mechanisms by modulating cell proliferation and cell death in cancer cells lacking COX [17,18], to apoptosis via activation of caspases [19] or via an unknown molecular mechanism where free radicals may also be involved [20,21]. Therefore, in an attempt to investigate the potential anticancer as well as antiinflammatory activity of NSAIDs and their complexes, their interaction with DNA as well as their antioxidant activity should be considered of great importance and further evaluated; it should be noted that only few relevant reports on the interaction of NSAIDs and their complexes with DNA have been published so far [22,23]. Furthermore, numerous copper(II) complexes with NSAIDs as ligands have been structurally characterized including those of diclofenac [8,24], diflunisal [25], flufenamic acid [26], ibuprofen [27,28], indomethacin [29], mefenamic acid [7], naproxen[27,28], suprofen [29,30] and tolmentin [27].

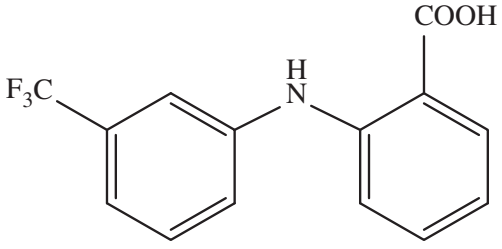
* Corresponding author. Tel.: +30 2310997790; fax: +30 2310997738.
*E-mail address:* gepsomas@chem.auth.gr (G. Psomas).

0162-0134/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jinorgbio.2013.02.009

Phenylalkanoic acids, anthranilic acids, oxicams, salicylate derivatives, sulfonamides and furanones constitute the chemical classes of NSAIDs [2]. The NSAID flufenamic acid (= Hfluf, Scheme 1) belongs to the derivatives of N-phenylanthranilic acid and resembles chemically to mefenamic and tolfenamic acids and other fenamates in clinical use [31]. Hfluf possesses analgesic, antiinflammatory and antipyretic properties and has been used in musculoskeletal and joint disorders and is administered orally and topically [32]. Its channel-regulating ability [33] has been long known since it has been used as a blocker of calcium-dependent cationic currents [34]. Additionally, Hfluf can inhibit nonselective cation channels [35]. On the other hand, it activates potassium channels [36] and has recently shown an interesting modulatory effect on neuronal sodium channels [32]. The number of the metal complexes of flufenamic acid is rather limited in comparison to other NSAIDs; few copper(II) complexes [26,37] have been found in the literature as well as a zinc complex recently reported by our group [38].

Having in mind the importance of NSAIDs in medicine and the enhanced activity of their metal complexes, we have recently initiated the synthesis, characterization and study of biological activity of Cu(II), Co(II) and Zn(II) with NSAIDs as ligands [7,8,25,31,38–42]. Within this context, we present herein the synthesis of Cu(II) complexes with the NSAID flufenamic acid in the presence of the O-donor ligand N,N-dimethylformamide (DMF) or the nitrogen-donor heterocyclic ligands 2,2′-bipyridylamine (bipyam), 1,10-phenanthroline (phen), 2,2′-bipyridine (bipy) or pyridine (py), resulting in the formation of complexes $[Cu_2(fluf)_4(DMF)_2]$, **1**, [Cu(fluf)(bipyam)Cl], **2**, [Cu(fluf)(phen)Cl], **3**, [Cu(fluf)(bipy)Cl], **4** and $[Cu(fluf)_2(py)_2]$, **5**, respectively. The complexes have been characterized with physicochemical and spectroscopic techniques and their electrochemical behavior has been also investigated. The crystal structures of $[Cu_2(fluf)_4(DMF)_2]$ **1** and [Cu(fluf)(bipyam)Cl] **2** have been determined by X-ray crystallography.

In an attempt to investigate the existence of potential anticancer and/or anti-inflammatory activity of the resultant complexes **1–5**, we have focused on (i) the interaction of the complexes with calf-thymus (CT) DNA studied by UV spectroscopy, cyclic voltammetry and viscosity measurements, (ii) the ability of the complexes to displace ethidium bromide (EB) from the EB-DNA complex in order to clarify the existence of a potential intercalation of the complexes to CT DNA in competition to the classical DNA-intercalator EB studied by fluorescence spectroscopy, (iii) the antioxidant capacity of the complexes by determining their ability to scavenge 1,1-diphenyl-picrylhydrazyl (DPPH), hydroxyl (•OH) and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) radicals as well as their inhibitory activity against soybean lipoxygenase (LOX)—since the use of NSAIDs in medicine as anagelsics and antiinflammatories may be related to free radicals scavenging. Furthermore, the affinity of the complexes to bovine (BSA) and human serum albumin (HSA) has been investigated by fluorescence spectroscopy since binding to such proteins that are involved in the transport of metal ions and metal–drug complexes through the blood stream may result in lower or enhanced biological properties of the original drug, or new paths for drug transportation [43].




**Scheme 1.** The NSAID flufenamic acid (=Hfluf).

## 2. Experimental

### 2.1. Materials—instrumentation—physical measurements

All chemicals ($CuCl_2 \cdot 2H_2O$, flufenamic acid, bipy, phen, bipyam, py, KOH, CT DNA, BSA, HSA, EB, NaCl and trisodium citrate) were purchased from Sigma-Aldrich Co and all solvents were purchased from Merck. The chemicals and solvents were 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 1.89, 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 $\varepsilon = 6600\ M^{-1}\ cm^{-1}$ [7,8].

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 $10^{-5}$ to $10^{-3}$ 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.

Cyclic voltammetry 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. $[Cu_2(fluf)_4(DMF)_2]$, 1

Potassium hydroxide (0.4 mmol, 22 mg) was added to a methanolic solution (15 mL) of flufenamic acid (0.4 mmol, 113 mg) and the solution was stirred for 1 h. The solution was added slowly to a methanolic solution (5 mL) of $CuCl_2 \cdot 2H_2O$ (0.2 mmol, 34 mg) followed by the addition of 2 mL of DMF. Dark green crystals of $[Cu_2(fluf)_4(DMF)_2]$ **1** suitable for X-ray structure determination were collected after twenty days. Yield: 90 mg, 65%. *Anal.* Calcd. for $[Cu_2(fluf)_4(DMF)_2]$ ($C_{62}H_{50}Cu_2F_{12}N_6O_{10}$) (MW = 1394.19): C 54.67, H 3.70, N 6.17; found C 54.25, H 3.59, N 5.95. IR (KBr disk): $\nu_{max}$, $cm^{-1}$; $\nu(C=O)_{DMF}$: 1667 (very strong (vs)); $\nu_{asym}(CO_2)$: 1590 (vs); $\nu_{sym}(CO_2)$: 1400 (vs); $\Delta = \nu_{asym}(CO_2) - \nu_{sym}(CO_2) = 190\ cm^{-1}$; UV-vis: λ, nm (ε, $M^{-1}\ cm^{-1}$) as nujol mull: 738, 410 (shoulder (sh)), 340, 295; in DMSO: 735 (120), 415(sh) (150), 335 (14,200), 296 (25,000). $\mu_{eff} = 1.45$ BM. The complex is soluble in DMF and DMSO ($\Lambda_M$ = 5 mho $cm^2\ mol^{-1}$, in 1 mM DMSO).