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Copper(II) diclofenac complexes: Synthesis, structural studies and interaction with albumins and calf-thymus DNA

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ABSTRACT

The reaction of the copper(II) diclofenac complex $[Cu(dicl)_2(H_2O)_2]$ (**1**) (*dicl* = deprotonated diclofenac (H*dicl*)) with the chelating N-donor ligands ethylenediamine (*en*), propan-1,3-diamine (*pn*), unsymmetrical dimethylethylene-diamine (*unsym-dmen*) and *N,N,N′,N′*-tetramethylethylene-diamine (*temed*) in methanol-water (4:1 v/v) yielded the novel copper(II) complexes $[Cu(en)_2(H_2O)_2](dicl)_2{\cdot}2H_2O$ (**2**), $[Cu(pn)_2(H_2O)_2](dicl)_2{\cdot}2H_2O$ (**3**), $[Cu(unsym\text{-}dmen)_2(H_2O)](dicl)_2{\cdot}H_2O$ (**4**) and $[Cu(temed)(dicl)_2]$ (**5**), respectively. All the synthesized complexes were characterized by spectroscopic (UV–vis, FT-IR) methods. The structures of complexes **2**, **3** and **5** were unambiguously determined by single-crystal X-ray crystallography. X-ray structures of complexes clearly revealed the ionic structure of complexes **2**, **3** and the covalent structure of complex **5**. The geometry of complex **4** was optimized by Density Functional Theory (DFT) calculations. The ability of the complexes **1–5** to bind to calf-thymus DNA was monitored *in vitro* by diverse techniques (UV–vis spectroscopy, cyclic voltammetry, viscosity measurements) and *via* competitive studies with ethidium bromide. The interaction of complexes **1–5** with bovine serum albumin was studied *in vitro* by fluorescence emission spectroscopy and the corresponding binding constants were calculated. The biological behavior of complexes **1–5** was compared with previously reported Cu(II), Mn(II) and Ni(II) complexes of diclofenac.

1. Introduction

It has been more than fifty years since Prof. Rosenberg discovered the activity of *cisplatin* ($[Pt(NH_3)_2Cl_2]$) against cancer cells [1–3]. This discovery provided a boost to “medicinal inorganic chemistry” which focuses on the design and discovery of metal-based drugs not only as anticancer agents but also for any possible therapeutic applications. Nowadays, metal complexes that are examined *in vitro* for their potential activity may contain diverse transition metal ions and a variety of ligands, and their potential applications cover a wide spectrum of activities against infections, inflammations and diseases [4–6].

Many of the used anticancer drugs are DNA-damaging agents [7]. Therefore, DNA is recognized as one of the most common biological targets of anticancer drugs. In general, transition metal complexes may bind to double-stranded DNA mainly in three fashions: (a) *via* covalent binding, *i.e.* replacement of a labile ligand of the complex by a nitrogenous DNA-base, (b) through noncovalent interactions, *i.e.* intercalation *via* $\pi \rightarrow \pi$ stacking of the complex in-between DNA-bases, electrostatic interactions resulting from Coulomb forces between metal complexes and the phosphate groups of DNA, and groove-binding occurring along major or minor groove of DNA helix upon the development of van der Waals forces or hydrogen-bonding or hydrophobic bonding and (c) cleavage of the DNA double-stranded helix which may occur along and/or across the strands [8–12].

The pioneering work of Sorenson provided a tremendous boost to research on copper(II) complexes for exploring their potential biological activity [13]. Copper is among the most abundant transition metals in the human body and plays several roles in human physiology and is involved in the active center of enzymes responsible for numerous redox processes while its excess or deficiency may cause diseases, such as Wilson's disease or Menkes syndrome [14,15]. As a result of its bacteriostatic activity, copper is used for the manufacturing of touch surfaces in hospitals and healthcare settings [16]. The mixture of ternary Cu(II) complexes called “Casiopeinas®” is in clinical trials for its

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cytostatic, cytotoxic and anti-neoplastic activities [17]. Copper(II) complexes are attractive as metal-based drugs because: (i) Cu(II) salts as starting material are cheaper than other metal salts, making treatment more affordable for the poor, (ii) the copper(II) complexes of several drugs (antibacterial [18], antifungal [19], anti-inflammatory [20], antiviral [21] agents) are more active than the parent drugs [22] and (iii) the biological activity of copper(II) complexes with nitrogen-donor ligands has been found to be higher than the original ligands [14,23–25]. In the context of medicinal inorganic chemistry, copper(II) complexes have shown noteworthy *in vitro* biological activity including anticancer [26], antifungal [27], anti-inflammatory [28], antimicrobial [29] and antioxidant [30].

Non-steroidal anti-inflammatory drugs (NSAIDs) constitute a commercially important class of drugs to cure pain and inflammation associated with diseases or injuries, including intestinal disease and migraine [31]. Furthermore, the mechanisms of action of the antitumorigenic effects of NSAIDs have not been yet completely revealed and may include cyclooxygenase (COX)-independent mechanisms, apoptosis, free radical involvement or other unknown molecular mechanisms [32–34].Within this context, the interaction of NSAIDs with DNA (which is also a biological target for anticancer drugs) is of great interest in order to try to explain the potential anticancer and antiinflammatory activities [35,36]. Moreover, from a metal-complex synthetic point of view, the carboxylic NSAIDs (*i.e.* salicylate derivatives, phenylalkanoic acids, and anthranilic acids) are particularly interesting since their carboxylic group –COOH may exhibit upon deprotonation a variety of coordination modes towards metal ions, *e.g.* monodentate, bidentate, bidentate chelating or bidentate bridging [37].

Diclofenac (H*dicl*) (Fig. 1(a)) is a widely used anti-inflammatory, analgesic and antipyretic agent. Sodium diclofenac (Na*dicl*) is an NSAID phenylalkanoic acid derivative used in the treatment of rheumatoid arthritis and osteoarthritis [38–41]. As for its metal complexes reported in the literature, the copper [41–44], manganese [45–47], cadmium [48], tin [49] and nickel [50] complexes have been studied.

In view to our long experience in structural chemistry of copper(II) carboxylates [51–56], we started a research program aiming at the synthesis and characterization of copper(II) complexes of NSAIDs, evaluation of their ability to interact with DNA and albumins and possibly assessment of their structure-activity relationship [30,42,44,57–68]. The characteristic features of our methodology are: (i) cheap and readily available starting materials, (ii) simple and convenient synthesis at room temperature, (iii) nearly quantitative yields. The understanding of the modes of action of potential metal-based drugs necessitates the study of interaction with possible biological targets including aminoacids, proteins, and biological macromolecules [69]. The study of the interaction with the potential biological target DNA as well as with the drug-carrier protein albumin is a first approach for further therapeutic applications.

As a continuation of our research project regarding the synthesis and biological evaluation of copper(II)/N-donors complexes [51–58], we report herein, the synthesis and characterization of four new copper (II)-diclofenac complexes in the presence of the N-donor ligands, such as ethylenediamine (*en*), propan-1,3-diamine (*pn*), unsymmetrical dimethylethylene-diamine (*unsym-dmen*) and *N,N,N′,N′*-tetramethylethylenediamine (*temed*) (Fig. 1(b)–(e)), namely, [Cu(*en*)$_2$(H$_2$O)$_2$](*dicl*)$_2$·2H$_2$O (**2**), [Cu(*pn*)$_2$(H$_2$O)$_2$](*dicl*)$_2$·2H$_2$O (**3**), [Cu(*unsym-dmen*)$_2$(H$_2$O)](*dicl*)$_2$·H$_2$O (**4**) and [Cu(*temed*)(*dicl*)$_2$] (**5**), along with previously reported [Cu(*dicl*)$_2$(H$_2$O)$_2$] (**1**) [39]. The *in vitro* biological activities of the resultant complexes have been also considered. More specifically, as a first approach for the potential use of the complexes as metallopharmaceutical agents, their *in vitro* affinity for bovine serum albumin (BSA) has been monitored by fluorescence emission spectroscopy and their *in vitro* interaction with calf-thymus (CT) DNA has been examined by UV–visible (UV–vis) spectroscopy, viscosity measurements, cyclic voltammetry and *via* their ability to displace ethidium bromide (EB) from the EB-DNA conjugate (which has been studied by fluorescence emission spectroscopy).

2. Experimental

2.1. Materials and physical measurements

Na*dicl*, CuSO$_4$·5H$_2$O CT DNA, BSA, EB, NaCl and trisodium citrate were purchased from Sigma-Aldrich Co and all solvents were purchased from Merck. All chemicals and solvents were reagent grade and were used as purchased without any further purification. 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 vigorous stirring for three days, and kept at 4 °C for no longer than ten days. This 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 [70]. The DNA concentration was determined by the UV absorbance at 260 nm after 1:20 dilution using $\varepsilon = 6600\ M^{-1}\ cm^{-1}$ [71].

Elemental analysis was performed using an automatic Perkin Elmer 2400 CHN element analyzer and copper content was gravimetrically determined as CuSCN by standard literature methods [72]. Attenuated Total Reflection-Fourier transform infrared spectra (ATR-FT-IR) were recorded on Perkin Elmer Spectrum RX FT-IR spectrometer (symbols used denote: m = medium, s = strong, w = weak). The UV–vis spectra of the complexes were recorded on a Hitachi U-2001 dual beam spectrophotometer using DMSO:water as solvent. The molar conductivity measurements were performed on 1 mM DMSO solution of the complexes with a Crison Basic 30 conductometer. The fluorescence spectra of all complexes **1–5** were recorded in solution on a Hitachi F-7000 fluorescence spectrophotometer. The viscosity experiments were carried out using an ALPHA L Fungilab rotational viscometer equipped with an 18-mL LCP spindle and the measurements were performed at 100 rpm. Cyclic voltammetry studies were performed on an Eco Chemie Autolab Potentiostat and the experiments were performed on 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. Oxygen was removed by purging the solutions with pure nitrogen. All electrochemical measurements were performed at 25.0 ± 0.2 °C.

2.2. Preparation of complexes

2.2.1. Synthesis of [Cu(dicl)$_2$(H$_2$O)$_2$], 1

CuSO$_4$·5H$_2$O (0.50 g, 2 mmol) was dissolved in 10 mL of distilled water and sodium diclofenac (1.27 g, 4 mmol) was also dissolved in minimum amount of water. On mixing the two solutions, a light-green precipitate of hydrated copper(II) diclofenac was formed immediately which was filtered through a fine filter paper, washed with water followed by methanol and dried at room temperature (yield 90%, 1.24 g). Complex **1** is insoluble in

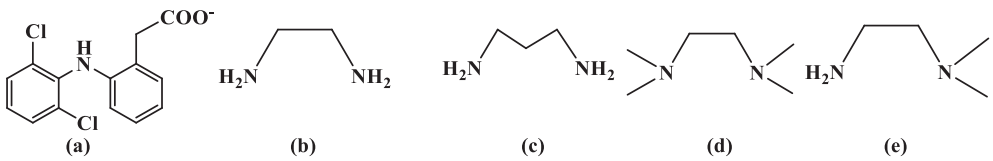

Fig. 1. Ligands used in this work: (a) diclofenac anion (*dicl*$^-$), (b) ethylenediamine (*en*), (c) propan-1,3-diamine (*pn*), (d) *N,N,N′,N′*-tetramethylethylenediamine (*temed*) and (e) unsymmetrical dimethylethylenediamine (*unsym-dmen*).