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Zinc complexes of flufenamic acid: Characterization and biological evaluation

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ABSTRACT

The reaction of $ZnCl_2$ with the non-steroidal anti-inflammatory drug flufenamic acid (Hfluf) led to the formation of complex $[Zn(fluf\text{-}O)_2(MeOH)_4]$, **1**. When the reaction takes places in the presence of a N,N′-donor heterocyclic ligand such as 2.2′-bipyridylamine (bipyam), 2.2′-bipyridine (bipy), 1.10-phenanthroline (phen) and 2.2′-dipyridylketone oxime (Hpko), the complexes $[Zn(fluf)_2(bipyam)]$, **2**, $[Zn(fluf)_2(bipy)]$, **3,** $[Zn(fluf)(phen)_2(H_2O)](fluf)\cdot 0.2MeOH$, **4**·0.2MeOH and $[Zn(fluf)_2(Hpko)_2]$, **5** were isolated, respectively. The complexes were characterized by physicochemical and spectroscopic techniques and the crystal structures of complexes **2** and **4** were determined by X-ray crystallography. The ability of the complexes to scavenge 1.1-diphenyl-picrylhydrazyl, 2.2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) and hydroxyl radicals and to inhibit soybean lipoxygenase was evaluated; the complexes were more active than free Hfluf. 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 employed to monitor the interaction of the complexes with calf-thymus DNA and revealed intercalation as the most possible mode of binding.

1. Introduction

As the second most abundant trace element in the human body, zinc has a significant role regulating the metabolism of cells [1,2] and is found in more than two hundred metalloenzymes having catalytic, structural or regulatory role [3]. The deficiency or changes in the metabolism of zinc may cause growth effects and disorders of the central nervous system [4,5]. The extensive use of zinc in the form of 'Baby Zinc' in the treatment of deadly diarrhea has saved the lives of many children in Asian and African countries [6]. Zinc has also demonstrated a unique role in nucleic acid chemistry since it is the only metal ion able to facilitate the rewinding of DNA [7]. Furthermore, many zinc complexes have been reported for their biological activity; zinc complexes have reported for their anticonvulsant [8], antidiabetic [9], anti-inflammatory [10], antimicrobial [11–16], antioxidant [17,18] and antiproliferative/antitumor [10,11,19–21] activity and other complexes have been tested for the treatment of Alzheimer disease [22].

Flufenamic acid (Hfluf, Fig. 1) is a non-steroidal anti-inflammatory drug (NSAID) and is a member of the anthranilic acid derivatives (fenamates) [23]. Similarly to other fenamates (e.g. mefenamic acid and tolfenamic acid) in clinical use, Hfluf is a cyclooxygenase inhibitor and prevents formation of prostaglandins [24]. Hfluf possesses analgesic, anti-inflammatory and antipyretic properties and has been used in musculoskeletal and joint disorders and is administered orally and topically [25] but it is not widely used for humans, since it has a high rate (30–60%) of gastrointestinal side -effects [26]. Flufenamic acid has been used as a cation-regulator [27], since it can activate potassium [28] and neuronal sodium channels [25], can block calcium-dependent cationic currents [29] and can inhibit nonselective cation channels [30]. In regard to the metal-flufenamato complexes, reports concerning few copper(II) [31–33], cobalt [34] and zinc [35] complexes were found in the literature.

Free radicals are species with an important role in the inflammatory process [36]. Compounds showing antioxidant activity, i.e. acting as inhibitors of free radical production and/or as radical scavengers, have also a potential anticancer and anti-inflammatory activity [36]. The radicals often used to evaluate the potential radical scavenging activity of the compounds are 1.1-diphenyl-picrylhydrazyl (DPPH), hydroxyl radicals (*OH) and 2.2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{+\bullet}$), since they are related with known inflammatory results. The DPPH scavenging is related to antiageing, anticancer and anti-inflammatory activity [37] and DPPH scavengers may offer potential protection against rheumatoid arthritis and inflammation. Hydroxyl radicals ($^{\bullet}OH$) are among the most reactive oxygen species (ROS) and their scavengers may serve as activators of the prostaglandin synthesis

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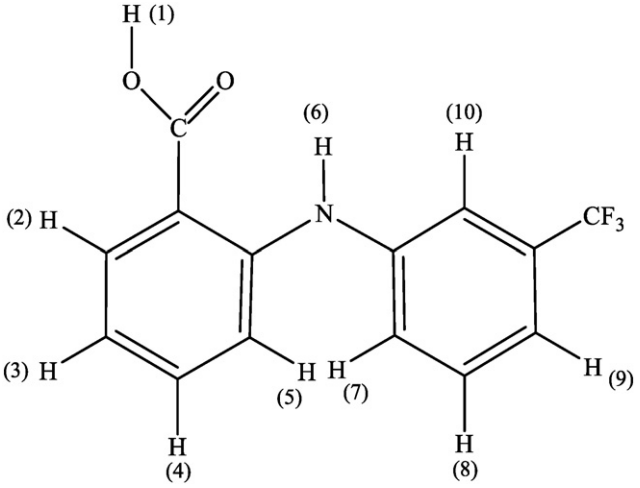

Fig. 1. The syntax formula of flufenamic acid and the labeling of the H atoms.

and subsequently as protectors from ROS [37]. On the other hand, the scavenging of the cationic radical ABTS ($ABTS^{+\bullet}$) is related to the total antioxidant activity [37]. Lipoxygenases (LOXs) are non-heme iron-containing dioxygenases involved in the transformation of arachidonic acid to leukotrienes and have an important role in the pathophysiology of several inflammatory and allergic diseases as well as rheumatoid arthritis, psoriasis and asthmatic responses [38]; most LOX inhibitors are considered potential antioxidants or free radical scavengers since lipoxygenation occurs via a carbon centered radical [39,40].

Taking into consideration the importance of NSAIDs in medicine, the enhanced activity of their metal complexes and the application of zinc in drugs and as a continuation of our research concerning the interaction of metal ions with NSAIDs [17,18,23,33–35,41–47], we present herein the synthesis of five novel Zn(II) complexes with flufenamic acid in the absence or presence of the nitrogen-donor ligands 2.2′-bipyridylamine (bipyam), 2.2′-bipyridine (bipy), 1.10-phenanthroline (phen) or 2.2′-dipyridylketone oxime (Hpko) (Fig. 2). The reaction of Zn(II) with deprotonated flufenamic acid resulted in the formation of complex $[Zn(fluf\text{-}O)_2(H_2O)_4]$, **1,** while in the presence of bipyam, bipy, phen or Hpko the mononuclear complexes $[Zn(fluf\text{-}O,O')_2(bipyam)]$, **2,** $[Zn(fluf\text{-}O,O')_2(bipy)]$, **3,** $[Zn(fluf\text{-}O)(phen)_2(H_2O)](fluf)\cdot 0.2MeOH$, **4**·0.2MeOH, and $[Zn(fluf\text{-}O)_2(Hpko\text{-}N,N')_2]$, **5**, respectively, were isolated. The complexes were characterized by physicochemical (elemental analysis and molecular conductivity measurements) and spectroscopic (IR, UV–vis and ^1H NMR) techniques, while the crystal structures of complexes $[Zn(fluf\text{-}O,O')_2(bipyam)]$, **2,** and $[Zn(fluf\text{-}O)(phen)_2(H_2O)](fluf)\cdot 0.2MeOH$, (**4**·0.2MeOH) were determined by X-ray crystallography. The antioxidant activity of the complexes was evaluated in regard to their ability to scavenge DPPH, hydroxyl and ABTS radicals and to inhibit *in vitro* the activity of soybean LOX. Additionally, the interaction of the complexes with calf-thymus (CT) DNA- and serum albumin (SA) was also investigated, since the interaction of the compounds with such biomolecules, which may also serve as biological targets, may reveal possible or alternative pathways for radical scavenging [48,49] and, subsequently the complexes could be considered as potentially effective drugs for the treatment of inflammation. Within this context, the interaction with CT DNA was studied directly by UV–vis spectroscopy and viscosity measurements and indirectly via the ethidium bromide (EB) displacement ability of the complexes from the EB-DNA compound by fluorescence emission spectroscopy, in order to determine the interaction mode and to calculate the DNA-binding constants of the complexes. The binding affinity of the complexes to bovine (BSA) and human serum albumin (HSA) was examined by fluorescence emission spectroscopy.

2. Experimental

2.1. Materials - instrumentation - physical measurements

Flufenamic acid, $ZnCl_2$, bipy, bipyam, phen, Hpko, KOH, trisodium citrate, NaCl, CT DNA, BSA, HSA, EB, DPPH, ABTS, sodium linoleate, butylated hydroxytoluene (BHT), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox) and nordihydroguaiaretic (NDGA) 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.

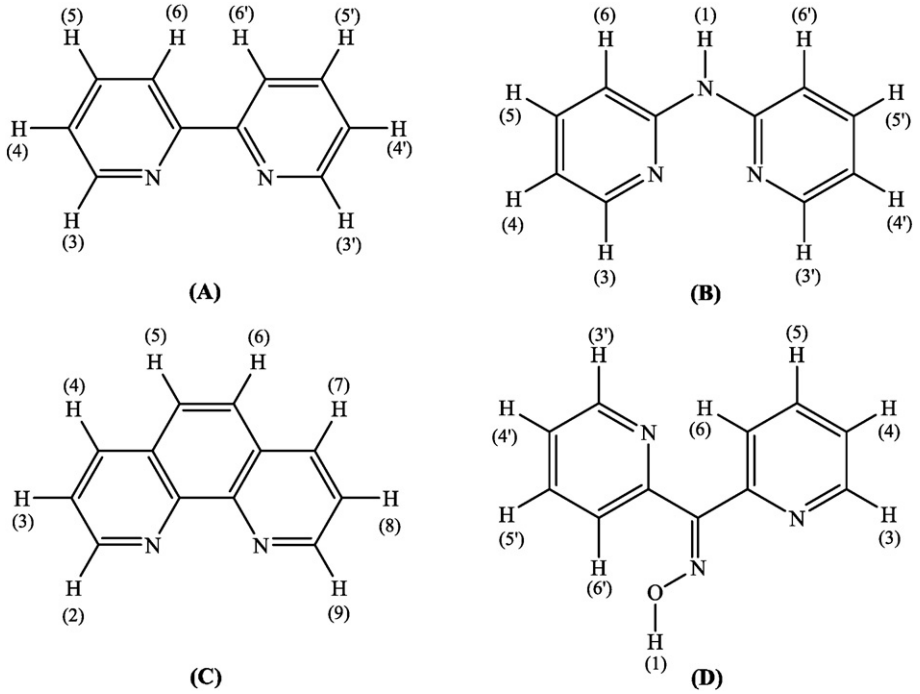

Fig. 2. The syntax formula and labeling of the H atoms of (A) bipy, (B) bipyam, (C) phen and(D) Hpko.