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# Antioxidant activity and interaction with DNA and albumins of zinc–tolfenamato complexes. Crystal structure of [Zn(tolfenamato)$_2$(2,2′-dipyridylketoneoxime)$_2$]

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ABSTRACT

The zinc(II) complex of the non-steroidal anti-inflammatory drug tolfenamic acid (=Htolf) in the presence of 2,2′-dipyridylketone oxime (=Hpko) as a *N*,*N*′-donor heterocyclic ligand, [Zn(tolf-O)$_2$(Hpko-*N*,*N*′)$_2$]·MeOH (=**1**·MeOH), has been synthesized and characterized by physicochemical techniques including X-ray crystallography. The complex exhibits good binding affinity to human or bovine serum albumin with high binding constant values. Complex **1** and previously reported Zn-tolfenamato complexes were tested for their free radical scavenging activity and *in vitro* inhibitory activity against soybean lipoxygenase and exhibited significant activity with [Zn(tolf)$_2$(1,10-phenantroline)] being the most active compound. The complexes interact with calf-thymus (CT) DNA via intercalation, and can displace the DNA-bound ethidium bromide with **1** exhibiting the highest binding constant to CT DNA.



## 1. Introduction

Zinc is the second most abundant trace element in the human body, has a major regulatory role in the metabolism of cells [1] and presents many beneficial effects to human health, while changes in its metabolism or trafficking are related to some diseases [2,3]. Zinc is extensively used to treat children suffering from deadly diarrhea resulting in significant reduction of child mortality in many countries of Asia and Africa [4]. The role of zinc in nucleic acid chemistry is noteworthy since zinc(II) ions are the only metal ions able to facilitate the rewinding of molten DNA [5]. In the literature diverse zinc compounds have exhibited a potential biological activity with zinc complexes with drugs being tested for the treatment of Alzheimer disease [6] and others showing antibacterial [7,8], anticonvulsant [9], antidiabetic [10], anti-inflammatory [11], antioxidant [12] and antiproliferative-antitumor [8,11] activity.

Non-steroidal anti-inflammatory drugs (NSAIDs)[†] are among the most frequently used drugs as analgesics, anti-inflammatories and antipyretics with known side-effects (mainly gastrointestinal such as gastric ulceration, nausea, diarrhea and renal including salt and fluid retention, hypertension, and in rare cases interstitial nephritis, acute renal failure) [13]. The inhibition of the cyclo-oxygenase (COX)-mediated production of prostaglandins is the main mode of action of the NSAIDs [14]. They have also shown a synergistic role on the activity of certain antitumor drugs [15] and have presented antitumor activity leading to cell death of a series of cancer cell lines via apoptotic pathways [16] or via diverse molecular

*Abbreviations:* ABTS, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cation; BHT, butylated hydroxytoluene; bipy, 2,2′-bipyridine; BSA, bovine serum albumin; COX, cyclo-oxygenase; CT, calf-thymus; DMF, *N*,*N*-dimethylformamide; DMSO, dimethylsulfoxide; DPPH, 1,1-diphenyl-picrylhydrazyl; EB, ethidium bromide, 3,8-diamino-5-ethyl-6-phenyl-phenanthridinium bromide; Hpko, 2,2′-dipyridylketone oxime; Htolf, tolfenamic acid, 2-[(3-chloro-2-methylphenyl)amino]benzoic acid; HSA, human serum albumin; LOX, soybean lipoxygenase; NDGA, nordihydroguaiaretic acid; NSAID, non-steroidal anti-inflammatory drug; phen, 1,10-phenanthroline; RA, DPPH radical scavenging activity; SA, serum albumin; tolf, tolfenamato, anion of tolfenamic acid.

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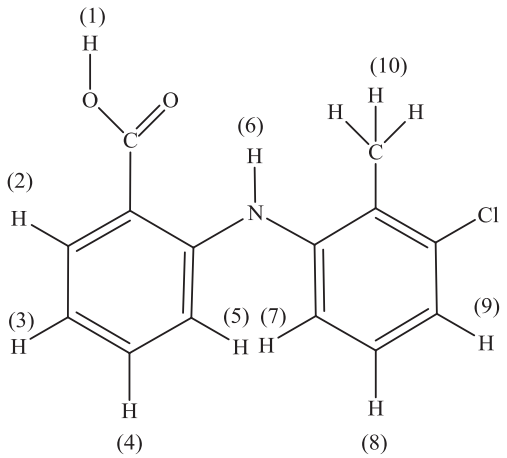
mechanisms [17] where free radicals have been also involved [18]. In an attempt to investigate potential mechanisms of the anticancer as well as the anti-inflammatory activity of the NSAIDs and their complexes, the interaction with DNA as well as the antioxidant activity are considered of great importance and should be further evaluated; it is noteworthy that only few relevant reports on the interaction of NSAIDs and their complexes with DNA have been published so far [19,20]. Furthermore, the number of structurally characterized zinc complexes with NSAIDs [21] is still expanding including an aspirinate complex [22], a flufenamato complex [23] and a series of complexes with indomethacin [8], mefenamic acid [12] and tolfenamic acid [24] as ligands.

Tolfenamic acid (=Htolf, Scheme 1) is a NSAID belonging to the *N*-phenylanthranilic acid derivatives with similar properties to mefenamic acid and flufenamic acid and other fenamates in clinical use [25]. Htolf is found in diverse analgesic, anti-inflammatory, antipyretic and antirheumatoid drugs and is also used for veterinary purposes [26]. The crystal structures of a series of tin(IV) [27], copper(II) [28,29], cobalt(II) [30], and zinc(II) [24] complexes with tolfenamato ligands have been reported in the literature.

Taking into consideration the significance of NSAIDs in medicine and the presence of zinc in diverse drugs, we have synthesized and characterized the structure and the spectroscopic (IR, UV and $^1$H NMR) properties of the neutral zinc(II) complexes with the NSAID tolfenamic acid in the presence of the *N*,*N*′-donor heterocyclic ligand 2,2′-dipyridylketone oxime (=Hpko). The crystal structure of the resultant complex [Zn(tolf-O)$_2$(Hpko-*N*,*N*′)$_2$]·MeOH, **1**·MeOH has been determined by X-ray crystallography. Furthermore, the $^1$H NMR spectra of the recently reported Zn-tolfenamato complexes [Zn(tolf)(phen)Cl] (**2**), [Zn(tolf)(bipy)Cl], (**3**), [Zn(tolf)$_2$(phen)], (**4**), [Zn(tolf)$_2$(bipy)], (**5**) and [Zn$_3$(tolf)$_6$(MeOH)$_2$] (**6**) (where phen and bipy are the *N*,*N*′-donor heterocyclic ligands 1,10-phenanthroline and 2,2′-bipyridine, respectively) [24] have been recorded and evaluated. In an attempt to further investigate the existence of potential anti-inflammatory activity of complexes **1**–**6**, the study of their biological properties has been focused on (i) their antioxidant capacity by investigating their scavenging ability of 1,1-diphenyl-picrylhydrazyl (DPPH) and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS$^{+}$•) radicals, their ability to antagonize DMSO in hydroxyl radicals (•OH) binding, as well as their *in vitro* inhibitory activity against soybean lipoxygenase (LOX), because the use of NSAIDs in medicine as anti-inflammatories is also related to free radicals scavenging, (ii) the affinity of the complexes to bovine (BSA) and human serum albumin (HSA), as characteristic serum proteins, investigated by fluorescence spectroscopy since the binding to these proteins which 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 may propose new paths for drug transportation [31], (iii) their binding properties with calf-thymus (CT) DNA investigated by UV spectroscopy and viscosity measurements and ethidium bromide (EB) displacement ability from the EB-DNA compound performed by fluorescence spectroscopy (EB is a typical DNA-intercalator and the competition with it for the DNA intercalation sites may serve as an indirect evidence of potential intercalation).




**Scheme 1.** Tolfenamic acid (=Htolf) with H atom numbering in accordance to $^1$H NMR proton's assignment.

## 2. Results and discussion

### 2.1. Synthesis and spectroscopic characterization

The synthesis of complex **1** was achieved in high yield via the aerobic reaction of tolfenamic acid, deprotonated by KOH, with $ZnCl_2$ in the presence of the *N*,*N*′-donor heterocyclic ligand Hpko in a ratio $Zn^{2+}$:tolf$^-$:Hpko of 1:2:2. The complex is stable in air, soluble in DMSO and DMF and non-electrolyte in DMSO (for 1 mM solution, 15 μS/cm). Complex **1** was characterized by elemental analysis, IR, UV and $^1$H NMR spectroscopic techniques and by X-ray crystallography. Additionally, the $^1$H NMR spectra of compounds **2**–**6** were recorded and evaluated.

IR spectroscopy is a useful tool to confirm the deprotonation and the binding mode of tolfenamic acid. In the IR spectrum of Htolf, the absorption band at 3355(br,m) cm$^{-1}$, attributed to the $\nu$(H–O) stretching vibration has disappeared upon binding to the zinc ion. The strong bands appearing at 1661 cm$^{-1}$ and 1265 cm$^{-1}$ which were attributed to $\nu$(C=O)$_{carboxylic}$ and $\nu$(C–O)$_{carboxylic}$ stretching vibrations of the carboxylic moiety (-COOH) of Htolf, respectively, have shifted, in the IR spectra of complex **1**, at 1584 cm$^{-1}$ and 1387 cm$^{-1}$ assigned to antisymmetric, $\nu_{asym}$(C=O), and symmetric, $\nu_{sym}$(C=O), stretching vibrations of the coordinated carboxylato group, respectively. The parameter Δ [=$\nu_{asym}$(C=O) − $\nu_{sym}$(C=O)] which is a useful characteristic tool for determining the coordination mode of the carboxylato ligands, has a value of 197 cm$^{-1}$ that is indicative of asymmetrically monodentate binding mode of the tolfenamato ligands [24,30].

$^1$H NMR spectroscopy can be considered a valuable tool to study the behavior of complexes in solution. The recorded $^1$H NMR spectra of compounds **1**–**6** in DMSO-$d_6$ solution confirm the formulae and the purity of the prepared compounds as well as the integrity of each complex in solution. More specifically, in the $^1$H NMR spectra of complexes **1**–**6**, the absence of the signal at 13.10 ppm assigned to carboxylic hydrogen of the free tolfenamic acid [32] confirms the deprotonated mode of the bound drug, while the rest signals of the tolfenamato ligand are slightly shifted downfield or upfield as expected upon binding to zinc metal ion [12,23,33,34]. Furthermore, all sets of signals related to the presence of the *N*-donor ligands are present and the ratios of integrated peaks confirm the ratio of ligands in the solid state, e.g. representatively for **1**, **2** and **4** in Fig. 1: (i) four signals for bipy or phen ligands and (ii) seven signals for Hpko ligands; six of them are attributed to aromatic hydrogen atoms and the seventh signal at 12.21 ppm is assigned to the oxime hydroxyl proton confirming that the Hpko ligand is bound in its non-deprotonated state. The absence of any additional sets of signals related to dissociated ligands is in agreement with the molecular conductance measurements and suggests that all complexes remain intact in solution [35–37].

The UV–vis spectra of complex **1** were recorded as nujol mull and in DMSO solution and were similar suggesting that it retains its structure in solution. Additionally, in order to explore the stability