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Nickel(II) complexes of the non-steroidal anti-inflammatory drug tolfenamic acid: Synthesis, structure, antioxidant activity and interaction with albumins and calf-thymus DNA

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ABSTRACT

The reaction of $NiCl_2{\cdot}6H_2O$ with the non-steroidal anti-inflammatory drug tolfenamic acid (Htolf) in the absence or presence of a nitrogen-donor heterocyclic ligand such as 2,2′-bipyridine (bipy), 1,10-phenanthroline (phen), 2,2′-bipyridylamine (bipyam), 2,2′-dipyridylketone oxime (Hpko) and pyridine (py) led to the formation of six novel Ni(II) mononuclear complexes. The complexes were characterized by physicochemical and spectroscopic techniques and the crystal structures of complexes $[Ni(tolf\text{-}O)_2(bipy)(MeOH)_2]$, **2** and $[Ni(tolf\text{-}O)_2(Hpko\text{-}N,N')_2]$, **5** were determined by X-ray crystallography. The *in vitro* investigation of 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 revealed their potential antioxidant activity. The interaction of the complexes to calf-thymus DNA was monitored by diverse techniques (UV spectroscopy, cyclic voltammetry, viscosity measurements) revealing intercalation as the most possible mode of binding. Competitive studies of the complexes with ethidium bromide were monitored by fluorescence emission spectroscopy. The interaction of the complexes with serum albumins was studied by fluorescence emission spectroscopy and the binding constants of the compounds to the albumins were calculated.

1. Introduction

Nickel was considered for many years an element without any important biological significance, despite its presence in the early stages of life evolution [1]. The identification of the presence of Ni in the active center of the enzyme urease in 1975 [2] was the initial incentive to draw the interest of bioinorganic chemists and make them reconsider the importance of nickel in biological systems. Since then, the biological significance of nickel was gradually established, especially after the verification of the existence of nickel in more enzymes [3,4] and the biological activity shown by diverse Ni(II) complexes; among them, Ni(II) complexes with vitamins [5], antitumor antibiotics [6], anticonvulsant [7] or antiepileptic agents [8] as ligands have been reported, while the antibacterial [9,10], antifungal [10,11], antioxidant [12–14], antileishmanian [15] and antiproliferative activity towards diverse cell lines [16,17] shown by diverse Ni(II) complexes has been also evaluated. Furthermore, diverse nickel complexes have been reported as DNA-intercalators [18,19] or DNA-cleaving agents [20,21]. Concerning the Ni(II) complexes with NSAIDs, Ni(II)–diclofenac [22] and Ni–mefenamato [23] compounds have been prepared, characterized and biologically evaluated.

Tolfenamic acid (Htolf, Fig. 1(A)), as N-phenylanthranilic acid derivative, is a non-steroidal anti-inflammatory drug (NSAID) resembling to other fenamates (e.g. mefenamic acid, flufenamic acid) in clinical use [24]. Htolf is a painkiller and is usually used

Abbreviations: ABTS, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cation; BHT, butylated hydroxytoluene; bipy, 2,2′-bipyridine; bipyam, 2,2′-bipyridylamine; BSA, bovine serum albumin; CT, calf-thymus; DMF, N,N-dimethylformamide; DPPH, 1,1-diphenyl-picrylhydrazyl; EB, ethidium bromide, 3,8-diamino-5-ethyl-6-phenyl-phenanthridinium bromide; Htolf, tolfenamic acid, 2-[(3-chloro-2-metylphenyl)amino]benzoic acid; Hpko, 2,2′-dipyridylketone oxime; HSA, human serum albumin; *K*, SA-binding constant; K_b, DNA-binding constant; k_q, quenching constant; K_{SV}, Stern–Volmer constant; LOX, lipoxygenase; m, medium; NDGA, nordihydroguaiaretic acid; NSAID, non-steroidal anti-inflammatory drug; phen, 1,10-phenanthroline; *r*, [compound]/[DNA] ratio; *r*′, [DNA]/[compound] ratio; s, strong; SA, serum albumin; sh, shoulder; TEAP, tetraethylammonium perchlorate; tolf, tolfenamato anion; trolox, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid; vs, very strong; $\Delta\nu(CO_2)$, $\nu_{asym}(CO_2)-\nu_{sym}(CO_2)$.

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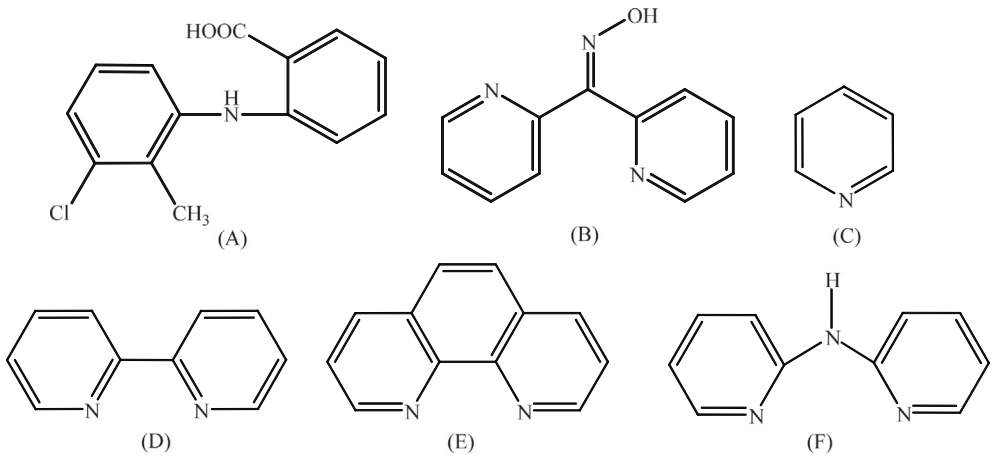

Fig. 1. The syntax formula of (A) Htolf, (B) Hpko, (C) py, (D) bipy, (E) phen and (F) bipyam.

to relief the pain of a migraine headache [25] and has been also approved for veterinary use [26]. Furthermore, the antiproliferative activity of tolfenamic acid has been also reported, including applications in colorectal, pancreatic, esophageal, and lung cancers [27–29]. The crystal structures of tin(IV) [30], copper(II) [31,32], cobalt(II) [33], zinc(II) [34,35] and manganese(II) [36] complexes with tolfenamato ligands have been already reported in the literature.

Free radicals are species having an important role in the inflammatory process [37] and are involved in mechanisms related to the antitumor activity exhibited by the NSAIDs [38]. 1,1-Diphenylpicrylhydrazyl (DPPH), hydroxyl (·OH) and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{+\cdot}$) radicals are related with known inflammatory results and are often used to investigate the potential antioxidant activity of compounds [39] which may have also a potential anticancer and anti-inflammatory activity [37]. DPPH scavengers may offer potential protection against rheumatoid arthritis and inflammation, since the scavenging of DPPH is mainly related to antiageing, anticancer and anti-inflammatory activity [39]. Hydroxyl radical (·OH) scavengers may activate the synthesis of prostaglandin and may be considered as protectors from reactive oxygen species and the scavenging of the $ABTS^{+\cdot}$ radicals is a marker of the total antioxidant activity [39]. Furthermore, the inhibitory activity of the enzyme lipoxygenase (LOX) is also an indirect index of the total antioxidant activity of the compounds and may serve as a preliminary step of the anti-inflammatory activity studies [40] and most LOX inhibitors are considered potential antioxidants or free radical scavengers [41,42].

Taking into consideration the importance of NSAIDs in medicine, the enhanced activity of their metal complexes and the continuous interest for nickel compounds and as a continuation of our research concerning the interaction of metal ions with NSAIDs [22,23,32–36,43–51], we present herein the synthesis of six novel Ni(II) complexes with tolfenamic acid in the absence or presence of the nitrogen-donor ligands 2,2′-bipyridine (bipy), 1,10-phenanthroline (phen), 2,2′-bipyridylamine (bipyam), 2,2′-dipyridylketone oxime (Hpko) or pyridine (py) (Fig. 1(B)–(F)). The reaction of Ni^{2+} with deprotonated tolfenamic acid resulted in the formation of complex $[Ni(tolf\text{-}O)_2(MeOH)_4]$ (**1**), while the co-existence of bipy, phen, bipyam, Hpko or py lead to the formation of the mononuclear complexes $[Ni(tolf\text{-}O)_2(bipy)(MeOH)_2]$ (**2**), $[Ni(tolf\text{-}O)_2(phen)(MeOH)_2]$ (**3**), $[Ni(tolf\text{-}O,O')_2(bipyam)]$ (**4**), $[Ni(tolf\text{-}O)_2(Hpko\text{-}N,N')_2]$ (**5**) and $[Ni(tolf\text{-}O)_2(py)_2(MeOH)_2]$ (**6**), respectively. The complexes were characterized by physicochemical (elemental analysis, molecular conductivity measurements and room temperature measurements) and spectroscopic (IR and UV–Vis) techniques, while the crystal structures of complexes $[Ni(tolf\text{-}O)_2(bipy)(MeOH)_2]$, **2** and $[Ni(tolf\text{-}O)_2(Hpko\text{-}N,N')_2]$, **5** were determined by X-ray crystallography.

The biological evaluation of complexes **1**–**6** includes: (i) the investigation of the *in vitro* ability of the complexes to scavenge DPPH, hydroxyl and ABTS radicals and to inhibit the activity of soybean LOX, (ii) the interaction of the complexes with calf-thymus (CT) DNA monitored by UV spectroscopy, cyclic voltammetry and viscosity measurements, in order to determine the interaction mode and to calculate the DNA-binding constants of the complexes, as well as their ability to displace ethidium bromide (EB) from the EB–DNA conjugate studied by fluorescence emission spectroscopy, (iii) the binding affinity of the complexes to bovine (BSA) and human serum albumin (HSA) examined by fluorescence emission spectroscopy. Such studies are usually closely related and may reveal novel biological targets or alternative pathways of the radical scavenging and the potential biological activity [38,52].

2. Materials, synthesis and methods

2.1. Materials – Instrumentation – Physical measurements

Tolfenamic acid, $NiCl_2{\cdot}6H_2O$, Hpko, bipy, phen, py, Hpko, bipyam, KOH, CT DNA, BSA, HSA, EB, NaCl, tetraethylammonium perchlorate (TEAP), trisodium citrate, DPPH, ABTS, linoleic acid sodium salt, 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 Chemlab. All chemicals and solvents were reagent grade and were used as purchased without any further purification. TEAP was recrystallized twice from ethanol and dried under vacuum, prior to its use.

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 1.85–1.88, indicating that the DNA was sufficiently free of protein contamination [53]. The DNA concentration was determined by the UV absorbance at 260 nm after 1:20 dilution using $\varepsilon = 6600\ M^{-1}\ cm^{-1}$ [54].