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Synthesis, crystal structures and spectroscopy of meclofenamic acid and its metal complexes with manganese(II), copper(II), zinc(II) and cadmium(II). Antiproliferative and superoxide dismutase activity

Dimitra Kovala-Demertzi [a,*], Malgorzata Staninska [a], Isabel Garcia-Santos [b], Alfonso Castineiras [b], Mavroudis A. Demertzis [a]

[a] Inorganic and Analytical Chemistry, Department of Chemistry, University of Ioannina, 45100 Ioannina, Greece
[b] Departamento de Quımica Inorganica, Universidad de Santiago de Compostela, E-15706 Santiago de Compostela, Spain

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ABSTRACT

Some new complexes of meclofenamic acid (N-(2,6-dichloro-m-tolyl)anthranilic acid), Hmeclo (**1**), with potentially interesting biological activities are described. Complexes [Mn(meclo)$_2$] (**2**), [Cu(meclo)$_2$(H$_2$O)$_2$] (**3**), [Zn(meclo)$_2$(H$_2$O)$_2$] (**4**) and [Cd(meclo)$_2$(H$_2$O)$_2$] (**5**) were prepared and structurally characterized by means of vibrational, electronic and ^{1}H and ^{13}C NMR spectroscopies. The crystal structure of complexes [Cu$_4$(meclo)$_6$(OH)$_2$(DMSO)$_2$]•2DMSO (**3a**) and [Cd(meclo)$_2$(DMSO)$_3$] (**5a**) have been determined by X-ray crystallography. Complex (**3a**) is a centrosymmetric tetramer built up around the planar cyclic Cu$_2$(OH)$_2$ unit. Complex **5a** is mononuclear seven-coordinated complex with the meclofenamato ligand behaving as a bidentate deprotonated chelating ligand. Intra and intermolecular hydrogen bonds stabilize these two structures, while the crystal packing is determined by π–π and C—H⋯π interactions. Meclofenamic acid and its metal complexes have been evaluated for antiproliferative activity *in vitro* against the cells of three human cancer cell lines, MCF-7 (breast cancer cell line), T24 (bladder cancer cell line), and A-549 (non-small cell lung carcinoma), and a mouse fibroblast L-929 cell line. Complex **5** exhibits the highest selectivity against MCF-7 and **4** shows the highest selectivity against T-24. Complexes **2**–**5** were found to be more potent cytotoxic agents against T-24 and complex **5** against MCF-7 cancer cell lines than the prevalent benchmark metallodrug, *cis-platin*. The superoxide dismutase activity was measured by the Fridovich test which showed that complex [Cu(meclo)$_2$(H$_2$O)$_2$] is a good superoxide scavenger.

1. Introduction

Meclofenamic acid (N-(2,6-dichloro-m-tolyl)anthranilic acid), HMeclo, is a non-steroidal anti-inflammatory drug (NSAID) from the family of fenamates with marked analgesic properties and is used in the treatment of osteoarthritis (OA), rheumatoid arthritis (RA) and other painful musculoskeletal diseases. Chemically, it resembles mefenamic and flufenamic acids and other fenamates in clinical use. The mode of action of the NSAIDs is attributed primarily to the inhibition of prostaglandins (PG) synthesis, and more specifically inhibition of the cyclo-oxygenase enzyme system, Cox, Cox-1 and Cox-2. Inhibition of the Cox-2 system results in anti-inflammatory action, while inhibition of the Cox-1 enzyme system results in anti-inflammatory action as well as gastric irritation [1]. However, accumulating evidence indicates that fenamates and meclofenamic acid also modulate a diversity of ion channels through a pathway that may be independent of the cyclooxygenase–prostaglandin mechanism [2]. It was found that meclofenamic acid results in the generation of potent and selective Cox-2 inhibitors [1]. New studies from the last years revealed that in addition to arthritis and pain, cancer and neurodegenerative diseases like Alzheimer's disease could potentially be treated with Cox-2 inhibitors [3,4]. Some researchers propose that NSAIDs, in addition to their inhibitory effects on the synthesis of PGs, may also inhibit the production, or act as scavengers of free radicals [5–8].

Metal complexes with active drugs as ligands are a research area of increasing interest for inorganic and medicinal chemistry and have concentrated much attention as an approach to new drug development [9,10]. The synthesis of metal complexes with NSAIDs as ligands has acquired new impetus in the past decade. The information collected from the preparative, structural and reactivity studies have high significance for several fields which span from the bio-sciences to the material sciences. The combination of two or more different species into the same compound may bring to a multi-therapeutic agent which can be expanded by the synergic action of the metal residue once the co-ordination compound dissociates inside the target tissue [12,13,15,17].

* Corresponding author. Tel.: +30 2651008425; fax: +30 2651008791.
E-mail address: dkovala@cc.uoi.gr (D. Kovala-Demertzi).

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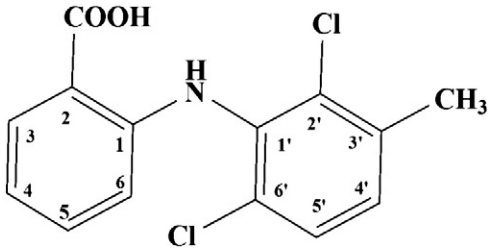
The coordination chemistry of NSAIDs has been studied by several groups worldwide. Some complexes have increased pharmaceutical or biological activity with respect to the drug or are interesting from a purely chemical point of view [10–17]. A review of the synthesis and crystal structures of a number of anti-inflammatory compounds as ligands in organotin complexes and a review of copper complexes with NSAIDs have been reported [6,10,14,16]. Crystal structures of organotin meclofenamic complexes were reported by our group. The [Ph_3Sn(Meclo)] complex exhibited high cytotoxic activity against cancer cell lines and was found to be a promising anti-mycobacterial lead compound, displaying high activity against M. tuberculosis H37Rv [17].

Copper(II)-complexes, including Cu-NSAIDs, exhibit significant anti-inflammatory activities as well as superoxide dismutase (SOD) mimetic activity. SODs, a family of metalloenzymes, catalyses the dismutation of superoxide radical anions to the non-radical products oxygen and hydrogen peroxide and protects living cells from damage induced by reactive oxygen species (ROS). The use of SOD as a pharmaceutical has been proposed for treatment of a number of diseases including, hyperoxia, reperfusion injury, acquired immune deficiency syndrome (AIDS) disease auto-immune deficiency disease (AIDS), ulcerative colitis, bronchopulmonary dysplasia in premature neonates, as well as inflammation and inflammation-associated diseases, such as rheumatoid arthritis (RA) and osteoarthritis (OA) [6]. Although there is much interest in the use of SOD for pharmaceutical purposes, its practical use in biological systems is problematic due to difficulties associated with the systematic infection of protein, i.e. the circulation lifetime, cell impermeability, immunogenicity, tissue targeting, antigenicity and high costs. To avoid such limitations, there has been considerable interest in developing synthetic SOD mimics that have low molecular weight, biological stability, and membrane permeability and are nontoxic and cost-effective [6,18,19]. However, the development of new synthetic compounds that act as mimetics of SOD and catalase has offered an alternative approach with some promise. Use of such compounds in mouse models has been effective in attenuating oxidative stress-associated disease processes and leads to extension of lifespan [20,21].

We have prepared novel complexes of meclofenamic acid, Scheme 1, with Mn(II), Cu(II), Zn(II) and Cd(II), [$Mn(meclo)_2$], [$Cu(meclo)_2(H_2O)_2$], [$Zn(meclo)_2(H_2O)_2$], and [$Cd(meclo)_2(H_2O)_2$] in order to obtain information on structure–activity relationships for systems involving metal atoms. The cytotoxic activity of meclofenamic acid and its metal complexes have been evaluated for antiproliferative activity *in vitro* against the cells of three human cancer cell lines: MCF-7 (human breast cancer cell line), T24 (bladder cancer cell line), A-549 (non-small cell lung carcinoma) and a mouse L-929 (a fibroblast-like cell line cloned from strain L). Also, the superoxide dismutase activity was measured and IC_{50} value was determined by the Fridovich test [22]. Herein, the goal is to define the probability to extend the pharmacological profile of meclofenamic acid, in order to discover new properties such as anti-cancer activity and SOD activity, to prepare new compounds, complexes of meclofenamic acid with essential metal ions, which probably would exhibit improved or different biological and pharmaceutical behaviour compared to the "parent drug", meclofenamic acid.



Scheme 1. The numbering scheme for Hmeclo, **1**.

2. Experimental section

2.1. General and instrumental

All reagents were commercially available (Aldrich or Merck) and used as supplied. Melting points were determined in open capillaries and are uncorrected. Infrared and far-infrared spectra were recorded on a Perkin Elmer Spectrum GX Fourier transform spectrophotometer using KBr pellets (4000–400 cm^{-1}) and nujol mulls dispersed between polyethylene disks (400–40 cm^{-1}). NMR spectra were recorded on a Bruker AV-400 spectrometer operating at 400 and 100 MHz for ^1H and ^{13}C acquisition, respectively, or on a Bruker AV-250 spectrometer operating at 250.13 and 62.90 MHz for ^1H and ^{13}C acquisition respectively. The spectra were acquired at room temperature (298 K). The splitting of proton resonances in the reported ^1H NMR spectra are defined as s = singlet, d = doublet, t = triplet, and m = multiplet. The chemical shifts are reported in ppm for ^1H and ^{13}C NMR. Samples were dissolved in $CDCl_3$ or DMSO-d^6 and spectra were obtained at room temperature with the signal of the free DMSO or $CHCl_3$ (at 2.49 and 7.24 ppm, respectively) as a reference. Elemental analyses, C, H, N and S were performed on a Carlo Erba EA (model 1108). Mass spectra were measured on an Agilent 1100 Series LC-MSD-Trap-SL spectrometer. Experimental and calcd. MS (ESI-MS) values (m/z) for each of the five compounds were identical to a significant last figure above the decimal point. Metal compositions were determined using an energy-dispersive X-ray fluorescence (EDXRF) spectroscopy arrangement.

2.2. Preparation of HMeclo and the complexes

2.2.1. Synthesis 2-[(2,6-Dichloro-3-methyl-phenyl)amino]benzoic acid (HMeclo); (**1**)

Meclofenamic was synthesized according to a published procedure from (2-carboxyphenyl)phenyliodonium and 2,6-dichloro-3-methylbenzenamine [23]. The white power was collected and recrystallized three times from ethanol to afford **1**, Yield 70%. M.p. 252–253 °C. IR (KBr): 3336 (ν(NH)); 3060br (ν(OH)); 2920, 2860 ($\nu(CH_3)$); 1656 (ν_{asym}(COO)); 1438(ν_{sym}(COO)); UV–visible (UV–vis): λ, nm (ε/logε) in DMF: 319 (3.66), 291 (3.64); in $CHCl_3$: 340 (3.53), 282 (3.49); ^1H NMR (DMSO): 13.14 s (OH); 9.15 (*s*, NH); 7.90 (*dd*, H–C(3), $J_{H(3)-H(4)} = 8.0$, $J_{H(3)-H(5)} = 1.0$); 6.77 (*t*, H–C(4), $J_{H(4)-H(5)} = 8.0$, $J_{H(4)-H(6)} = 1.1$); 7.31 (m, H–C(5)), 6.19 (*d*, H–C(6), $J_{H(6)-H(5)} = 8.4$); 7.35 (d, H4′), $J_{H(4')-H(5')} = 8.3$); 7.50 (d, H5′), $J_{H(5')-H(4')} = 8.3$); 2.38 (s, 3′ Me); ^{13}C NMR: 170.0 (COOH); 147.1 (C(1)); 111.7 (C(2)); 131.5 (C(3)); 117.3 (C(4)); 134.6 (C(5)); 112.9 (C(6)); 134.1 (C(1′)); 133.5 (C(2′)); 130.5 (C(3′)); 129.3 (C(4′)); 128.8 (C(5′)); 130.6 (C(6′)); 20.1 (3′ Me); ^1H NMR ($CDCl_3$): 9.21 (*s*, NH); 8.07 (*d*, H–C(3), $J_{H(3)-H(4)} = 6.7$); 6.79 (*t*, H–C(4), $J_{H(4)-H(5)} = 7.6$, $J_{H(4)-H(6)} = 1.1$); 7.22 (m, H–C(5)), 6.33 (*d*, H–C(6), $J_{H(6)-H(5)} = 8.4$); 7.14 (d, H4′), $J_{H(4')-H(5')} = 8.3$); 7.32 (d, H5′), $J_{H(5')-H(4')} = 8.2$); 2.41 (s, 3′ Me); ^{13}C NMR: 172.1 (COOH); 148.5 (C(1)); 110.4 (C(2)); 132.3 (C(3)); 117.4 (C(4)); 134.9 (C(5)); 113.1 (C(6)); 135.1 (C(1′)); 134.7 (C(2′)); 131.6 (C(3′)); 128.8 (C(4′)); 127.8 (C(5′)); 136.6 (C(6′)); 20.6 (3′ Me); Mass spectrum, MS (electrospray ionization, ESI, m/z): 297 $[\mathbf{1}+H]^+$, 612$[\mathbf{1_2}+H_3O]^+$. Anal. calc. for $C_{14}H_{11}Cl_2NO_2$ (296.13 g mol^{-1}): C, 56.8; H, 3.7; N, 4.7; found: C, 56.2, H, 3.9; N, 4.3%.

2.2.2. Synthesis of [Mn(meclo)$_2$]; (**2**)

Anhydrous manganese(II) acetate ([$Mn(CH_3COO)_2$]) (0.0216 g, 0.125 mM) was dissolved in methanol (2 mL) and this solution was added to a solution of meclofenamic acid (0.0740 g, 0.25 mM) in methanol (2 mL). Drops of triethylamine ($N(eth)_3$) were added to the solution till the apparent pH value was ~7. The reaction mixture was stirred for 4 h at room temperature and then it was left to the refrigerator overnight. Drops of cold distilled water were added to the solution and a whitish precipitate was collected by filtration. The powder was filtered and washed with cold MeOH/H_2O 1/1 (1:1), and