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In vitro and *in silico* study of the biological activity of manganese(III) inverse-[9-MC-3]-metallacrowns and manganese(II) complexes with the anti-inflammatory drugs diclofenac or indomethacin

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ABSTRACT

In the present contribution, the biological properties of four manganese complexes with the non-steroidal anti-inflammatory drugs sodium diclofenac (Nadicl) or indomethacin (Hindo) in the presence or absence of salicylaldoxime (H_2sao), *i.e.* $[Mn_6(O)_2(dicl)_2(sao)_6(CH_3OH)_6]$ **1**, $[Mn_6(O)_2(indo)_2(sao)_6(H_2O)_4]$, **2**, $[Mn(dicl)_2(CH_3OH)_4]$, **3**, and $[Mn(indo)_2(CH_3OH)_4]$, **4** are presented. More specifically, the *in vitro* cytotoxic effects of the complexes were evaluated against three cancer cell lines (HeLa, MCF-7 and A549 cells) as well as their combinatory activity with the well-known chemotherapeutic drugs irinotecan, cisplatin, paclitaxel and 5-fluorouracil. The biological activity of the complexes was investigated *in vitro* by studying their affinity to calf-thymus DNA and their binding towards bovine or human serum albumin (HSA). Molecular docking simulations on the crystal structure of HSA and human estrogen receptor alpha (hERa) were employed in order to study *in silico* the ability of the studied complexes to bind to these proteins.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used as analgesic, anti-inflammatory and antipyretic medicaments [1], although they may induce renal and gastrointestinal side-effects, such as ulceration and hemorrhage [2,3]. The main mode of action of the NSAIDs is the inhibition of the production of prostaglandins which is mediated by the enzymes cyclooxygenase-1 and cyclooxygenase-2 [4,5]. Additionally, the NSAIDs have presented synergetic activity with diverse antitumor drugs [5] and have also exhibited themselves noteworthy antitumor activity inducing the cell death of cancer cell lines [6–8]. Within this context, DNA is also a biological target of the NSAIDs and their compounds and the study of this interaction is an initial approach of potential anti-inflammatory and anticancer activity [9–11]. Indomethacin (Hindo, Fig. 1(A)) and sodium diclofenac (Nadicl, Fig. 1(B)) are commonly used anti-inflammatory, analgesic and antipyretic agents that belong to the phenylalkanoic acid derivatives used as NSAIDs [12–14]. Indomethacin and its copper(II) complex are clinically used mainly in the treatment of acute inflammation [14]. In the literature, the structures of copper(II) [15–18], nickel(II) [19] and tin(IV) complexes [20] with indomethacin have been found. The sodium salt of diclofenac is used in the treatment of rheumatoid arthritis and osteoarthritis [12]. Considering the metal-diclofenac complexes, reports on the Cu(II) [21–24], Mn(II) [25,26], Cd(II) [27], Sn(IV) [28] and Ni(II) [29] complexes have been found in the literature.

Since the discovery of cytotoxic properties of cisplatin by Prof. Rosenberg fifty years ago [30], the research concerning metallopharmaceuticals has been mainly focused on the platinum complexes which have been proven to be among the most effective chemotherapeutic agents, despite their known side-effects [31–35]. In parallel, mononuclear Ru complexes such as NAMI-A ([HIm][*trans*-$RuCl_4$(dmso-S)(Im)], Im = imidazole), KP1019 (*trans*-[Ru(III)Cl_4(1H-indazole)$_2$]) and those of RAPTA (Ru(II)-arene 1,3,5-triaza-7-phosphaadamantane) series have been thoroughly investigated for the cytotoxic efficacy [36,37]. In addition, polynuclear metal-based drugs have been also examined for their potential cytotoxicity [38–41], *e.g.* the platinum chemotherapeutic AP5346. Furthermore, there are many recent reports in the literature regarding the cytotoxic properties of diverse polynuclear or polymeric complexes with diverse metal ions, such as Mn(II) [42], Co(II) [43], Cu(II) [44], Zn(II) [45,46], Sn(IV) [47] and Ru(II) [48].

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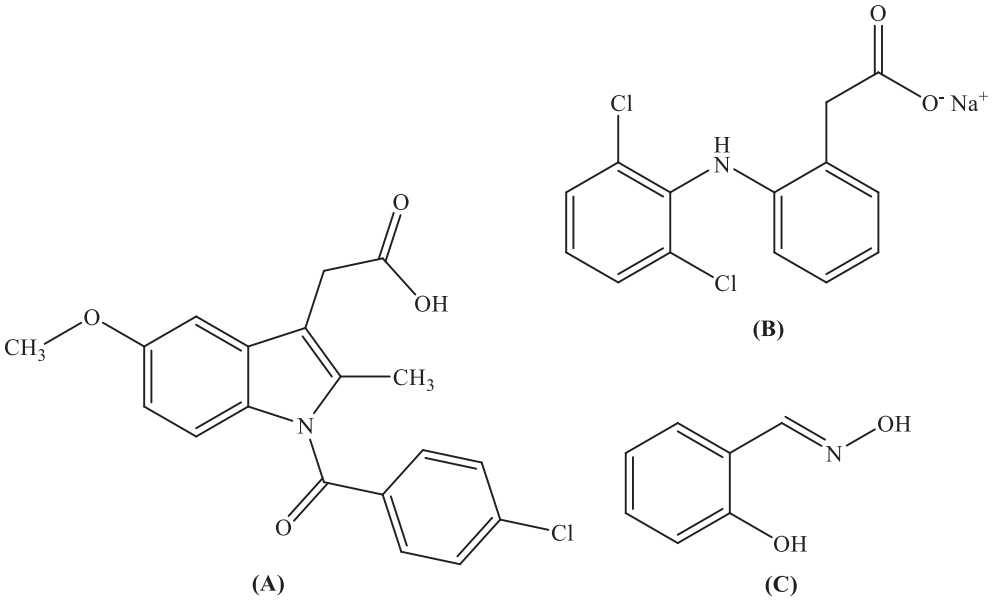

Fig. 1. The syntax formula of (A) indomethacin (Hindo), (B) sodium diclofenac (Nadicl) and (C) salicylaldoxime (H_2sao).

Manganese is present in the active centre of many important enzymes; thus, it is considered a necessary bioelement [49, 50]. Additionally, the anticancer agent SC-52608 and the MRI (magnetic resonance imaging) contrast agent Teslascan are the most common manganese compounds used in medicine [51]. There are many reports in the literature with promising results concerning the *in vitro* anticancer [51–57], antimicrobial [58–61], antifungal [62] and antioxidant [26,63] activity of diverse manganese complexes. In regard to the reported manganese complexes with NSAIDs as ligands, these refer to its complexes with the NSAIDs the anthranilic acid derivatives tolfenamic acid [63], mefenamic acid [64,65], niflumic acid [66] and the phenylalkanoic acids diclofenac [25,26,67] and indomethacin [67].

Metallacrowns (MCs) are a class of polynuclear complexes bearing a cyclic structure formed by a repeating [-O-N-M-] pattern and are similar to crown ethers with the methylene carbons being replaced by transition metal ions (M) and nitrogen atoms [68,69]. The metallacrowns are classified to: (i) inverse MCs, when the metal atoms are located towards the centre of the MC cavity and so MCs may encapsulate anions and (ii) regular MCs, when the oxygen atoms are found towards the centre of the MC cavity and so MCs may host metal cations [68,69]. Ligands able to contribute to the formation of the MC ring include hydroxamic acids and/or oximes. Salicylaldoxime (H_2sao, Fig. 1(C)) is an oxime which, when it is doubly-deprotonated, may act as a tridentate binucleating ligand providing its nitrogen atom and its oximato oxygen atom for the formation of the metallacrown ring [65,67].

In a previous contribution [67], we have reported the synthesis, the structure, the spectroscopic and magnetochemical properties of the hexanuclear complexes $[Mn_6(O)_2(dicl)_2(sao)_6(CH_3OH)_6]$, **1** and $[Mn_6(O)_2(indo)_2(sao)_6(H_2O)_4]$, **2** which can be also considered as *inverse*-[9-MC-3]$_2$ metallacrowns and the mononuclear complexes $[Mn(dicl)_2(CH_3OH)_4]$, **3** and $[Mn(indo)_2(CH_3OH)_4]$, **4**. We report herein the *in vitro*: (i) cytotoxic activity of complexes **1–4** against HeLa (cervical), MCF-7 (breast) and A549 (lung adenocarcinoma) cancer cell lines as well as their combination effect with a series of well-known chemotherapeutic drugs (irinotecan, cisplatin, paclitaxel and 5-fluorouracil), (ii) interaction of complexes **1–4** with calf-thymus (CT) DNA investigated by UV–vis spectroscopy, DNA-viscosity measurements and competitive studies with ethidium bromide (EB) and (iii) interaction of complexes **1–4** with bovine (BSA) or human (HSA) serum albumins studied by fluorescence emission spectroscopy. The interaction of complexes **1–4** with CT DNA, BSA and HSA has been studied in an attempt to evaluate the interaction of the complexes with biomolecules. Furthermore, *in silico* approaches with the employment of molecular docking were adopted in an attempt to provide information for the understanding of the ability of complexes **1–4** for transportation through HSA and, thus, possible interaction with other protein targets involved in various diseases, such as hERa (human estrogen receptor alpha) which is present in MCF-7 hormone-dependent human breast cancer cells that was proved to be the most sensitive of the studied cancer cells.

2. Experimental

2.1. Materials and instrumentation

Sodium diclofenac, indomethacin, salicylaldoxime, CT DNA, BSA, HSA, ethidium bromide (EB), NaCl and trisodium citrate were purchased from Sigma-Aldrich Co and all solvents were purchased from Chemlab. All chemicals and solvents were reagent grade and were used as purchased without any further purification. The synthesis of complexes **1–4** has been already reported in the literature [67].

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 1.89, indicating that the DNA was sufficiently free of protein contamination [70]. The DNA concentration was determined by the UV–vis absorbance at 260 nm after 1:20 dilution using $\varepsilon = 6600\ M^{-1}\ cm^{-1}$ [71].

UV–vis spectra were recorded on a Hitachi U-2001 dual beam spectrophotometer. Fluorescence spectra were recorded in solution on a Hitachi F-7000 fluorescence spectrophotometer. Viscosity experiments were carried out using an ALPHA L Fungilab rotational viscometer equipped with an 18 mL LCP spindle.

Infusion experiments were performed by using a ThermoFisher Scientific (Bremen, Germany) model LTQ Orbitrap Discovery MS. All solutions introduced into the ESI source of the MS at a flow rate of 3 μL/min by using an integrated syringe pump. The infusion experiments were conducted using a standard ESI source operating in positive ionization mode. Source operating conditions were: 3.7 kV spray voltage, 275 °C heated capillary temperature, 5 psi sheath gas pressure. Full scan MS analysis was performed in profile mode using the Orbitrap mass