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DNA binding, nuclease activity, DNA photocleavage and cytotoxic properties of Cu(II) complexes of N-substituted sulfonamides

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ABSTRACT

Ternary copper(II) complexes $[Cu(NST)_2(phen)]$ (**1**) and $[Cu(NST)_2(NH_3)_2]\cdot H_2O$ (**2**) [HNST = N-(4,5-dimethylthiazol-2-yl)naphthalene-1-sulfonamide] were prepared and characterized by physico-chemical techniques. Both **1** and **2** were structurally characterized by X-ray crystallography. The crystal structures show the presence of a distorted square planar CuN_4 geometry in which the deprotonated sulfonamide, acting as monodentate ligand, binds to the metal ion through the thiazole N atom. Both complexes present intermolecular π–π stacking interactions between phenanthroline rings (compound **1**) and between naphthalene rings (compound **2**). The interaction of the complexes with CT DNA was studied by means of thermal denaturation, viscosity measurements and fluorescence spectroscopy. The complexes display good binding propensity to the calf thymus DNA giving the order: **1** > **2**. Complex **1**, which has a higher capability for binding to DNA, showed better nuclease activity than **2** in the presence of ascorbate/H_2O_2. Both the kinetics and the mechanism of the DNA cleavage reaction were investigated. Furthermore, complex **1** showed efficient photo-induced DNA cleavage activity on irradiation with UV light in the absence of any external reagent. The UV light induced DNA cleavage follows a photo-redox pathway with generation of hydroxyl radicals as reactive species. In addition, the cytotoxic properties of both complexes (**1** and **2**) were evaluated in human cancer cells (HeLa, Caco-2 and MDA-468). The low IC_{50} values, in particular those against Caco-2, have indicated that the compounds can be considered as promising chemotherapeutic agents.

1. Introduction

Since DNA was identified as the primary molecular target of metal based anticancer drugs, the interaction mode of metal complexes with DNA and the potential of these complexes to act as chemotherapeutic agents have been analyzed [1]. Adducts formed between metal complexes and DNA range from covalently to no covalently bound. Non-covalent DNA binding metal complexes, particularly those that are able to intercalate between DNA base pairs, have received much attention in the development of efficient anticancer drugs [2].

Metal complexes can promote the cleavage of DNA by targeting its basic constituents (base and/or sugar) by an oxidative pathway or by hydrolysis of phosphodiester linkages. Oxidative cleavage of DNA can take place by chemical or photochemical means. Metal complexes that act as oxidative chemical nucleases require a reducing agent (i.e. ascorbic acid or 3-mercaptopropionic acid) and/or H_2O_2 to produce reactive oxygen species (ROS) after the reduction of the metallic center. Photo-sensitization of transition metal complexes can promote DNA damage following the singlet oxygen (type-2) or/and photo-redox pathways [3]. In some cases, photo-reduction of metal complexes is an important step in DNA cleavage reactions and constitutes the first step in the photocleavage process [4–6].

Transition metal complexes, in particular Cu(II) compounds, with tunable coordination environments and versatile spectral and electrochemical properties offer a greater scope of design for species that are suitable for the photocleavage of DNA. In this sense the rational synthesis of copper complexes able to be photo-activated with UV or visible light represents an interesting tool for photodynamic therapy (PDT) [7]. PDT is used as a noninvasive treatment of cancer that involves a photoexcitation of a drug at cancer cells producing ROS that cause oxidative cellular damage, leaving the normal cells unaltered [8]. Topical PDT has been approved by regulatory authorities in 18 countries worldwide for use in nonmelanoma skin cancer, actinic keratosis, Bowen's disease, and superficial and nodular basal

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cell carcinomas. It could be used as a preventive therapy for cutaneous malignancy processes, acne and photo-rejuvenation [9].

Sulfonamides have been used for years due to their diverse biological activities (i.e. bactericide, antiviral, antidiabetic, and diuretic) [10–14]. These last years, some sulfonamide compounds are being clinically evaluated for cancer treatment. For example, the N-(3-chloro-7-indolyl)-1,4-benzendisulfonamide and N-[2-[(4-hidroxyphenyl)amino]-3-piridinyl]-4-metoxobenzene sulfonamide compounds block the cell cycle progression and can activate apoptosis signals in tumoral cells [15–19]. In the last years, our group has published several works describing the prominent oxidative nuclease activity of Cu–sulfonamide complexes [20–26]. As far as we know, sulfonamide copper complexes have not been used in photo-cleavage experiments although the photo-induced DNA cleavage activity of several copper(II) complexes has been reported [8].

In this sense, the present work stems from our interest to explore for the first time the ability of new Cu(II)–sulfonamide compounds as photocleavers of DNA. Herein, we report the synthesis, physicochemical characterization, DNA binding and nuclease activity of the two new copper sulfonamide complexes $[Cu(NST)_2(phen)]$ (**1**) and $[Cu(NST)_2(NH_3)_2]\cdot H_2O$ (**2**) [HNST = N-(4,5-dimethylthiazol-2-yl) naphthalene-1-sulfonamide; phen = 1,10-phenanthroline] (Chart 1). Ammonia and *o*-phenanthroline have been chosen as coligands in order to investigate the effect of the nature of the coligand on the binding affinity and nuclease efficiency of Cu–sulfonamide complexes. Studies have been made to explore the mechanistic pathways involved in the cleavage reactions. The photo-induced DNA cleavage activity of **1** has also been investigated. A significant result of this study is our observation that **1** was able to promote DNA damage via a photo-redox mechanism involving the formation of hydroxyl radicals as reactive species. The cytotoxicity of both compounds has been tested in human cancer cells (HeLa, Caco-2 and MDA-468). The low IC_{50} values have suggested that the complexes could be encouraging chemotherapeutic agents.

2. Experimental

2.1. Materials and methods

Copper salts and other chemicals and solvents were commercially available (Sigma) in high purity and used as such. Plasmid pUC18 (0.25 μg/μL, 750 μM in nucleotides) in TE (tris 10 mM and EDTA 1 mM, pH = 8.0) was purchased from Roche Diagnostics, Germany. Calf thymus DNA (CT DNA) type XV was provided by Sigma. Solutions of the metal complexes and other reagents for the strand scission experiments were prepared fresh daily. Chemical analyses for carbon, nitrogen, hydrogen and sulfur were performed with a CE Instrument EA 1110 CHNS analyzer. Infrared spectra were recorded with a Mattson Satellite FT-IR spectrophotometer from 4000 to 400 cm^{-1} using KBr disks. Diffuse reflectance spectra (Nujol mulls) were carried out on a Shimadzu 2101-PC UV–visible instrument. UV–visible (UV–vis) spectra of the complexes solutions were carried out with an HP 8453 spectrophotometer. Fast atomic bombardment (FAB) mass spectra were obtained on a VG Autospec spectrometer with 3-nitrobenzyl alcohol as matrix. Electrospray ionization-mass spectrum (ESI) (+ mode) analyses were performed on a Bruker Esquire 3000 plus liquid chromatography–mass spectrometry (LC–MS) system. EPR spectra at room temperature were collected with a Bruker ELEXSYS spectrometer operating at the X-band frequency.

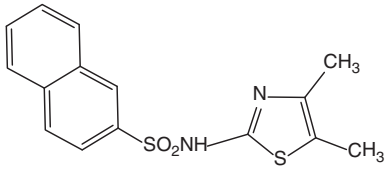

Chart 1. N-(4,5-dimethylthiazol-2-yl)naphthalene-1-sulfonamide (HNST).

2.2. Synthesis of N-2-(4,5-dimethylthiazol)naphthalenesulfonamide (HNST)

The sulfonamide ligand was prepared by reaction of 1 g of 2-amino-4,5-dimethylthiazole (7.8 mmol) with 2.77 g of naphthalenesulfonyl chloride (12.2 mmol) in 6 mL of pyridine at reflux (50 °C) for 2 h. Afterwards, the mixture was added to 10 mL of cold water and stirred for 30 min in an ice bath. The resulting solid was crystallized from ethanol, and then it was filtered off, washed with water, and dried until constant weight. Data for HNST (yield 87%, 2.16 g): anal. calc. for $C_{15}H_{14}N_2S_2O_2$ (318.39): C, 56.58; H, 4.43; N, 8.79; S, 20.14. Found: C, 56.51; H, 4.47; N, 8.68; S, 19.61. ^1H NMR (300 MHz, DMSO-d_6, δ/ppm): 12.4 [s (singlet), 1H, NHsulfonamide]; 8.4 [s, 1H, naphthalene]; 8.2–7.9 [m (multiplet), 3H, naphthalene]; 7.8–7.6 [m, 3H, naphthalene]; 2.0 [s, 3H, CH_3]; 1.9 [s, 3H, CH_3]. FAB: m/z^+ 319 [M^+]. IR (KBr) (ν_{max}/cm^{-1}): 3210 [ν(N–H)]; 1536 [ν(thiazole)]; 1300–1284 [$\nu(SO_2)$ asymmetric]; 1146 [$\nu(SO_2)$ symmetric]; 954 [ν(S–N)].

2.3. Synthesis of the complex $[Cu(NST)_2(phen)]$ (1)

A solution of 0.25 mmol of $Cu(CH_3COO)_2\cdot H_2O$ (20.1 mg) in 5 mL of methanol was added to 40 mL of a methanolic solution containing 0.5 mmol of the ligand (159 mg) and 0.5 mL of an aqueous solution of 1 M NaOH. To this mixture, 0.25 mmol (49.5 mg) of 1,10-phenanthroline was then added. The dark green resulting solution was left to stand at room temperature. Well-shaped prismatic green crystals suitable for X-ray diffraction were formed after a few days. Crystals were isolated by means of filtration, washed with methanol, and dried under vacuum.

Data for $[Cu(NST)_2(phen)]$ (**1**): anal. calc. for $CuC_{42}H_{34}N_6O_4S_4$ (878.53): C, 57.42; H, 3.90; N, 9.56; S, 14.59. Found: C, 57.36; H, 3.83; N, 9.39; S, 14.06. IR (KBr) (ν_{max}/cm^{-1}): 1448 [ν(thiazole)]; 1283 [$\nu(SO_2)$ asymmetric] and 1145 [$\nu(SO_2)$ symmetric]; 957 [ν(S–N)]; 1425 (phen). Solid UV–vis (λ_{max}/nm): 430 sh, 558. Mass (ESI^+) (DMF:cacodylate buffer 0.1 M, pH = 6.0, 1:10): m/z^+ 965.0 {[Cu(NST-2CH_3)(NST-CH_3)(phen)], DMF, H_2O, K^+}$^+$ and 583.1 {[Cu(NST-CH_3)(phen)], 2H_2O}$^+$. UV–vis (DMF:cacodylate buffer 0.1 M, pH = 6.0; 1:10) (λ_{max}/nm): 430sh, 656 (ε = 130 cm^{-1} M^{-1}).

2.4. Synthesis of the complex $[Cu(NST)_2(NH_3)_2]\cdot H_2O$ (2)

A solution of 0.5 mmol (120.8 mg) of $Cu(NO_3)_2\cdot 3H_2O$ in 20 mL of methanol was added slowly to 30 mL of a methanolic solution containing 1 mmol (318.4 mg) of the ligand and 5 mL of an aqueous solution of NH_3 (35%). The green resulting solution was left to stand at room temperature. After a few days well-shaped needle violet crystals suitable for X-ray diffraction were obtained. Crystals were isolated by means of filtration, washed with methanol, and dried under vacuum.

Data for $[Cu(NST)_2(NH_3)_2]\cdot H_2O$ (**2**): anal. calc. for $CuC_{30}H_{34}N_6O_5S_4$ (750.41): C, 47.97; H, 4.53; N, 11.19; S, 17.06. Found: C, 47.85; H, 4.51; N, 11.13; S, 16.96. IR (KBr) (ν_{max}/cm^{-1}): 1448 [ν(thiazole)]; 1279 [$\nu(SO_2)$asymmetric] and 1145 [$\nu(SO_2)$symmetric]; 957 [ν(S–N)]; 3487, 3360 [$\nu(NH_3)$]. Solid UV–vis (λ_{max}/nm): 555. Mass (ESI+) (DMF): m/z^+ 800.9 {[Cu(NST-2CH_3)$_2$(NH_3)$_2$], DMF, H_2O, K^+}$^+$. UV–vis (DMF) (λ_{max}/nm): 633 (ε = 100 cm^{-1} M^{-1}).

2.5. X-ray crystallography

A brown prismatic crystal of **1** and a violet prismatic crystal of **2** were mounted on a glass fiber and used for data collection. For **1** crystal data were collected at 100(2) K, using a Bruker X8 KappaAPEXII diffractometer. Graphite monochromated Mo-Kα radiation (λ =