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Sulfonamide-containing copper(II) metallonucleases: Correlations with in vitro antimycobacterial and antiproliferative activities☆

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ABSTRACT

The bis-(1,10-phenanthroline)copper(I) complex, $[Cu(I)(phen)_2]^+$, was the first copper-based artificial nuclease reported in the literature. The biological and ligand-like properties of sulfonamides make them good candidates for fine-tuning the reactivity of the $[Cu(phen)_2]$ motif with biomolecules. In this context, we developed three novel copper(II) complexes containing the sulfonamides sulfameter (smtrH) and sulfadimethoxine (sdmxH) and (N^N)-bidentate ligands (2,2′-biyridine or 1,10-phenantroline). The compounds were characterized by chemical and spectroscopic techniques and single-crystal X-ray crystallography. When targeting plasmid DNA, the phen-containing compounds $[Cu(smtr^-)_2(phen)]$ **(1)** and $[Cu(sdmx^-)_2(phen)]$ **(2)** demonstrated nuclease activity even in the absence of reducing agents. Addition of ascorbic acid resulted in a complete cleavage of DNA by **1** and **2** at concentrations higher than 10 μM. Experiments designed to evaluate the copper intermediates involved in the nuclease effect after reaction with ascorbic acid identified at least the $[Cu(I)(N^N)_2]^+$, $[Cu(I)(sulfa)(N^N)]^+$ and $[Cu(I)(sulfa)_2]^+$ species. The compounds interact with DNA via groove binding and intercalation as verified by fluorescence spectroscopy, circular dichroism (CD) and molecular docking. The magnitude and preferred mode of binding are dependent on the nature of both N^N ligand and the sulfonamide. The potent nuclease activity of compounds **1** and **2** are well correlated with their antiproliferative and anti-*M. tuberculosis* profiles. The results presented here demonstrated the potential for further development of copper(II)-sulfonamide-(N^N) complexes as multipurpose metallodrugs.

1. Introduction

Artificial nucleases mimic the function of nucleases due to their capability to cleave the DNA phosphate ester bonds. The bis-(1,10-phenanthroline)copper(I) complex, $[Cu(I)(phen)_2]^+$ was the first copper-based artificial nuclease reported in the literature [1]. The mode of action of $[Cu(phen)_2]^{2+}$ is based on the generation of reactive oxygen species (ROS) upon reduction in the presence of molecular oxygen or hydrogen peroxide [1–4].

The remarkable nuclease properties of $[Cu(I)(phen)_2]^+$ are related to its high DNA binding constants, directly related to the properties of the phen ligand, which gives to the compound the ability to interact with DNA via minor groove-binding [5–7]. The reaction mechanism involves H atoms C1' and C4' of the deoxyribose, which are located within the minor groove, as the main targets for the oxidative attack [8]. The (bpy)-copper complex (bpy-bipyridine), however, interacts mainly via electrostatic interactions with the negatively charged phosphate backbone [5, 9]. For this reason, the series of Cu(II) compounds explored in this work was designed to compare the DNA-binding properties of phen and bpy and the effects on the nuclease

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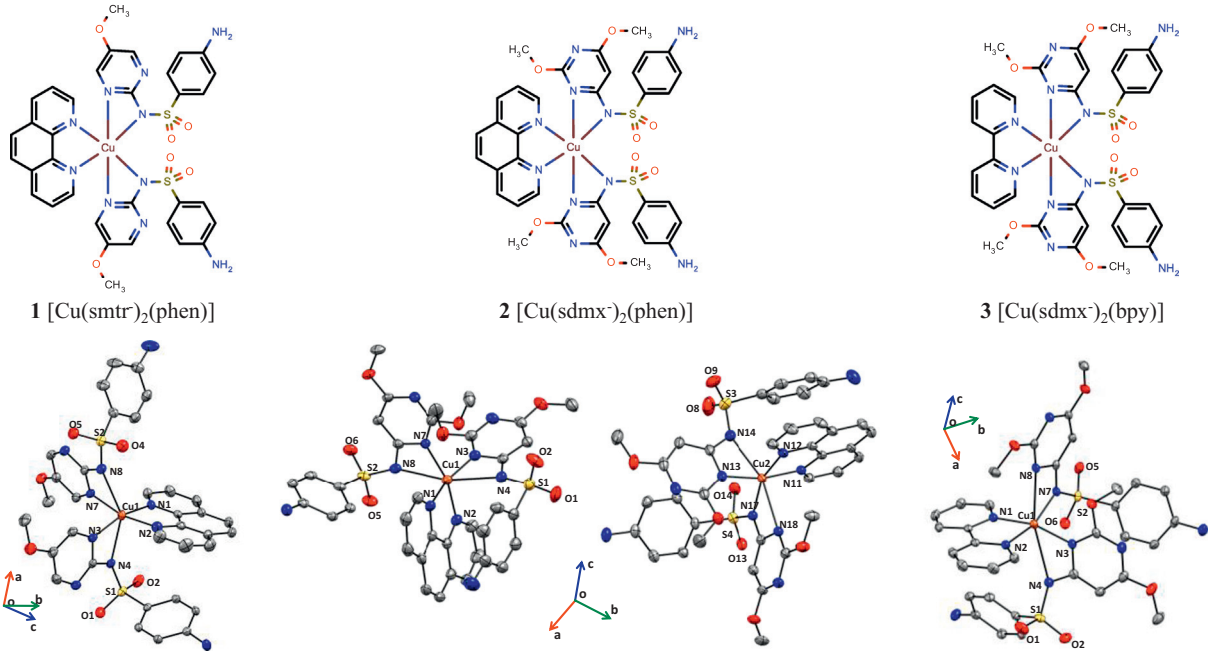

Fig. 1. Crystal structures for the copper(II) compounds with molecular formulas [Cu(sulfa$^-$)$_2$(N^N)] synthesized in this work. Two coordination isomers are present in the asymmetric unit of **2**. Displacement ellipsoids are drawn at the 40% probability level. The hydrogen atoms and water molecules were omitted for clarity.

activity of the compounds.

Since the introduction of $[Cu(phen)_2]^{2+}$, much effort has been dedicated to the development of novel artificial metallonucleases, since metal complexes are endowed with several properties that can be explored to fine-tune the desired nuclease activity. This includes the number and nature of the ligands, structural diversity and electronic properties [5, 7, 10]. For reviews of artificial copper metallonucleases, the reader is directed to the works of McGivern et al. [7] and Pratviel et al. [11]. Mixed chelate copper(II) complexes known as Casiopeinas® are also based on phen and bipy derivatives and possess remarkable antiproliferative activities [12, 13]. Casiopeinas are an excellent example of compounds built around the Cu(N^N) moiety with variable co-ligands and demonstrate the importance of the co-ligand on the cytotoxic activity. In the same context, the nuclease activity of [Cu(II)(phen)] and [Cu(II)(bpy)] complexes are also expected to be affected by the introduction of additional ligands, including sulfonamides.

Sulfonamides represent an optimal class of drugs to be used as ligands following this approach since they have interesting structural variety, containing N, O and S-donor moieties. In terms of biological activities, sulfonamides are studied in the development of antimicrobial, anticancer and anti-inflammatory drugs [14]. Sulfameter (also known as sulfamethoxidiazine, smtrH), one of the sulfonamides investigated in this work, is used in the treatment of infections of the respiratory and urinary tracts in animals [15]. Sulfadimethoxine (sdmxH), on the other hand, is a long-acting sulfonamide, which is specially used for the treatment of coccidiosis, a parasitic disease of animals [16].

One of the areas of interest for the application of artificial nucleases resides on nucleic acid-targeting therapies. Two DNA-targeting applications are explored in this work. The first one is tuberculosis, which is still among the top 10 causes of death worldwide, according to the latest World Health Organization report on tuberculosis [17]. Copper complexes have been gaining attention as candidates for the development of novel agents for tuberculosis treatment. It has been reported that copper resistance is an important factor for *Mycobacterium tuberculosis* virulence. In this sense, increasing the intracellular content of bactericidal copper via administration of compounds (named "copper-boosting" compounds) that can cross the mycobacterial outer membrane barrier is one approach for the development of novel anti-tuberculosis drugs [18, 19]. The second biological endpoint tackled here, cancer, is the second largest cause of death worldwide [20]. It is estimated that 75% of cancer cases occur in low to middle-income countries. For that reason, the development of effective and low cost cancer treatments is of major interest. Since copper is an essential metal for human health, its compounds have been developed as anticancer agents based on the hypothesis that they may be more selective and less toxic [21–23].

Copper complexes with sulfonamides have been explored for a variety of applications, including their superoxide dismutase (SOD)-like and nuclease properties, and antibacterial and anticancer activities. The copper(II) complex containing sdmxH and two ammonia molecules presented antibacterial activity, and it was more active over Gram-positive *Staphylococcus aureus* strain than over Gram-negative *Escherichia coli* [24]. However, there are no reports of copper-sulfonamide complexes active over *M. tuberculosis*. Several papers have described the anticancer activities of copper-sulfonamide complexes. González-Álvarez et al., for example, reported copper(II)-bpy complexes with thiazol-sulfonamide derivatives with nuclease activities that correlated with their cytotoxicity on the low micromolar range against human Jurkat T lymphocytes and Caco-2 cell lines. The complexes induced cell death by an apoptotic mechanism [10]. The same research group reported copper complexes of N-substituted sulfonamides that presented DNA photocleavage and remarkable cytotoxicity on sub-micromolar range against Caco-2 cell lines [25]. Such results stimulate the synthesis of new copper(II) sulfonamide complexes and strengthen the potential of application of such compounds also as anticancer drugs.

Within this context, here we report three Cu(II) coordination compounds with activity over both mycobacteria and cancer cells of general formula [Cu(sulfa$^-$)$_2$(N^N)], where sulfa$^-$ is the anionic form of a sulfonamide ligand (sulfameter or sulfadimethoxine) and the (N^N)-bidentate ligand is 2,2′-bipyridine (bpy) or 1,10-phenanthroline (phen), see Fig. 1. The bpy and phen ligands are explored to compare the effect of different DNA-binding modes (electrostatic interaction vs. minor groove binding) on the nuclease activity of the designed copper(II) compounds.