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Crystal structure, spectroscopic characterization and antibacterial activities of a silver complex with sulfameter

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ABSTRACT

A silver complex with the sulfonamide sulfameter, also known as sulfamethoxydiazine (SMTR), was prepared and characterized. Chemical analyses were consistent with the [Ag($C_{11}H_{11}N_4O_3S$)] composition (AgSMTR), while conductivity measurements in DMSO indicated a non-electrolyte behavior of the complex in this solvent. High-resolution ESI(+)-QTOF mass spectrometric experiments revealed the presence of the [Ag($C_{11}H_{11}N_4O_3S$)+H]$^+$ and [Ag_2($C_{11}H_{11}N_4O_3S$)$_2$+H]$^+$ species in solution. Infrared and NMR spectroscopies indicated coordination of the ligand to the metal by the nitrogen atoms of the sulfonamide group and of the pyrimidine ring. The structure of AgSMTR was solved by powder X-ray diffraction technique using the Rietveld method. The solved structure confirms the formation of a dimer, where each silver ion is coordinated by one of the nitrogen atoms of the pyrimidine ring, the nitrogen of the sulfonamide group and by an oxygen atom from the sulfonyl group. An argentophilic interaction of 2.901(1) Å is present in this dimeric structure. The AgSMTR complex was assayed over Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) bacterial strains, and it was found that the compound is 8 times more active over the Gram-negative bacteria in DMSO solution, with MIC values in the micromolar range.

1. Introduction

Silver compounds have been used as antimicrobial agents in the treatment of human and animal infections for a long time [1]. Silver vessels were applied for water and food preservation, while diluted solutions of silver(I) nitrate were used, for example, in the prevention of gonorrhoeal eye infections in newborns [2,3]. However, the fast liberation of silver(I) ions from silver nitrate may lead to tissue toxicity and loss of selectivity. In addition, since there is no residual activity, silver nitrate has to be applied many times a day [3]. According to the literature, the mechanism of action of silver ions include bacterial cell membrane damage and/or interaction with biomolecules, such as proteins and DNA, leading to bacterial death [4–6].

Even though silver(I) complexes are mostly used as antibacterial agents, there are some recent examples in the literature of complexes that show *in vitro* activity against many cancer cell lines. Silver complexes with carboxylate ligands, for example, show selective activity against adenocarcinoma and sarcoma cells [6]. Also, some N-heterocyclic carbene silver complexes showed antiproliferative activity toward carcinoma and leukemia cancer cell lines in the nanomolar range [7].

Sulfonamides are an important class of molecules in medicinal chemistry. This class of compounds exhibits antibacterial, anti-inflammatory, diuretic, hypoglycemic and antitumor activities, among others [8]. The antibacterial activity of sulfonamides was first reported by Domagk, who found that Prontosil – an azo compound – was active over *Streptococci* strains due to the *in vivo* release of sulfanilamide [9]. Antibacterial sulfonamides act as inhibitors of the enzyme dihydropteroate synthase, which is involved

Abbreviations: AgSDZ, Ag(I) complex with sulfadiazine; AgSMTR, Ag(I) complex with sulfameter; ATCC, American Type Culture Collection; ATR, Attenuated Total Reflectance; BHI, Brain-Heart Infusion; CFU, Colony Forming Unit; CP/MAS, Cross-Polarization and Magic Angle Spinning; CSD, Cambridge Structural Database; DMSO, Dimethylsulfoxide; ESI-QTOF-MS, Electrospray Ionization Quadrupole Time-of-flight Mass Spectrometry; FTIR, Fourier Transform Infrared; HMBC, Heteronuclear Multiple Bond Correlation; HSQC, Heteronuclear Single Quantum Coherence; MIC, Minimum Inhibitory Concentration; NMR, Nuclear Magnetic Resonance; SA, Simulated Annealing; SMTR, Sulfameter; UV-Vis, Ultraviolet-Visible.

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in the folic acid metabolism. However, the indiscriminate use of antibiotics led to the development of resistance to common sulfonamides due to structural changes in the active site of this enzyme, which lowers enzyme-sulfonamide interactions [10]. Examples of some sulfonamides with pharmacological activities are presented in Scheme 1.

The investigation of the bioactivity of metal complexes with sulfonamides began with the report of silver sulfadiazine (AgSDZ) by Fox in 1968 [11]. AgSDZ is a silver-sulfonamide complex and it is approved by the US FDA for topical use as a cream in the treatment of burns and skin ulcers [1,3,6]. The mechanism of action of silver sulfadiazine is related to the slow release of silver ions, while the sulfonamide acts as a carrier, preventing silver precipitation with chlorides, thus preventing hypochloremia in burns [12,13]. Indeed, it is also possible to occur an additive effect between the antibacterial activities of the sulfonamide and the metal ion - in this case, silver(I) – over bacterial strains. AgSDZ has a broad spectrum of action over the bacteria that most commonly infect burn wounds, though the true efficacy of topical silver on burn wounds healing has been recently questioned [1,14].

Our group has been reevaluating the structures and antibacterial effectiveness of several silver and copper complexes with sulfonamides, such as sulfadoxine [15], sulfathiazol and sulfamethoxazole [16,17]. There are also reports in the literature about silver complexes with the sulfonamides sulfamoxole [18], sulfachloropyridazine [19] and sulfamethazine [20] (see Scheme 1). Such complexes show dimeric structures, but the coordination sites seem to vary depending on the sulfonamide. The antibacterial profiles of the silver-sulfonamide complexes are structure-related as shown from the different activities of these compounds over Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* and *Pseudomonas aeruginosa* strains.

Sulfamethoxydiazine (SMTR), also known as sulfameter, is a long-acting sulfonamide, which is characterized by slow excretion and it can be administered once a day [21,22]. Sulfameter is structurally similar to sulfadiazine, the difference being an extra methoxy group in the pyrimidine ring, as shown in Scheme 1. It is used in the treatment of infections of the respiratory and urinary tracts, though it is more restricted for animals [22,23]. Recently, it was shown that sulfameter is effective and possess antibacterial activities over Gram-negative *Borrelia burgdorferi* strains, which causes Lyme's disease [24]. Sulfameter complexes with metals from the first transition series and cadmium were reported by Yang et al. [25]. The authors reported the antimicrobial activities of zinc and copper complexes over *S. aureus* bacterial strains. A dimeric copper complex with sulfameter was also reported by Ellena et al. [26], though no antibacterial activity was reported.

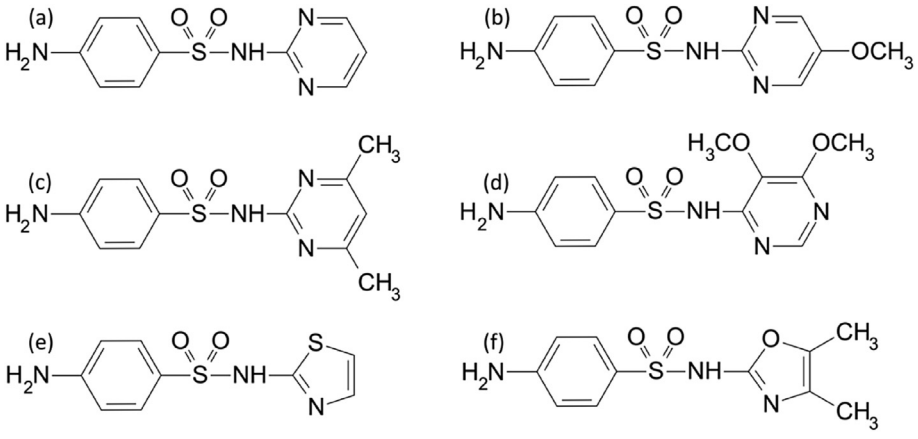
A silver(I) complex with sulfamethoxydiazine was first described by Bult and Klasen [27]. However, only infrared spectroscopic analysis was used for the proposition of the coordination mode of the sulfonamide to the metal center. Inspired by our recent works with metal complexes of sulfonamides, and also in the potential of application of such compounds in skin infections, we describe the synthesis, crystal structure, spectroscopic characterization and *in vitro* antibacterial assays of the silver complex with sulfameter.

2. Experimental

2.1. Materials and methods

Silver nitrate (≥99%) and sulfameter (sulfamethoxydiazine, or 2-sulfanilamido-5-methoxypyrimidine, 99%) were purchased from Sigma-Aldrich laboratories. Potassium hydroxide (≥85%) was acquired from Fluka.

Elemental analyses were performed on a Perkin Elmer 2400 CHNS/O Analyzer. Thermogravimetric analyses were performed on a simultaneous TGA/DTA SEIKO EXSTAR 6000 thermoanalyzer, using the following conditions: temperature range from 25 °C to 900 °C, heating rate of 10 °C min^{-1} and synthetic air flow. Conductivity measurements of AgSMTR in DMSO (concentration $1.0 \cdot 10^{-3}$ mol L^{-1}) were performed using a LAB1000 conductivity meter. Infrared spectroscopy measurements were performed on an Agilent Cary 630 FTIR spectrometer, using the Attenuated Total Reflectance (ATR) method, with a diamond cell. Electronic absorption spectra were obtained on a HP Agilent 8453 spectrophotometer, using a quartz cuvette with an optical path length of 1 cm equipped with a HP 89090 A Peltier. The measurements were performed at 37 °C. The IR spectra were recorded from 4000 to 400 cm^{-1}, with 64 scans and resolution of 2 cm^{-1}. Electrospray ionization quadrupole time-of-flight mass spectrometric measurements (ESI-QTOF-MS) were performed using a Waters Synapt HDMS instrument (Manchester, UK). The AgSMTR sample was evaluated in the positive mode, starting from a 1:1 acetonitrile:-water, 0.10% (v/v) formic acid and 10 μL of DMSO solution, which was further diluted 100-fold. The diluted solution was directly infused into the instrument's ESI source and analyzed with capillary potential of 4 kV, source temperature of 140 °C and nitrogen gas at 150 °C for desolvation. The NMR spectra in DMSO-d_6 solutions of both SMTR and AgSMTR were recorded in a Bruker Avance III 500 MHz spectrometer (11.7 T), operating at 499.87 MHz for ^{1}H. The ^{13}C and ^{15}N solid-state NMR spectra were recorded in a Bruker



Scheme 1. Chemical structures of the sulfonamides (a) sulfadiazine, (b) sulfameter, (c) sulfamethazine, (d) sulfadoxine, (e) sulfathiazole and (f) sulfamoxole.