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Silver sulfadoxinate: Synthesis, structural and spectroscopic characterizations, and preliminary antibacterial assays *in vitro*

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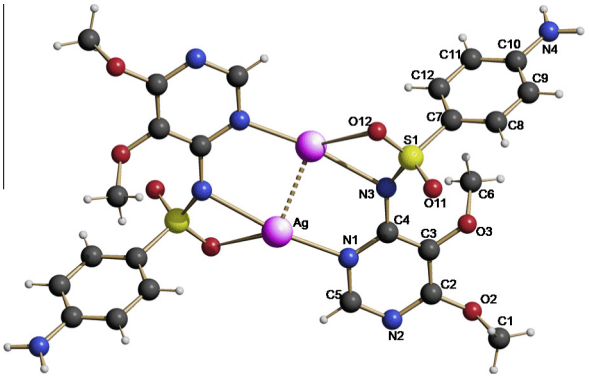
HIGHLIGHTS

- A novel silver(I) complex with sulfadoxine.
- IR and NMR data indicate coordination of the ligand to Ag(I) by the nitrogen and oxygen atoms.
- Structural characterization of the complex was based on X-ray powder diffraction data.
- Antibacterial activities of the complex were observed over Gram-negative and Gram-positive strains.

GRAPHICAL ABSTRACT

Structure of the AgSFX dimer. Colour code: silver in pink, nitrogen, oxygen, sulphur, carbon and hydrogen atoms in blue, red, yellow, black and white, respectively.



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Dedicated to Professor Antonio C. Massabni in the occasion of his 70th birthday.

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ABSTRACT

The sulfa drug sulfadoxine (SFX) reacted with Ag^+ ions in aqueous solution, affording a new silver(I) complex (AgSFX), which was fully characterized by chemical, spectroscopic and structural methods. Elemental, ESI-TOF mass spectrometric and thermal analyses of AgSFX suggested a $[Ag(C_{12}H_{13}N_4O_2S)]$ empirical formula. Infrared spectroscopic measurements indicated ligand coordination to Ag(I) through the nitrogen atoms of the (deprotonated) sulfonamide group and by the pyrimidine ring, as well as through oxygen atom(s) of the sulfonamide group. These hypotheses were corroborated by ^{13}C and ^{15}N SS-NMR spectroscopy and by an unconventional structural characterization based on X-ray powder diffraction data. The latter showed that AgSFX crystallizes as centrosymmetric dimers with a strong Ag···Ag interaction of 2.7435(6) Å, induced by the presence of *exo*-bidentate *N*,*N*′ bridging ligands and the formation of an eight-membered ring of $[AgNCN]_2$ sequence, nearly planar. Participation of oxygen atoms of the sulfonamide residues generates in the crystal a 1D coordination polymer, likely responsible for its very limited solubility in all common solvents. Besides the analytical, spectroscopic and structural

Abbreviations: SFX, Sulfadoxine (4-Amino-*N*-(5,6-dimethoxy-4-pyrimidinyl)benzenesulfonamide); AgSFX, Ag(I) complex with sulfadoxine; AgSFD, Ag(I) complex with sulfadiazine; NMR, nuclear magnetic resonance; HSQC, Heteronuclear Single Quantum Coherence; HMBC, Heteronuclear Multiple Bond Coherence; SS-NMR, Solid State NMR spectroscopy; ESI-TOF-MS, Electrospray Ionization Time-of-flight Mass Spectrometry; IR, Infrared Spectroscopy; TGA/DTA, Thermogravimetric and Differential Thermal Analysis; XRPD, X-ray Powder Diffraction; ATCC, American Type Collection Cell; MH, Mueller-Hinton agar; BHI, Brain-Heart Infusion Medium; CFU, Colony Forming Unit.

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X-ray powder diffraction
Antibacterial activities

description, the antibacterial properties of AgSFX were assayed using disc diffusion methods against *Escherichia coli* and *Pseudomonas aeruginosa* (Gram-negative), and *Staphylococcus aureus* (Gram-positive) bacterial strains. The AgSFX complex showed to be active against Gram-positive and Gram-negative bacterial strains, being comparable to the activities of silver sulfadiazine.

Introduction

Metal complexes have been widely used in pharmacology and medicine worldwide for the treatment of many diseases. The medicinal use of metals in China dates back to *ca.* 2500 b.C [1], being silver one of the most used metals. As early as 1000 b.C., silver was used to make water potable and, nearly two millennia later, in the 19th century (well before the advent of antibiotics), silver compounds were popular drugs [2]. The main use of silver was for the treatment of burns and wounds, efficiently limiting bacterial infection. Initially, silver dosage was made through solid silver nitrate by the use of instruments as the so-called *lapis infernalis*, followed by the use of solutions and foils [3]. Recently, the advent of penicillin and sulfa drugs made silver-based antibacterial agents fall into disuse.

The interest in silver was recovered by Moyer, who proposed the use of silver nitrate solutions to treat burns [4]. Unfortunately, the fast delivery of silver(I) ions into the blood system causes severe toxicity, and it is accompanied by the inhibition of the epithelial growth. In the 1970's, Fox studied [5] the combination of different sulfonamides with silver nitrate, aiming for improving the antibacterial activity, while diminishing the undesired effects of silver nitrate solutions. It was then demonstrated [6] that Fox's antibacterial agent was a silver complex with sulfonamide. Among the many sulfonamides, silver sulfadiazine (AgSFD) was the one that showed the highest activity against bacteria, and was soon introduced in the clinic for the treatment of burn infections and skin ulcers as a topical cream [2,4–8]. The good performance of silver sulfadiazine is mainly attributed to the presence of slowly released Ag^+ ions [9–11], with no sulfadiazine molecules being ever found inside of the bacterial cells. In this case, sulfadiazine acts as a carrier of silver ions, and avoids the readily precipitation of Ag^+ as silver chloride, or oxide/hydroxide, maintaining the electrolyte levels of the body fluids as well as the antibacterial activity due to the constant concentration gradient of Ag^+ [9].

Nowadays silver is known as a broad-spectrum antimicrobial agent and diverse formulations are commercially available. For example, colloidal silver, silver salts, silver complexes, nanocrystalline silver, silver oxide, and silver zeolite formulations are known, all possessing good antimicrobial activity [2,8]. However, the widespread use of silver has caused the isolation of some resistant bacterial strains. The studies on silver resistance are sparse, but they suggest that the resistance is mediated by plasmid. These evidences increased the concerns about the misuse of silver and the development of novel antibacterial silver compounds became of great interest [9].

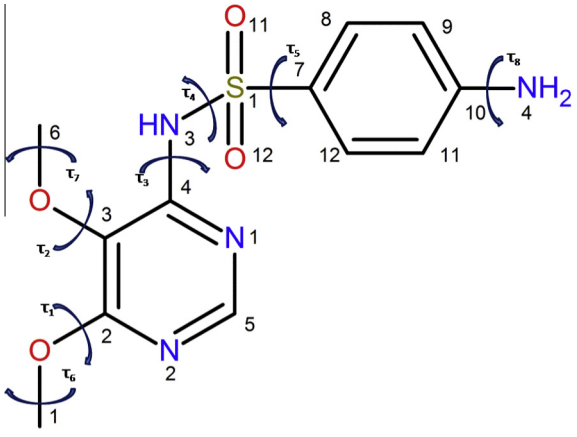

Fig. 1. Sketch of SFX molecule. The SFX molecular conformation was defined by eight torsion (τ_i) angles indicated by arrows and used in the powder diffraction study (*vide infra*).

For this reason, novel silver complexes with enhanced, or tailored, antimicrobial activity are continuously being investigated. For example, a silver complex with N-acetyl-L-cysteine was recently synthesized, and showed a broad spectrum of activity against microorganisms [12]. In addition, a silver complex with the anti-inflammatory drug nimesulide (another sulfonamide) [13] was shown to be active against Gram-positive and Gram-negative bacteria. Moreover, the Ag(I) derivatives are currently being investigated as anticancer drugs [12,14].

Sulfadoxine (SFX, $C_{12}H_{14}N_4SO_4$, Fig. 1) is a sulfonamide widely used in association with pyrimethamine as an antimalarial drug. The associated drug is indeed active against *Plasmodium falciparum*, the main human malaria parasite [15–18]. Besides the antimalarial activity, the sulfadoxine-pyrimethamine hybrid is also active against the parasite *Toxoplasma gondii* [15] and is considered as primary prophylaxis of pneumonia caused by *Pneumocyctis carinii* [16–18] and toxoplasmatic encephalitis in patients infected with HIV [17,18]. Sulfadoxine is also used in combination with trimethoprim to treat different bacterial infections in animals as horses and calves, being active against Gram-negative and Gram-positive bacteria [19–21].

Metal complexes of sulfadoxine were also recently studied and Fe(II), Fe(III), Co(II), Cu(II), Cr(III) derivatives were synthesized [22–24]. Ogunniran et al. synthesized Cu and Fe complexes with sulfadoxine and pyrimethamine, and showed that these species are active against *E. coli*, *S. aureus*, *P. aureginosa* and *S. typhi*. The complexes have shown to have an enhanced activity in comparison with the free ligands [23]. In the current manuscript, inspired by these works and by the desirable properties of many silver derivatives, we present the synthesis, spectroscopic and structural characterization, and the antibacterial activity of the novel silver-sulfadoxine (AgSFX) complex.

Experimental

Materials and methods

Sulfadoxine (SFX 95%) and silver nitrate ($AgNO_3$ 99%) were purchased from Sigma Aldrich Laboratories. Potassium hydroxide (85%) was obtained from Fluka. Elemental analyses for carbon, hydrogen, and nitrogen were performed using a CHNS/O Perkin Elmer 2400 Analyzer. Infrared (IR) spectra from 4000 cm^{-1} to 400 cm^{-1} of SFX and the AgSFX complex were measured using a Bomen MB Series Model B100 spectrometer with resolution of 4 cm^{-1}; samples were prepared as KBr pellets. The ^1H nuclear magnetic resonance (NMR) spectrum of SFX was recorded on a Bruker Avance 400 MHz spectrometer operating at 400.1 MHz. The ^{13}C