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Synthesis, characterization and *in vitro* biological assays of a silver(I) complex with 5-fluorouracil: A strategy to overcome multidrug resistant tumor cells

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ABSTRACT

A silver(I) complex with the antitumor drug 5-fluorouracil was synthesized and characterized by a set of chemical and spectroscopic techniques. Elemental, thermogravimetric and mass spectrometric analyses indicate a 3:2 metal/ligand composition, with minimal formula $Ag_3(C_4HFN_2O_2)(C_4H_2FN_2O_2)$. Solid-state NMR and IR spectroscopic studies suggest that coordination to silver(I) occurs by the nitrogen atoms N1 and N3, and by oxygen atom O2 of the ligand. The *in vitro* antiproliferative assays show the higher activity of the silver(I) complex with 5-fluorouracil when compared to the free drug against ovarian multidrug resistant (NCI/ADR-RES) and colon (HT29) tumor cell lines, with 50% growth inhibition (GI_{50}) values of 0.36 and 0.34 $\mu g\,mL^{-1}$, respectively. Gel electrophoresis assay indicated that the silver(I) complex does not interact with pIRES DNA plasmid. The compound also presented higher activity than cisplatin against a variety of tumor cell lines. The compound was also assayed over Gram-positive (*Staphyloccocus aureus*) and −negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacterial strains and the MIC values show its activity over the considered microorganisms at high concentrations.

1. Introduction

Cisplatin was one of the first metal complexes used in clinics. It was discovered in 1844 by Peyrone [1] and had its structure described by Werner in 1893 [2], but only in the 1960's its biological properties were described by Rosenberg et al. [3]. In the 1970's cisplatin had its antitumor activity tested and it was finally approved for clinical use by the FDA (U.S. Food and Drug Administration) in 1978 [4]. Since then, many antitumor metallodrugs have been developed. Second generation of platinum compounds based on cisplatin, like carboplatin and oxaliplatin, have been synthesized seeking drugs with reduced side-effects and to overcome tumor resistance [5]. Since the approval of these drugs for clinical use by FDA, cisplatin has been used mainly in the

Abbreviations: [Ag_3(fu)(fu-H)], silver(I) complex with 5-fluorouracil; ATCC, American type culture collection; BHI, Brain and heart infusion; bp, base pair; CFU, colony-forming units; CLSI, Clinical and Laboratory Standards Institute; CP/MAS, cross-polarization magic angle spinning; DMSO, dimethylsulfoxide; DNA, deoxyribonucleic acid; *E. coli*, *Escherichia coli*; ESI-QTOF-MS, electrospray ionization quadrupole time-of-flight mass spectrometry; FBS, fetal bovine serum; FDA, U.S. Food and Drug Administration; fu, 5 fluorouracil with both nitrogen atoms deprotonated; fu-H, 5-fluorouracil with one nitrogen deprotonated; fu-H_2, 5-fluorouracil with both nitrogen atoms protonated; GI_{50}, concentration that inhibits 50% cell growth; IR, infrared; MIC, minimal inhibitory concentration; NHC, N-heterocyclic carbenes; NMR, nuclear magnetic resonance; pIRES, internal ribosome entry site plasmid; RNA, ribonucleic acid; RPMI, Roswell Park Memorial Institute, medium for cell culture; SSD, silver sulfadiazine; *S. aureus*, *Staphylococcus aureus*; SYBR Green, Nucleic acid electrophoresis stain: *N*′,*N*′-dimethyl-*N*-[4-[(*E*)-(3-methyl-1,3-benzothiazol-2-ylidene)methyl]-1-phenylquinolin-1-ium-2-yl]-*N*-propyl-propane-1,3-diamine; Tumor human cell lines 2, U251 (glioma); M, MCF-7 (breast); A, NCI-ADR/RES (multidrug resistant ovarian); 7, 786-0 (renal); 4, NCI-H460 (lung, non-small cells); P, PC-3 (prostate); O, OVCAR-3 (ovarian); H, HT29 (colon); K, K562 (leukemia); Non-tumor human line: Cat, HaCat (immortal keratinocyte).

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treatment of testicular, ovarian, lung and breast tumors, while carboplatin and oxaliplatin have been used for ovarian and colorectal tumors, respectively [4,5].

Although platinum(II) compounds are the most used metallodrugs in the clinics for cancer treatment, they present disadvantages such as extended toxicity and tumor resistance even considering the new generations of these compounds [6,7]. Therefore, other metal ions have been evaluated as antitumor agents in a search for different targets and modes of actions when compared to the platinum(II) analogs [8–10]. Palladium(II) and gold(III) metal complexes are interesting because of their structural similarities with platinum(II) [11,12].

Historically, silver(I) compounds were first explored as antibacterial agents [13]. For instance, silver-sulfadiazine (SSD) is clinically used in the treatment of bacterial infections in burn wounds [14], as well as colloidal silver solutions, silver dressings and gels [15]. The mechanism of antibacterial activity of SSD relies mainly on the interaction of silver(I) ions with the bacterial DNA, further triggering the bacterial death [16]. Silver(I) can also bind cell receptors of microorganisms and biomolecules containing sulfur, oxygen and nitrogen, interfering in bacterial metabolism [17]. The peculiar interactions of silver(I) ions with cellular contents has also stimulated the evaluation of compounds based on this nuclei as antitumor agents [18].

Recently, an extensive review on the antiproliferative and antitumor activities of silver compounds was published by Bantis and Hadjikakou [19]. In this way, silver(I) complexes with antitumor activities have been studied by many research groups. Haque et al. described the synthesis and anticancer activities over human colorectal carcinoma (HCT 116) and promyelocytic leukemia (HL-60) cells of p-xylyl linked bis-benzimidazolium salts and the corresponding silver(I) N-carbene heterocyclic (NHC) complexes. The compounds exhibited significant cytotoxicity against the considered cells with IC_{50} values in the range 0.01–18.7 μmol L^{-1} for HCT 116 and 0.7–55.7 μmol L^{-1} for HL-60. The complexes have shown to be more active than the free ligands [20]. In another article, Haque et al. described the synthesis and structural characterization of two new silver(I) imidazole and benzimidazole complexes stabilized by pyridine based NHC ligands. The dinuclear silver(I) complexes exhibited antibacterial activities against *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) bacterial strains, with Minimum Inhibitory Concentration (MIC) values in the range 75–25 μg mL^{-1} [21]. In addition, the authors studied the nuclease activities of the complexes by gel electrophoresis assay. The results indicated that the compounds were able to degrade plasmid pTS414 showing nuclease activity against DNA and RNA [21].

Sandtorv et al. showed Ag(I) complexes with *N*-heterocyclic carbenes of compositions $C_{11}H_{12}N_2AgI$ and $C_{17}H_{24}N_2AgI$, which are potentially cytotoxic against human leukemia cell lines (MOLM-13 and HL60) [22]. In the same way, Sanchez et al. showed an Ag(I) complex with coordination formula $[Ag_2(\mu\text{-}\kappa^2C,C'\text{-}C_{10}H_{14}N_4)Br_2]$, which is active at similar concentrations when compared to cisplatin against prostate (PC-3) and colon tumor (HT-29) human cell lines [23].

Silver(I) complexes with dicarboxylate ligands and 1,10-phenantroline, $[Ag_2(phen)_x(OOC\text{-}(CH_2)_y\text{-}COO)]\cdot zH_2O$ (phen = 1,10-phenantroline, x = 2 or 3, y = 1–10, z = 1–4) were also prepared. Such compounds presented *in vitro* activity against breast (MCF-7) and ovarian (SKOV-3) human tumor cells [24]. In addition, a recent silver(I) complex with the anti-inflammatory drug nimesulide and a triaryl derivative was obtained by Banti et al. Such compound showed higher *in vitro* antiproliferative activity than cisplatin against human breast adenocarcinoma cell lines (MCF-7 and MDA-MB-231) [25]. Previously, a silver(I) complex with nimesulide was reported considering its antibacterial activity [26,27]. The observed results support the investigation of silver(I) compounds as antitumor agents.

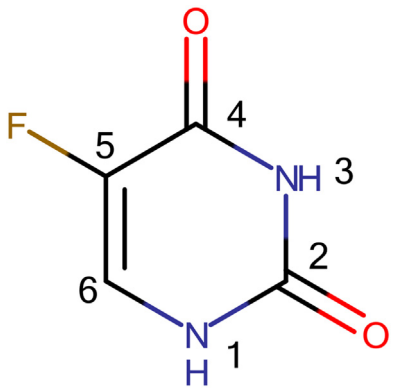

Fig. 1. Molecular structure of fu-H_2, with atom numbering.

5-Fluorouracil (fu-H_2, $C_4H_3FN_2O_2$, Fig. 1) is a fluoropyrimidine analogue to uracil and an important fluorine anticancer agent [28]. It acts by misincorporating itself into ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), and also inhibiting the nucleotide synthetic enzyme thymidylate synthase, leading to DNA damage [29–31]. According to the literature, fluorination of nucleosides is of great interest. It was suggested that the fluorine atom may mimic a hydrogen atom and then turn the substrate (uracil) into an inhibitor (5-fluorouracil) [32]. 5-Fluorouracil is widely used in the treatment of cancer, particularly for colorectal, breast, and aerodigestive tract tumors [29]. In the viewpoint of coordination chemistry, 5-fluorouracil is a versatile ligand and its coordination to metal ions may provide synergistic effects among the antiproliferative activities of the metal and the free drug. The first Ag(I) complexes with 5-fluorouracil were reported in the literature in the 1970's [33]. At that time, only the synthetic route and elemental and potentiometric studies of the obtained complexes were presented. No spectroscopic, structural or biological studies were discussed so far.

Herein, we describe the synthesis and spectroscopic characterization of a silver(I) complex with 5-fluorouracil. The compound had its antiproliferative activities evaluated *in vitro* and the obtained results were compared to free 5-fluorouracil and cisplatin. In addition, the complex was investigated about its antibacterial activities and the results are also described.

2. Results and discussion

2.1. Synthesis

Free 5-fluorouracil (fu-H_2) is slightly soluble in water (12.2 mg mL^{-1}) and the pk_a values, according to the literature, are 8.0 and 13.0 [34]. Considering this information and based on the paper of Gel'fman and Kustova [33], the synthesis was performed in alkaline medium, by using KOH for deprotonation of the ligand. Gel'fman and Kustova described three different methodologies, in which they obtained three different compositions, two of them containing potassium as a counter ion. In addition, the authors suggested different charges for the 5-fluorouracil ligand, with one or two nitrogen atoms deprotonated (here described as fu-H and fu, respectively).

In our case, flame atomic emission spectrometric measurements were performed to determine the presence or absence of potassium in the complex. No potassium content was found. Considering this information and elemental analysis results (see