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Article

EVALUATION OF THE CYTOTOXICITY AND GENOTOXICOLOGICAL SAFETY PROFILE OF BIOACTIVE SILVER(I) COMPLEXES WITH AMINOADAMANTANE LIGANDS

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Silver(I) complexes of amantadine (atdH), memantine (mtnH) and rimantadine (rtdH), named Ag-atd, Ag-mtn and Ag-rtd, respectively, were recently synthesized and described in the literature as promising antibacterial agents. In the present study, the cytotoxicity of such complexes was evaluated against cultures of primary epidermal keratinocytes (HaCaT) and murine melanoma tumor cells (B16-F10), and mutagenicity was studied by the Ames test to investigate their abilities to induce gene mutations. The Ames test was performed using *Salmonella* Typhimurium strains (TA98, TA100, TA102 and TA97a) capable of detecting frameshift and base pair substitution gene mutations, in experiments with and without metabolic activation (microsomal fraction S9). This study revealed significant cytotoxic activity against tumor cells and selectivity of Ag-atd and Ag-rtd complexes when compared to non-tumor human keratinocyte cells. Moreover, the Ag(I) complexes did not induce a significant growth in the number of revertant colonies when comparing with the negative control, both in the experiments without and with metabolic activation, indicating absence of mutagenic activity. The results are encouraging and collaborate in the genotoxicological investigations necessary for understanding the interaction and ability of the silver complexes to induce mutations and contribute to ensure their uses as future antibacterial or antitumor agents.

Keywords: silver(I) complexes; antiproliferative activities; mutagenicity; Ames test.

INTRODUCTION

The discovery of penicillin in the early 1920's and the growth of synthetic and analytical methods of characterization of organic and/or inorganic compounds led to an extraordinary advance of medicinal chemistry worldwide. Today, synthetic drugs with antibacterial, antifungal, and antiviral activities, among others, are well established in the clinics.[1,2] In special, medicinal inorganic chemistry encompass the synthesis of new metal-based drugs, which can be used for diagnostic or treatment of several diseases. Some examples are the platinum(II) complexes, such as cisplatin, carboplatin and oxaliplatin, which have been used for the treatment of head and neck, testicular and ovarian cancers, among others,[3-5] gold complexes as antiarthritic drugs,[6] technetium and gadolinium complexes in diagnostics,[5,7] and so on.

In this context, silver-based compounds deserve a special attention mainly due to their promising antibacterial activity. The best example is silver sulfadiazine (SSD), which has been used to treat skin infections in burns and wounds since the 1970's.[8-11] Silver is a non-essential metal, and it can be toxic to humans depending on its form of application and dosage.[12-14] Nevertheless, the effects of metals may be distinct when mammalian and microorganisms' targets are compared. This enables the synthesis of compounds based on this metal as therapeutic agents for external uses with little negative effects to the host.[13,14]

Due to the growth of bacterial resistance, a renewed interest has been observed in the study about how the antimicrobial activities of silver-based compounds can be improved and directed to the treatment of bacterial infections as, for example, in skin lesions.[15] According to the literature,[16-18] Ag(I) ions are able to form pores and puncture the bacterial cell wall or enter the bacterial cell inhibiting cellular respiration and disrupting metabolic pathways. Inside the bacterial cell, Ag(I) ions can also attach to DNA and block the bacterial replication. Considering the relevance of silver and its compounds in medicinal chemistry, it becomes necessary evaluate and describe the genotoxicological evaluation of such compounds, to guarantee the safety of its use in therapy.

Mutations in the genetic material can occur spontaneously or induced by physical, chemical and/or biological factors.[19,20] The Ames test is one of the most used to detect mutagens among pure substances, mixtures and environmental samples and uses *Salmonella* Typhimurium strains that are sensitive to substances capable of inducing different types of mutation. Each type of bacterial strain has different mutations in the histidine operon, which makes it possible to differentiate the mechanisms of action. Mutations can be substitutions of bases or of the frameshift type.[21,22] The Ames test is used as an initial screening and is widely accepted by the scientific community, governmental agencies, and corporations for regulatory approval.[23]

Here, the cytotoxicity of three silver complexes with the aminoadamantane ligands amantadine (atdH), memantine (mtnH) and rimantadine (rtdH) was evaluated for the first time in the present manuscript against cultures of primary epidermal keratinocytes (HaCaT) and murine melanoma tumor cells (B16-F10), in addition to silver nitrate ($AgNO_3$) and free ligands. The mutagenicity of the three silver-based compounds was evaluated by the Ames test to investigate their abilities to induce gene mutations. $AgNO_3$ and free ligands were also evaluated since they were used as precursors in the synthesis of the complexes and served as controls.

The silver complexes with amantadine (Ag-atd) and memantine (Ag-mtn), of compositions $C_{20}H_{34}AgN_3O_3$ and $C_{24}H_{42}AgN_3O_3{\cdot}H_2O$, respectively, had structural characterizations determined by single-crystal X-ray diffraction[24] and are presented in Figures 1a and 1b, while the structure of the silver complex with rimantadine (Ag-rtd, Figure 1c), of composition $C_{24}H_{42}AgN_3O_3{\cdot}H_2O$ was proposed

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by a combination of nuclear magnetic resonance spectroscopic measurements and molecular modeling.[25] The previous biological assays showed the promising antibacterial action of the complexes against Gram-negative and Gram-positive strains, with minimum inhibitory concentration (MIC) values in the micromolar range.[24,25]

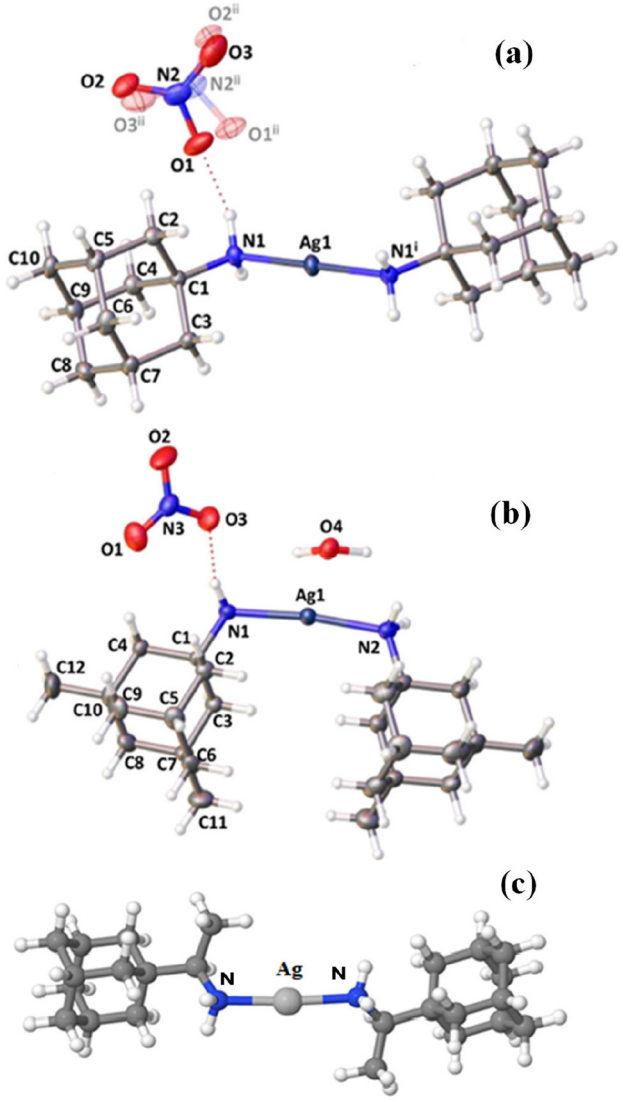

Figure 1. *Crystal structures of the silver(I) complexes with (a) amantadine, (b) memantine, and (c) proposed structure for the silver complex with rimantadine based on DFT studies (adapted from Santos Pereira et al. and Sucena et al.)*[24,25]

EXPERIMENTAL

Materials

Dimethylsulfoxide (DMSO), potassium chloride (KCl), magnesium chloride ($MgCl_2$), resazurin sodium salt, nicotinamide adenine dinucleotide phosphate sodium salt (NADP), D-glucose-6-phosphate disodium salt, L-histidine monohydrate, D-biotin, 4-nitro-*o*-phenylenediamine (NPD), mitomycin C (MMC), 2-aminofluorene (2-AF), sodium azide (SA) and 2-anthramine (2-AA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). D-Glucose, magnesium sulfate, citric acid monohydrate, anhydrous dibasic potassium phosphate, sodium ammonium phosphate, monobasic sodium phosphate, dibasic sodium phosphate and sodium chloride were purchased from Merck (Whitehouse Station, NJ, USA).

Bacto Agar (BD Bacto™, Sparks, MD, USA) and Oxoid Nutrient Broth No. 2 (Oxoid, Basingstoke, HAM, UK) were purchased and applied for bacterial medium. Microsomal fraction (S9 fraction) was purchased from Moltox Molecular Toxicology Inc. (Boone, USA). Fetal bovine serum (FBS), Dulbecco's Modified Eagle's Medium (DMEM), trypsin-EDTA 5X, L-glutamine, antibiotic-antimycotic mix 100X were acquired from Gibco-Invitrogen® Thermo Fisher Scientific (Waltham, MA, USA).

The strains TA98, TA100, TA102 and TA97a of *Salmonella* Typhimurium were provided by Dr. B. N. Ames (Berkeley, CA, USA). $AgNO_3$, amantadine hydrochloride (atdH) and rimantadine hydrochloride (rtdH) were obtained Sigma-Aldrich laboratories, while memantine hydrochloride (mtnH) was obtained from Acros Organics.

Ag-atd, Ag-rtd and Ag-mtn were synthesized following the synthetic procedures developed by our group and recently described in the literature.[24,25] Briefly, the complexes were prepared as follows: first, the chloride ions from atdH, mtnH and rtdH ligands were removed preventing interferences in the preparation of the complexes. Afterwards, the aminoadamantanes were suspended in water and a diluted solution of nitric acid was added to a complete solubilization of the ligands. After 1 h of constant stirring, $AgNO_3$ (aqueous solution) was added to the respective aminoadamantane solution in a metal: ligand molar proportion of 1:1. Finally, a diluted potassium hydroxide solution was added to the reaction, leading to the formation of light grey solids. The silver complexes were filtered off, washed with water, dried, and stored for biological analysis.

Cytotoxicity

Cytotoxicity tests were carried out by standard resazurin reduction method.[26] The primary epidermal keratinocytes (HaCaT) and murine melanoma tumor cells (B16-F10) were maintained in DMEM with 10% v/v of FBS in a humidified atmosphere of 5% CO_2 and 95% air, at 37 ºC, in a 75 cm^2 culture flask (Corning). For the test, the cells were seeded into the wells of a 96-well microplate (1.0×10^4 cells $well^{-1}$) and incubated for 24 h for adhesion.

Complexes and ligands were evaluated, for 24 h, in concentrations from 0.9 to 100 μmol L^{-1}, while $AgNO_3$ was tested at concentrations of 0.7 to 25 μmol L^{-1}. Cells were also treated with DMSO 0.1% (v/v) to control the solvent of the complexes and cells without any treatment were used for negative control of the experiments. After incubation, 50 μL of resazurin (0.01% m/v) was added to each well, and the plates were incubated for 4 h, at 37 °C, in the dark. The fluorescence reading was performed as reported in the literature.[27] Data were checked for normality using the K-S test (Kolmogorov-Smirnov test) and submitted to analysis of variance (ANOVA) followed by Dunnett's comparison post-test, with the negative control as a reference. Statistical analysis was performed using the GraphPad Prism 7.0 program (Intuitive Software for Science, San Diego, CA, USA),[28] as well as the calculation of the IC_{50} (inhibitory concentration), which represents the concentration required to reduce the viability of cells to 50%. The selectivity indices (SI) were calculated using the following: $SI = IC_{50}$ [non-cancer cells]/IC_{50} [cancer cells].[27]

Mutagenicity (Ames test)

The *Salmonella* Typhimurium strains TA98, TA97a, TA100 and TA102 were used for mutagenic analysis of the three new Ag(I) complexes (Ag-atd, Ag-mtn and Ag-rtd), in experiments with and without metabolic activation (S9 mix), according to the preincubation method described in Maron and Ames.[21]

The strains from frozen cultures were grown overnight for 12-14 h in Oxoid Nutrient Broth No. 2. The metabolic activation mixture (S9) was freshly prepared before each test and consisted of 4% of S9 fraction, 1% of 0.4 mol L^{-1} $MgCl_2$, 1% of 1.65 mol L^{-1} KCl, 0.5% of 1 mol L^{-1} D-glucose-6-phosphate disodium and 4% of 0.1 mol L^{-1} NADP, 50% of 0.2 mol L^{-1} phosphate buffer and 39.5% sterile distilled water.[21]

Five different doses of silver-based compounds (1.25 to 10 μg $plate^{-1}$), diluted in DMSO, were assayed. The concentrations were selected based on a preliminary toxicity test with strain TA98. Toxicity was apparent either as a reduction in the number of His + revertants, or as an alteration in the auxotrophic background (i.e., background lawn). The various concentrations of compounds to be tested were added to 0.5 mL of 0.2 mol L^{-1} phosphate buffer (pH 7.4) or with 0.5 mL of 4% S9 mixture and 0.1 mL of bacterial culture and then incubated at 37 °C for 20-30 min. $AgNO_3$ and the ligands atdH, mtnH and rtdH were also tested separately only at the maximum concentration of 10 μg $plate^{-1}$.

After this time, 2 mL of top agar supplemented with L-histidine and D-biotin was added to the mixture and poured onto a plate containing minimal agar. The plates were incubated at 37 °C for 48 h and the revertant colonies were counted manually. The assay was performed in triplicate. Salanal statistical software package was applied as reported in the literature.[29] The data (revertants/plate) were assessed by analysis of variance (ANOVA), followed by linear regression. Calculation of the mutagenic index (MI) was performed for each concentration. Compounds are considered mutagenic when a dose-response relationship is detected and a MI ≥ 2 is observed with at least one concentration.[22]

Mutagens NPD (24 μmol L^{-1}, for TA98 and TA97a), SA (7 μmol L^{-1}, for TA100) and MMC (0.53 μmol L^{-1}, for TA102) were the positive controls used in the experiments without metabolization, while for the experiments with metabolic activation, 2-AA (2.3 μmol L^{-1}) was used for the strains TA98, TA97a, TA100, and 2-AF (20 μmol L^{-1}) for TA102. DMSO served as the negative (solvent) control (100 μL $plate^{-1}$).

RESULTS

The cytotoxic activities of the complexes of Ag(I) with aminoadamantanes, their respective ligands and $AgNO_3$ were tested against the tumor cell line B16F10 (metastatic mouse melanoma) and non-tumor cell line HaCaT (human keratinocyte cells). The IC_{50} and SI values are presented in Table 1.

The results showed that the free ligands did not induce a reduction in cell viability against the examined cell lines (IC_{50} > 100 μmol L^{-1}), but the Ag(I) complexes have potential cytotoxic activities against tumor cells. IC_{50} values were 39.9 ± 1.4, 47.4 ± 3.0 and 51.2 ± 2.3 μmol L^{-1} for Ag-atd, Ag-rtd and Ag-mtn, respectively.

Despite inducing high cytotoxicity against B16F10 cells with a IC_{50} of 2.4 ± 0.3 μmol L^{-1}, $AgNO_3$ showed its lack of selectivity. The IC_{50} value against HaCaT cells was 2.5 ± 0.1 μmol L^{-1} and SI = 1.07. It is important to note that Ag-atd and Ag-rtd complexes are less active against normal cells (HaCaT), which is of high significance in the search for new chemotherapeutic agents. The IC_{50} values against HaCaT cells were 83.0 ± 3.8 (SI = 2.08), 71.9 ± 3.4 (SI = 1.52) and 49.8 ± 2.5 μmol L^{-1} (SI = 0.97) for Ag-atd, Ag-rtd and Ag-mtn, respectively.

On Ames test, the Ag(I) complexes did not induce direct mutagenicity (Table 2), since an increase in the number of revertants in relation to the negative control was not observed in any of the concentrations and strains of *S.* Typhimurium analyzed. When tested in experiments with the S9 fraction (xenobiotic metabolization system), the mutagenic response of the complexes was also negative (Table 3).

Table 1. Mean and standard deviation of IC_{50} values and selectivity indexes (SI) for B16F10 and HaCaT cells

Compound	IC_{50} B16F10 / (μmol L^{-1})	IC_{50} HaCaT / (μmol L^{-1})	SI
Ag-atd	39.9 ± 1.4	83.0 ± 3.8	2.08
Ag-mtn	51.2 ± 2.3	49.8 ± 2.5	0.97
Ag-rtd	47.4 ± 3.0	71.9 ± 3.4	1.52
$AgNO_3$	2.4 ± 0.3	2.5 ± 0.1	1.07

IC_{50}: inhibitory concentration required to reduce the viability of cells to 50%. Each value represents the mean derived from at least three individual experiments in triplicate (mean ± SD).

The highest MI obtained (another parameter evaluated in the test) was 1.43 for Ag-atd and 1.39 for Ag-rtd, both in the TA98 strain (-S9); for Ag-mtn, maximum MI was 1.36 in the TA102 strain (-S9), that is, values lower than 2 prove the absence of mutagenic activity of the tested complexes, in the experimental conditions used in this study.

The ligands (atdH, mtnH and rtdH) also did not show mutagenic potential. MtnH tested at higher concentration (50 μg $plate^{-1}$; 85.83 μmol L^{-1}), as previously reported,[30] also was not able to induce genetic mutations. Finally, $AgNO_3$ which was used in the synthesis of the complexes, was evaluated at a concentration of 10 μg $plate^{-1}$. This salt completely inhibited bacterial growth in the strains TA98, TA100 and TA97a, and caused a substantial reduction in the revertant colonies of strain TA102, proving to be toxic to *Salmonella* strains in experiments without S9. In the evaluation with the metabolic activation system, the mean number of revertants was close to the value obtained from the negative control, showing that $AgNO_3$ does not indirectly induce genetic mutations by the Ames test.

DISCUSSION

Metals provide chemical functionalities that are not accessible to purely organic compounds, in addition to several other promising characteristics and, therefore, they have been used in drug therapies and as diagnostic agents.[31,32] Silver is a metal used to treat human diseases since ancient times. Although its use has been empirical for a long time, nowadays, the applications of this metal in medicine are mainly related to its antibacterial activity.[33] Studies also show the antitumor potential of both the Ag(I) ion and its complexes,[34,35] which is associated with oxidative stress-induced apoptosis of several cancer cells.[36] This feature is highly relevant since opportunistic bacteria and fungi usually develop during cancer treatments due to the immunosuppressive effects of anticancer drugs.[14]

However, silver has a side effect of changing skin color, which is unpleasant, reaching bluish or grayish tones,[37] in addition to blood and lymphatic system disorders, gastrointestinal and allergic reactions.[38] These effects can be explained by the toxicity of the metal, which when internalized inside cells can release Ag(I) ions causing oxidative stress, resulting in cell death.[39] Therefore, it is necessary to investigate the beneficial and harmful effects of new molecules, before considering them as promising drugs.[40]

In the present study, we confirmed the lack of selectivity of $AgNO_3$, which, despite being highly active against tumor cells, also induces high cytotoxic activity against non-tumor cells. It has already been mentioned that this salt is toxic to tissues at concentrations above 1%,[35,41] but it was possible to observe that when combined with aminoadamantanes, the toxicity of the metal was reduced in mammalian cell lines (*in vitro* tests).

Table 2. Mean (M) and standard deviation (SD) of the number of revertants/plate and mutagenicity index (MI) in *S.* Typhimurium strains TA98, TA100, TA102 and TA97a for the Ag-atd, Ag-mtn and Ag-rtd complexes after treatment with variable concentrations, in experiments without (-S9) metabolic activation

Number of revertants (M ± SD)/plate and MI					
		TA98	TA100	TA102	TA97a
	C–	14 ± 4	104 ± 8	221 ± 26	168 ± 15
	C+	628 ± 87*,[a]	1776 ± 102*,[b]	1406 ± 99*,[c]	1118 ± 153*,[a]
	$AgNO_3$	toxic	toxic	34 ± 8 (0.15)	toxic
Ag-atd					
μmol L^{-1}	μg $plate^{-1}$				
1.0	1.25	20 ± 3 (1.43)	114 ± 15 (1.10)	262 ± 26 (1.18)	177 ± 12 (1.05)
2.0	2.5	14 ± 0 (1.00)	102 ± 10 (0.98)	286 ± 18 (1.29)	166 ± 22 (0.99)
4.0	5.0	14 ± 1 (1.00)	93 ± 8 (0.89)	254 ± 32 (1.15)	173 ± 16 (1.03)
5.9	7.5	19 ± 4 (1.32)	105 ± 14 (1.00)	213 ± 20 (0.96)	160 ± 10 (0.96)
7.8	10.0	18 ± 1 (1.25)	112 ± 11 (1.08)	170 ± 15 (0.77)	155 ± 8 (0.93)
	atdH	14 ± 2 (0.96)	107 ± 5 (1.03)	194 ± 12 (0.88)	139 ± 11 (0.83)
Ag-mtn					
μmol L^{-1}	μg $plate^{-1}$				
0.9	1.25	15 ± 2 (1.07)	83 ± 2 (0.79)	282 ± 14 (1.28)	207 ± 9 (1.24)
1.7	2.5	18 ± 6 (1.25)	98 ± 8 (0.94)	300 ± 29 (1.36)	208 ± 12 (1.24)
3.5	5.0	17 ± 9 (1.18)	101 ± 18 (0.97)	295 ± 33 (1.33)	177 ± 5 (1.06)
5.1	7.5	18 ± 3 (1.29)	93 ± 6 (0.89)	281 ± 26 (1.27)	179 ± 25 (1.07)
6.8	10.0	12 ± 3 (0.86)	92 ± 4 (0.88)	247 ± 16 (1.12)	158 ± 18 (0.94)
	mtnH	15 ± 1 (1.04)	116 ± 4 (1.11)	255 ± 25 (1.15)	190 ± 21 (1.13)
Ag-rtd					
μmol L^{-1}	μg $plate^{-1}$				
0.9	1.25	18 ± 4 (1.25)	93 ± 5 (0.89)	279 ± 19 (1.26)	171 ± 9 (1.02)
1.7	2.5	14 ± 1 (1.00)	106 ± 9 (1.01)	282 ± 23 (1.28)	180 ± 18 (1.07)
3.5	5.0	20 ± 4 (1.39)	93 ± 4 (0.89)	272 ± 37 (1.23)	174 ± 11 (1.03)
5.1	7.5	13 ± 0 (0.93)	94 ± 2 (0.90)	243 ± 20 (1.10)	170 ± 11 (1.01)
6.8	10.0	15 ± 1 (1.07)	85 ± 7 (0.82)	209 ± 12 (0.95)	157 ± 14 (0.93)
	rtdH	12 ± 2 (0.82)	102 ± 8 (0.98)	216 ± 28 (0.98)	152 ± 15 (0.90)

Ag-atd: silver(I) complex with amantadine (atdH, 10 μg $plate^{-1}$ = 24.5 μmol L^{-1}). Ag-mtn: silver(I) complex with memantine (mtnH, 10 μg $plate^{-1}$ = 20.7 μmol L^{-1}). Ag-rtd: silver(I) complex with rimantadine (rtdH, 10 μg $plate^{-1}$ = 20.7 μmol L^{-1}). $AgNO_3$: silver nitrate (10 μg $plate^{-1}$ = 21.8 μmol L^{-1}). *$p < 0.05$ (ANOVA). M ± SD: mean and standard deviation. MI: mutagenicity index. C–: Negative control: dimethylsulfoxide (DMSO, 100 μL $plate^{-1}$). C+: Positive control: [a]4-nitro-*o*-phenylenediamine (TA98 and TA97a, 24 μmol L^{-1}). [b]Sodium azide (TA100, 7 μmol L^{-1}). [c]Mitomycin (TA102, 0.53 μmol L^{-1}). Values in parentheses (MI) ≥ 2 indicate mutagenicity.

The Ag-atd and Ag-rtd complexes showed antitumor potential and selective characteristic, which confers less toxicity to the molecules, which is important to avoid side effects. The lowest cytotoxic effect against HaCaT cells showed positive gesture for moving ahead to the next level of screening. Although it also has antitumor activity, HaCaT cells were more sensitive to Ag-mtn than B16F10 cells. This behavior seems to be dependent on the type of the ligand coordinated to the Ag(I) ions, which can modulate the hydrophilicity-lipophilicity of the complexes. Adamantane derivatives are known for their antibacterial, antifungal, antiviral, trypanocidal and anti-Parkinson properties,[42-46] and here we show new antiproliferative agents combining the drug with Ag(I) ions.

On mutagenicity, a preliminary toxicity test without metabolization by the pre-incubation method[21] for the future Ames test of the complexes was carried out using the strain TA98 of *S.* Typhimurium in experiments without metabolic activation, to know the concentrations capable of killing prokaryotic cells. The TA98 strain has a mutation in the hisD gene (hisD3052) that codes for histidinol dehydrogenase, presenting as a preferential point for the reversal of eight repetitive residues of G:C. This behavior can be of high importance for detection of mutagenic compounds that cause displacement of the DNA reading frame.[22]

The toxicity of the three complexes was confirmed by the absence of revertant colonies at concentrations of 22.5 and 30 μg $plate^{-1}$ and by a substantial reduction (approximately 2 times less) in the number of colonies compared to the negative control (DMSO) caused by treatment with 15 μg $plate^{-1}$ of Ag-atd. A similar drop in the mean number of revertants was not observed after treatment with Ag-mtn and Ag-rtd (15 μg $plate^{-1}$). Based on these data and to guarantee the testing of non-cytotoxic concentrations, the Ames test was performed with treatments ranging from 1.25 to 10 μg $plate^{-1}$ of the complexes. The absence of mutagenic potential of the Ag-atd, Ag-mtn and Ag-rtd complexes, evaluated in this study by the *Salmonella*/microsome system, is a positive and encouraging point aiming at the continuity of the investigation in pre-clinical and clinical studies.

All strains used in the assay are histidine-dependent due to mutations in several genes in the histidine operon. These mutations act as "hot spots" for mutagens that cause DNA damage through different mechanisms. In addition, other mutations made the strains even more sensitive to chemical mutagens, such as, for example, the deletion mutation through the uvrB-bio genes, the mutation (rfa) that leads to a defective layer of lipolysaccharide (LPS) and the introduction of plasmid pKM101 that increases chemical and UV-induced mutagenesis.[22]

In the literature, other works show the absence of mutagenicity of silver(I) complexes by the Ames test, such as the complex with antibacterial and antitumor activities obtained from Ag(I) with 4-aminobenzoic acid (Ag-pABA) tested in assays with and without metabolic activation.[35] Manzano *et al.*[47] evaluated three

Table 3. Mean (M) and standard deviation (SD) of the number of revertants/plate and mutagenicity index (MI) in *S.* Typhimurium strains TA98, TA100, TA102 and TA97a for the Ag-atd, Ag-mtn and Ag-rtd complexes after treatment with variable concentrations, in experiments with (+S9) metabolic activation

Number of revertants (M ± SD)/plate and MI					
		TA98	TA100	TA102	TA97a
	C–	19 ± 5	118 ± 15	279 ± 47	173 ± 6
	C+	1134 ± 73*,a	1488 ± 68*,a	1645 ± 89*,b	2158 ± 97*,a
	$AgNO_3$	20 ± 4 (1.05)	159 ± 18 (1.35)	251 ± 28 (0.90)	212 ± 18 (1.23)
Ag-atd					
μmol L^{-1}	μg $plate^{-1}$				
1.0	1.25	23 ± 8 (1.18)	140 ± 28 (1.18)	268 ± 36 (0.96)	183 ± 16 (1.06)
2.0	2.5	21 ± 5 (1.08)	129 ± 19 (1.09)	256 ± 28 (0.92)	185 ± 11 (1.07)
4.0	5.0	21 ± 1 (1.11)	134 ± 15 (1.13)	264 ± 32 (0.95)	177 ± 15 (1.02)
5.9	7.5	19 ± 3 (1.00)	133 ± 16 (1.13)	284 ± 41 (1.02)	184 ± 26 (1.07)
7.8	10.0	23 ± 9 (1.18)	124 ± 22 (1.05)	275 ± 15 (0.99)	190 ± 21 (1.10)
	atdH	21 ± 4 (1.11)	107 ± 10 (0.91)	275 ± 17 (0.99)	163 ± 9 (0.94)
Ag-mtn					
μmol L^{-1}	μg $plate^{-1}$				
0.9	1.25	20 ± 4 (1.05)	114 ± 12 (0.97)	270 ± 21 (0.97)	180 ± 22 (1.04)
1.7	2.5	22 ± 4 (1.13)	116 ± 8 (0.98)	251 ± 15 (0.90)	176 ± 31 (1.02)
3.5	5.0	22 ± 6 (1.16)	106 ± 20 (0.90)	276 ± 28 (0.99)	183 ± 15 (1.06)
5.1	7.5	19 ± 8 (1.00)	107 ± 14 (0.91)	230 ± 14 (0.83)	163 ± 17 (0.94)
6.8	10.0	18 ± 2 (0.92)	92 ± 5 (0.78)	194 ± 26 (0.69)	132 ± 9 (0.77)
	mtnH	17 ± 1 (0.89)	88 ± 16 (0.74)	268 ± 20 (0.96)	176 ± 18 (1.02)
Ag-rtd					
μmol L^{-1}	μg $plate^{-1}$				
0.9	1.25	26 ± 7 (1.37)	133 ± 15 (1.13)	353 ± 29 (1.26)	205 ± 12 (1.18)
1.7	2.5	26 ± 3 (1.37)	123 ± 9 (1.04)	344 ± 13 (1.23)	183 ± 14 (1.06)
3.5	5.0	24 ± 0 (1.26)	94 ± 14 (0.80)	322 ± 25 (1.15)	218 ± 11 (1.26)
5.1	7.5	20 ± 4 (1.05)	107 ± 12 (0.91)	321 ± 17 (1.15)	160 ± 5 (0.92)
6.8	10.0	21 ± 2 (1.10)	118 ± 7 (1.00)	349 ± 25 (1.25)	149 ± 11 (0.86)
	rtdH	17 ± 4 (0.89)	90 ± 13 (0.76)	256 ± 37 (0.92)	184 ± 13 (1.06)

Ag-atd: silver(I) complex with amantadine (atdH, 10 μg $plate^{-1}$ = 24.5 μmol L^{-1}). Ag-mtn: silver(I) complex with memantine (mtnH, 10 μg $plate^{-1}$ = 20.7 μmol L^{-1}). Ag-rtd: silver(I) complex with rimantadine (rtdH, 10 μg $plate^{-1}$ = 20.7 μmol L^{-1}). $AgNO_3$: silver nitrate (10 μg $plate^{-1}$ = 21.8 μmol L^{-1}). *$p < 0.05$ (ANOVA). M ± SD: mean and standard deviation. MI: mutagenicity index. C–: Negative control: dimethylsulfoxide (DMSO, 100 μL $plate^{-1}$). C+: Positive control: [a]2-antramine (TA98, TA100 and TA97a, 2.3 μmol L^{-1}). [b]2-aminofluorene (TA102, 20 μmol L^{-1}). Values in parentheses (MI) ≥ 2 indicate mutagenicity.

Ag(I) complexes with fluoroanthranilic acid (fa) isomers, named Ag4fa, Ag5fa, and Ag6fa, which were also not mutagenic by the Ames test. As well as a complex of Ag(I) with cycloserine obtained by Ciol *et al.*,[34] active against *Mycobacterium tuberculosis* (minimum inhibitory concentration of 79.1 μmol L^{-1}), and bacterial strains of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, in addition to presenting antitumor potential and selectivity against breast cancer and leukemia cells, had no mutagenic effect on *Salmonella* strains by the Ames test.

In view of the genotoxicological context and the various applications of silver-based complexes and materials in general, health and industrial products, such as medical devices, dressings, food storage materials, cosmetics, among others, the data from the present study have implications, important for the analysis of Ag risks associated with its use as potential metallopharmaceuticals.

CONCLUSIONS

The Ag-atd, Ag-mtn and Ag-rtd complexes did not induce gene mutations by Ames test and, therefore, can be considered safe under the experimental conditions tested, increasing its acceptance, and minimizing the risks associated with its use. Moreover, the tested Ag(I) complexes expressed cytotoxic activity against B16-F10 cells (murine melanoma tumor), and Ag-atd and Ag-rtd were less toxic toward nontumor cells. The promising *in vitro* results obtained in this work support future studies in the design of more effective metal-based aminoadamantane complexes as antiproliferative agents over tumor cells.

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The authors declare no conflict of interest.

REFERENCES

1. Paiva, R. E. F.; Marçal Neto, A.; Santos, I. A.; Jardim, A. C. G.; Corbi, P. P.; Bergamini, F. R. G.; *Dalton Trans.* **2020**, *49*, 16004. [Crossref]
2. Hutchings, M. I.; Truman, A. W.; Wilkinson, B.; *Curr. Opin. Microbiol.* **2019**, *51*, 72. [Crossref]
3. Jurgens, S.; Kuhn, F. E.; Casini, A.; *Curr. Med. Chem.* **2018**, *25*, 437. [Crossref]
4. Zhang, C. X.; Lippard, S. J.; *Curr. Opin. Chem. Biol.* **2003**, *7*, 481. [Crossref]

5. Gaynor, D.; Griffith, D. M.; *Dalton Trans.* **2012**, *41*, 13239. [Crossref]
6. Abdalbari, F. H.; Telleria, C. M.; *Discover Oncol.* **2021**, *12*, 42. [Crossref]
7. Varol, S.; Öktem, F.; Koçyiğit, A.; Demir, A. D.; Karataş, E.; Aydın, M.; Göknar, N.; İpekçi, T.; *Int. J. Clin. Pract.* **2021**, *75*, e14810. [Crossref]
8. Miller, A. C.; Rashid, R. M.; Falzon, L.; Elamin, E. M.; Zehtabchi, S.; *J. Am. Acad. Dermatol.* **2012**, *66*, e159. [Crossref]
9. Fox, C. L.; Modak, S. M.; *Antimicrob. Agents Chemother.* **1974**, *5*, 582. [Crossref]
10. Medici, S.; Peana, M.; Crisponi, G.; Nurchi, V. M.; Lachowicz, J. I.; Remelli, M.; Zoroddu, M. A.; *Coord. Chem. Rev.* **2016**, *327-328*, 349. [Crossref]
11. Medici, S.; Peana, M.; Nurchi, V. M.; Zoroddu, M. A.; *J. Med. Chem.* **2019**, *62*, 5923. [Crossref]
12. Hadrup, N.; Lam, H. R.; *Regul. Toxicol. Pharmacol.* **2014**, *68*, 1. [Crossref]
13. Godoy-Gallardo, M.; Eckhard, U.; Delgado, L. M.; Roo Puente, Y. J. D.; Hoyos-Nogués, M.; Gil, F. J.; Perez, R. A.; *Bioact. Mater.* **2021**, *6*, 4470. [Crossref]
14. Leitão, J.; Sousa, S.; Leite, S.; Carvalho, M.; *Antibiotics* **2018**, *7*, 65. [Crossref]
15. Sim, W.; Barnard, R.; Blaskovich, M. A. T.; Ziora, Z.; *Antibiotics* **2018**, *7*, 93. [Crossref]
16. Jung, W. K.; Koo, H. C.; Kim, K. W.; Shin, S.; Kim, S. H.; Park, Y. H.; *Appl. Environ. Microbiol.* **2008**, *74*, 2171. [Crossref]
17. Morones-Ramirez, J. R.; Winkler, J. A.; Spina, C. S.; Collins, J. J.; *Sci. Transl. Med.* **2013**, *5*, 190ra81. [Crossref]
18. Yakabe, Y.; Sano, T.; Ushio, H.; Yasunaga, T.; *Chem. Lett.* **1980**, *9*, 373. [Crossref]
19. Santos, F. V.; Colus, I. M. S.; Silva, M. A.; Vilegas, W.; Varanda, E. A.; *Food Chem. Toxicol.* **2006**, *44*, 1585. [Crossref]
20. Chatterjee, N.; Walker, G. C.; *Environ. Mol. Mutagen.* **2017**, *58*, 235. [Crossref]
21. Maron, D. M.; Ames, B. N.; *Mutat. Res., Environ. Mutagen. Relat. Subj.* **1983**, *113*, 173. [Crossref]
22. Mortelmans, K.; Zeiger, E.; *Mutat. Res., Fundam. Mol. Mech. Mutagen.* **2000**, *455*, 29. [Crossref]
23. Zeiger, E.; *Mutat. Res., Genet. Toxicol. Environ. Mutagen.* **2019**, *841*, 43. [Crossref]
24. Santos Pereira, A. K.; Nakahata, D. H.; Manzano, C. M.; Simoni, D. A.; Pereira, D. H.; Lustri, W. R.; Formiga, A. L. B.; Corbi, P. P.; *Polyhedron* **2019**, *173*, 114116. [Crossref]
25. Sucena, S.: *Síntese, Caracterização e Estudo das Atividades Antibacterianas de Complexos de Au(I), Ag(I) e Pd(II) com Rimantadina*; Dissertação de Mestrado, Universidade Estadual de Campinas, Campinas, Brasil, 2013. [Link] acessed in November 2023
26. Page, B.; Page, M.; Noel, C.; *Int. J. Oncol.* **1993**, *3*, 473. [Crossref]
27. de Grandis, R. A.; Costa, A. R.; Moraes, C. A. F.; Sampaio, N. Z.; Cerqueira, I. H.; Marques, W. G.; Guedes, A. P. M.; Araujo-Neto, J. H.; Pavan, F. R.; Demidoff, F. C.; Daher Netto, C.; Batista, A. A.; Resende, F. A.; *J. Inorg. Biochem.* **2022**, *237*, 112005. [Crossref]
28. Motulsky, H. J.; *GraphPad Statistics Guide*, GraphPad Software Inc., San Diego, CA, USA, 2016. [Link] accessed in November 2023
29. Bernstein, L.; Kaldor, J.; McCann, J.; Pike, M. C.; *Mutat. Res., Environ. Mutagen. Relat. Subj.* **1982**, *97*, 267. [Crossref]
30. Pereira, A. K. S.; Santos, I. A.; Silva, W. W.; Nogueira, F. A. R.; Bergamini, F. R. G.; Jardim, A. C. G.; Corbi, P. P.; *Pharmacol. Rep.* **2021**, *73*, 954. [Crossref]
31. Miethke, M.; Pieroni, M.; Weber, T.; Brönstrup, M.; Hammann, P.; Halby, L.; Arimondo, P. B.; Glaser, P.; Aigle, B.; Bode, H. B.; Moreira, R.; Li, Y.; Luzhetskyy, A.; Medema, M. H.; Pernodet, J. L.; Stadler, M.; Tormo, J. R.; Genilloud, O.; Truman, A. W.; Weissman, K. J.; Takano, E.; Sabatini, S.; Stegmann, E.; Brötz-Oesterhelt, H.; Wohlleben, W.; Seemann, M.; Empting, M.; Hirsch, A. K. H.; Loretz, B.; Lehr, C. M.; Titz, A.; Herrmann, J.; Jaeger, T.; Alt, S.; Hesterkamp, T.; Winterhalter, M.; Schiefer, A.; Pfarr, K.; Hoerauf, A.; Graz, H.; Graz, M.; Lindvall, M.; Ramurthy, S.; Karlén, A.; van Dongen, M.; Petkovic, H.; Keller, A.; Peyrane, F.; Donadio, S.; Fraisse, L.; Piddock, L. J. V.; Gilbert, I. H.; Moser, H. E.; Müller, R.; *Nat. Rev. Chem.* **2021**, *5*, 726. [Crossref]
32. Anthony, E. J.; Bolitho, E. M.; Bridgewater, H. E.; Carter, O. W. L.; Donnelly, J. M.; Imberti, C.; Lant, E. C.; Lermyte, F.; Needham, R. J.; Palau, M.; Sadler, P. J.; Shi, H.; Wang, F. X.; Zhang, W. Y.; Zhang, Z.; *Chem. Sci.* **2020**, *11*, 12888. [Crossref]
33. Candido, T. X.; Paiva, R. E. F.; Figueiredo, M. C.; Coser, L. O.; Frajácomo, S. C. L.; Abbehausen, C.; Cardinalli, I. A.; Lustri, W. R.; Carvalho, J. E.; Ruiz, A. L. T. G.; Corbi, P. P.; Lima, C. S. P.; *Pharmaceutics* **2022**, *14*, 462. [Crossref]
34. Ciol, M. R.; Manzano, C. M.; Cuin, A.; Pavan, F. R.; Ribeiro, C. M.; Ruiz, A. L. T. G.; Oliveira, E. C. S.; Lustri, W. R.; Fregonezi, N. F.; Nogueira, F. A. R.; Corbi, P. P.; *ChemistrySelect* **2018**, *3*, 1719. [Crossref]
35. Aquaroni, N. A. S.; Nakahata, D. H.; Lazarini, S. C.; Resende, F. A.; Cândido, A. L. P.; Silva Barud, H.; Claro, A. M.; Carvalho, J. E.; Ribeiro, C. M.; Pavan, F. R.; Lustri, B. C.; Ribeiro, T. R. M.; Moreira, C. G.; Cândido, T. Z.; Lima, C. S. P.; Ruiz, A. L. T. G.; Corbi, P. P.; Lustri, W. R.; *J. Inorg. Biochem.* **2020**, *212*, 111247. [Crossref]
36. Żyro, D.; Śliwińska, A.; Szymczak-Pajor, I.; Stręk, M.; Ochocki, J.; *Cancers* **2020**, *12*, 3848. [Crossref]
37. Sterling, J. P.; *Burns* **2014**, *40*, S19. [Crossref]
38. Kalinowska-Lis, U.; Felczak, A.; Chęcińska, L.; Zawadzka, K.; Patyna, E.; Lisowska, K.; Ochocki, J.; *Dalton Trans.* **2015**, *44*, 8178. [Crossref]
39. Durán, N.; Rolim, W.; Durán, M.; Fávaro, W.; Seabra, A.; *Quim. Nova* **2019**, *42*, 206. [Crossref]
40. Resende, F. A.; Vilegas, W.; dos Santos, L. C.; Varanda, E. A.; *Molecules* **2012**, *17*, 5255. [Crossref]
41. Fiori-Duarte, A. T.; Nakahata, D. H.; Cuin, A.; Lustri, W. R.; Corbi, P. P.; *Polyhedron* **2017**, *121*, 172. [Crossref]
42. Warda, E. T.; El-Ashmanwy, M. B.; Habib, E. S. E.; Abdelbaky, M. S. M.; Garcia-Granda, S.; Thamotharan, S.; El-Eman, A. A.; *Sci. Rep.* **2022**, *12*, 21058. [Crossref]
43. Kolocouris, N.; Kolocouris, A.; Foscolos, G. B.; Fytas, G.; Neyts, J.; Padalko, E.; Balzarini, J.; Snoeck, R.; Andrei, G.; De Clercq, E.; *J. Med. Chem.* **1996**, *39*, 3307. [Crossref]
44. Kelly, J. M.; Quack, G.; Miles, M. A.; *Antimicrob. Agents Chemother.* **2001**, *45*, 1360. [Crossref]
45. Shiryaev, V. A.; Skomorohov, M. Y.; Leonova, M. V.; Bormotov, N. I.; Serova, O. A.; Shishkina, L. N.; Agafonov, A. P.; Maksyutov, R. A.; Klimochkin, Y. N.; *Eur J. Med. Chem.* **2021**, *221*, 113485. [Crossref]
46. Butterworth, R. F.; *Drugs R&D* **2021**, *21*, 267. [Crossref]
47. Manzano, C. M.; Nakahata, D. H.; Tenorio, J. C.; Lustri, W. R.; Resende Nogueira, F. A.; Aleixo, N. A.; Gomes, P. S. S.; Pavan, F. R.; Grecco, J. A.; Ribeiro, C. M.; Corbi, P. P.; *Inorg. Chim. Acta* **2020**, *502*, 119293. [Crossref]