Antimicrobial, *In Vitro* and *In Vivo* Antineoplastic Activities, Mechanism of Action, Structural and Thermal Properties of a Small-Novel Pharmaceutical Organometallic Chelate

Kareem I. Batarseh*

Alpha-OmegaBiologicals, 8610 Larkview Lane, Fairfax Station, VA 22039, USA; NovaGene, LLC, 1209 Macomb Street, Morgantown, WV 26501, USA

Abstract: The antimicrobial, the *in vitro* and *in vivo* antineoplastic activities, mechanism of action, the structural and thermal properties of a pharmaceutically novel and small organometallic silver (I)-tartaric acid complex synthesized by the author given the title name Aliargentumycine or AAgM are reviewed here.

Key Words: Silver(I)-tartaric acid, Aliargentumycine (AAgM), antimicrobial, antineoplastic, solid and disseminated tumors, *in vitro*, *in vivo*, apoptosis, structural and thermal properties.

1. INTRODUCTION

The *oligodynamic action* or the antimicrobial activity of small amounts of metal has been known for a long time and is the basis for the development of many metal coordination therapeutic agents [1-7]. For example, the water-insoluble Ag(I) compound silver sulphadiazine (AgSD) has been used extensively for the treatment of burn patients [8]. This compound has also been reported to exhibit antiherpes, antitreponemal and antifungal actions [9, 10]. Recently, silver nanoparticles have been employed in the disinfection of medical devices [11].

Besides the use of heavy metals as antibiotic agents, they are also employed in the design of cytotoxic compounds. The current treatments for cell proliferative diseases such as cancer employ metal coordination complexes where such complexes inhibit DNA replication and cell division. The most prominent and promising family of cytotoxic agents that proved to have clinical benefits that is used in combination with other antitumor drugs is these that employs platinum as the heavy metal [12, 13]. One of the most active and broad-spectrum cytotostatic drugs belonging to this family is cisplatin, cis-diaminedichloroplatinum (II), which is used to treat epithelial malignancies such as bladder, head and neck tumors, testicular and ovarian carcinomas [12-14]. A growing interest in other metal coordination complexes has also been explored [15-17].

Ionic silver substances are resurging again in popularity due to the fact that silver at low concentrations has no toxicity, mutagenicity or carcinogenic activities compared to other heavy metals [18-22]. Additionally, ubiquitous metalothioneins have the property of binding silver and other metals in metal thiolate cluster structures to transport, store, and detoxify essential and nonessential trace metals that may enter the body [23]. Surprisingly, despite the intense interest in the use of heavy metals in the treatment of cancer, silver (I) remains unexplored as an anticancerous agent [24-27].

The interest in the potential clinical implementation of silver as an antimicrobial and anticancer agent is very promising. Hence, this review summarizes and discusses the antimicrobial, *in vitro* and *in vivo* cytotoxic activities, mechanism of action, structural and thermal properties of a pharmaceutically novel silver (I) complex, synthesized by the author, and is given the title name Aliargentumycine (AAgM).

2. ANTIMICROBIAL ACTIVITIES OF AAgM

In an effort to ascertain whether silver complexes or silver ions are more effective as therapeutic agents and to examine silver's mode of action, efficacy studies were conducted with AAgM at different concentrations, and compared with free silver ions on *Pseudomonas aeruginosa,* ATCC # 15442 [28]. Results have revealed that AAgM gives significantly better efficacies than that of silver alone and as silver ion concentration increases lesser efficacy was observed [28]. Accordingly, the effect of silver atom on unwinding of cellular DNA, thereby inhibiting its replication might constitute the mode of action of silver. This emanates from the fact that silver is transported intracellularly as a package-a protected complex-and not as a free ion, precluding silver from binding with a host of substances present on the cell membrane or the intracellular space of microorganisms such as thiol groups, enzymes or any other negatively charged moiety.

3. *IN VITRO* ANTINEOPLASTIC ACTIVITIES OF AAgM

In vitro antineoplastic or cytotoxic activities of AAgM were determined on both solid and hematopoietic human tumor cell lines [29-34]. For instance, the results of cytotoxicity in terms of % inhibition as a function of AAgM concentration in μM obtained on T-47D (a human ductal breast carcinoma) and on Jurkat (a human T-cell lymphoblast leukemia) are depicted in Figs. (**1a**) and (**1b**), respectively. On the average, AAgM induced one order of magnitude reduction of

*Address correspondence to this author at the Alpha-OmegaBiologicals, 8610 Larkview Lane, Fairfax Station, VA 22039, USA; Tel: 703-976-8720; Fax: 703-690-0785; E-mail: fractals00@yahoo.com

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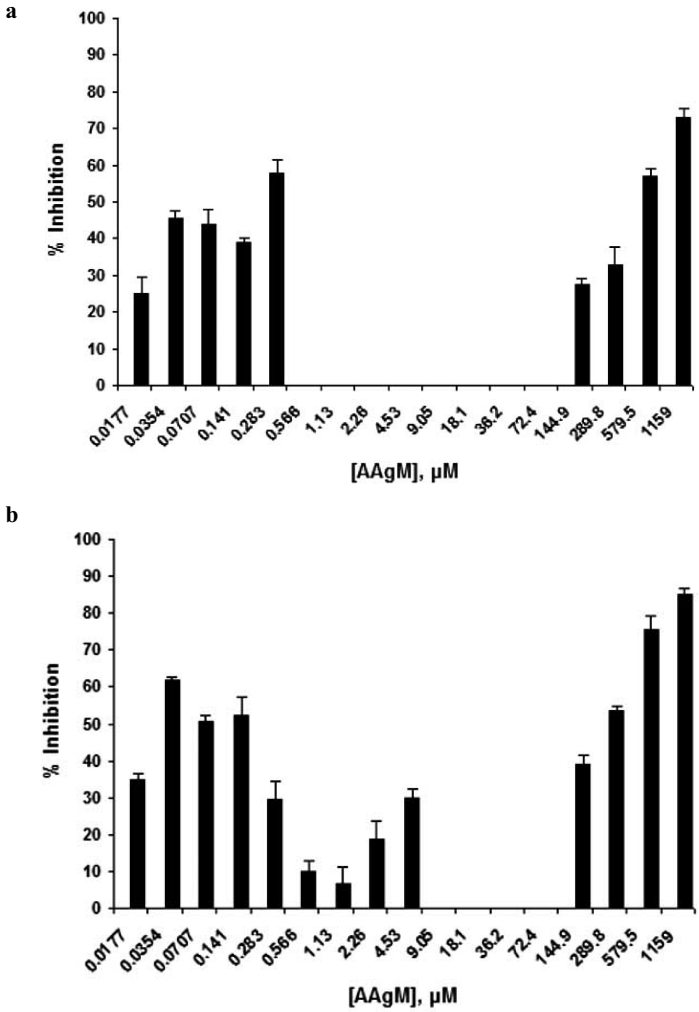

Fig. (1). % Inhibition of AAgM on (a) T- 47D and (b) Jurkat tumor cell lines. The results shown are the means ±S.E. of three independent experiments.

the original cell count on both T-47D and Jurkat when compared with free silver ions, and at lower concentrations, free silver ions did not exhibit any cytotoxic effects when compared to AAgM [29-34]. Similar results were obtained on the antimicrobial activities of this chelate [28].

Careful examination of the data on AAgM depicted in Figs. (**1a**) and (**1b**) reveals that the salient features of inhibition for T-47D and Jurkat are similar in that both profiles are triphasic. In general, Phase I is characterized by cytotoxicity in the nanoconcentration range, Phase II is characterized by the absence of cytotoxicity in the intermediate range, and finally Phase III is characterized by cytotoxicity in the microconcentration range. This behavior is more pronounced for T-47D in comparison with that for Jurkat. These phases occur abruptly as the concentration of AAgM increases. For the range of concentrations studied, the initiation of these phases is different for each tumor cell line examined (compare Figs. (**1a**) and (**1b**)). Such a triphasic behavior is also encountered in hormone co-activators [33].

The ratio of the area-under-the-concentration-curve for the time period over 24 h ($AUC_{t>24}$) to the 50% inhibitory concentration (IC_{50}) was calculated for the data presented in Figs. (**1a**) and (**1b**). Only the $AUC_{t>24}/IC_{50}$ values for Phase I were calculated since interest in drug development lies mainly within the nanoconcentration range. Results have revealed that the same conclusion can be deduced regarding the activities of AAgM on these tumor cell lines; namely, the cytotoxicity of AAgM on Jurkat is more superior to that of T-47D in Phase I. It can be concluded therefore that AAgM has profound activities on disseminated Jurkat tumor cells in comparison with solid T-47D cell line.

4. *IN VIVO* ANTINEOPLASTIC ACTIVITIES OF AAgM

The *in vivo* cytotoxic activities at different concentrations of AAgM were studied on human solid hepatocellular HepG-2 carcinoma and disseminated HL-60 acute myeloid leukemia (AML) tumors grown subcutaneously (s.c.) in female athymic nude mouse xenograft models [35]. Hepatocellular HepG-2 carcinoma or malignant hepatoma was chosen because it has a very poor prognosis and the survival rate of patients is very low because often the tumor is discovered at later stages of the disease. On the other hand, HL-60 cell line

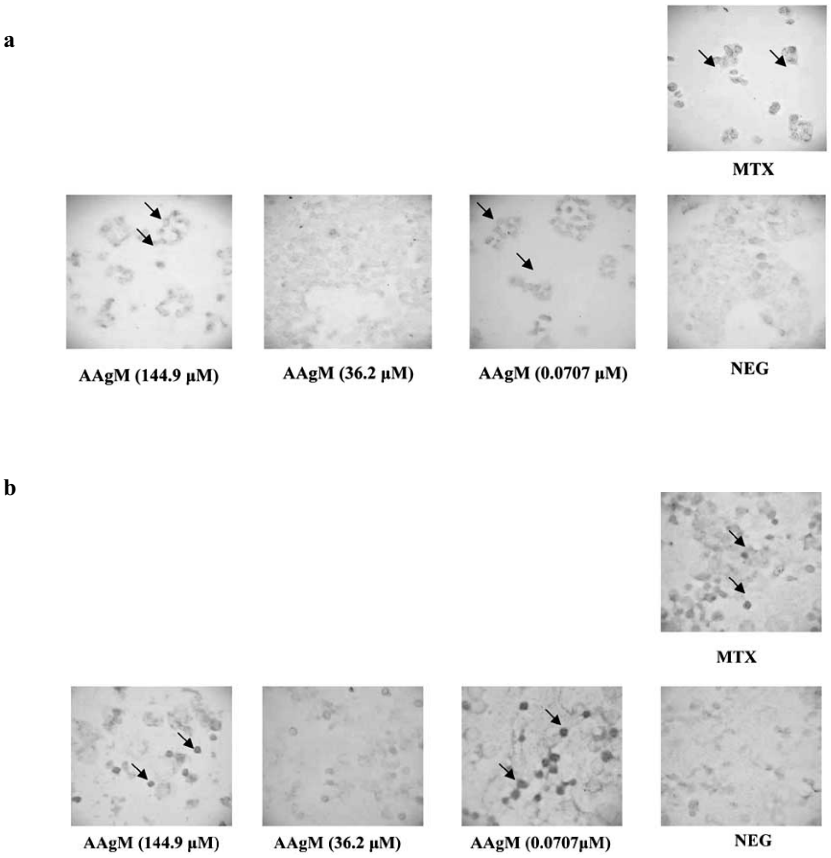

Fig. (2). Photomicrographs showing *in-situ* apoptotic staining depicted by arrows on (a) T-47D and (b) Jurkat. T-47D and Jurkat cells were treated with AAgM to induce apoptosis at three different concentrations, 0.0707, 36.2, and 144.9 µM. Positive and negative controls were used by using methotrexate (MTX) and by keeping the cells in the media without treatment (NEG). Detection of apoptosis was done by the DeadEnd Colorimetric TUNEL (terminal deoxynucleotidyltransferase nick end labeling) assay for the detection of apoptosis in cultured cells.

was chosen because it is a very fast growing disseminated leukemia tumor cell line.

Statistical analyses on the results on HepG-2 and HL-60 have shown that AAgM resulted in significant antitumor activities against both tumor cell lines. Particularly, it induced a 76% tumor inhibition on HepG-2 in the first 14 days of treatment and by the end of the course of the experiment or after 38 days of treatment, tumor inhibition reached almost 85% while for HL-60 the tumor inhibition at the end of the experiment (16 days of treatment) reached almost 70% [35]. Additionally, regression analyses on the data showed that AAgM *in vivo* treatment on HepG-2 and HL-60 resulted in 30 and 20% of the mice being tumor-free, respectively, and tumors did not re-grow even after treatment was stopped. Calculations have revealed that AAgM exhibited significant tumor growth delay of about 81 days for HepG-2 and 58 days for HL-60 [35]. Equally important is the fact that throughout the duration of the *in vivo* study on HepG-2 and HL-60, all animals treated did not show any signs of toxicity such as body weight loss or other commonly observed signs of toxicity.

5. MECHANISM OF ACTION OF AAgM

Living Cells can die by either necrosis "cell murder" [36, 37] or apoptosis "cell suicide" [38]. It is well established that cytotoxic drugs induce cytotoxic activities through a number of mechanisms, including necrosis (cytotoxic agents overdose), apoptosis, genotoxin (DNA damage), cell membrane damage, or free radical formation.

To examine the mode of AAgM inherent cytotoxic activities, *in-situ* apoptosis was detected by the DeadEnd Colorimetric TUNEL (terminal deoxynucleotidyltransferase nick end labeling) assay for Phases I, II and III identified in Figs. (**1a**) and (**1b**) [29-34]. Three concentrations, one for each phase, were chosen for the apoptotic studies and compared with the control, 0.0707, 36.2, and 144.9 µM for Phases I, II and III, respectively.

The TUNEL assay results after 18 hours of incubation for T-47D and 6 hours for Jurkat are shown in Figs. (**2a**) and (**2b**), respectively. The 18 and 6-hour incubation periods were chosen because it was determined in previous experiments of this work that induction of apoptosis of AAgM was within these times for these two cell lines.

For both cell lines studied, it can be concluded from Figs. (**2a**) and (**2b**) that apoptotic staining, depicted by arrows, is evident for AAgM at these concentrations. As can be seen, there are apparent alterations in cell morphology and detachment from the culture surface and cells became sparse

and rounded, which are classical characteristics of apoptosis. However, in Phase II for both T-47D and Jurkat treated with AAgM apoptosis is absent, and the cells are adherent to the surface of the slides, resembling that of the negatives (NEG), corroborating the previous results, viz., the lack of cytotoxicity of AAgM in Phase II, Figs. (**1a**) and (**1b**). It is also of significance to note here that the frequency of apoptosis is also reflected in the % inhibition results. For example, in the case of Jurkat treated with AAgM, the concentration at 0.0707 μM of AAgM corresponds to higher inhibition than that at 144.9 μM of AAgM, compare 50.5% with 38.9% of Fig. (**1b**), which is fingerprinted in the apoptotic results. For both cell lines examined, Phase II might be considered a region where AAgM is nontoxic at these concentrations. This finding could be interpreted in terms of AAgM lack of cytotoxicity, signaling cell dedifferentiation where cells might have reverted back to becoming nonmalignant, similar to experiments done on salamander cancers [39, 40].

Silencing of the proliferation-associated *p15* gene is commonly associated with myelodysplastic syndrome (MDS) [41]. Hence, drugs that inhibit DNA methylation are becoming very important in the fight of cancer. One such drug that has promising clinical results is decitabine or 5-Aza-2'-deoxycytidine. *In vitro* and *in vivo* studies have shown that decitabine was found to revert hypermethylation of *p15* gene, and has the ability of correcting leukemic cells and revert them back to normal [42-44]. The underlying mechanism responsible for the antineoplastic activity against MDS at low-dose of decitabine involves demethylation and the reversal of silencing of the *p15* gene promoter hypomethylation [41]. Hypermethylation was also found to be responsible for colorectal carcinomas [41, 45]. A recent *in vitro* study has shown that in colon cancer cells, promoter of hypermethylation is reversed as well [45]. The observed noncytotoxicity of AAgM at moderate concentrations in Phase II might be indicative that reversal of hypermethylation through *p15* gene is present. It is also possible that the observed lack of cytotoxicity in Phase II is related to the loss of ability of cells to divide, leading to growth arrest [33].

6. STRUCTURAL PROPERTIES OF AAgM

X-ray Crystallography (XRC) was employed to determine the structural properties of the title compound [29-32, 34]. A view of AAgM including the scheme of atomic numbering is presented in Fig. (**3**), while comprehensive crystal data and values of geometrical bond lengths and angles are given elsewhere [29-32, 34]. The coordination sphere of the crystalline state of AAgM revealed a polymeric chain hydrate structure, and is constructed from silver complex molecules and water solvent molecules, with no imposed symmetry on it [29-32, 34]. The stereochemical structure of AAgM is basically a two-bidentate and two-unidentate chelate residue of tartrate rings and water, $[Ag(bidentate)_2(unidentate)_2]^{\pm}$. Thus, AAgM is composed of lipophilic and hydrophilic moieties, making it miscible in both lipids *via* the tartrate rings and ionic solutions *via* the water molecules, Fig. (**3**). The water molecule is present stoichiometrically with respect to the complex, which is coordinated to the silver atom, Fig. (**3**). The tartaric acid residue coordinates to the Ag(1) center, forming 5-member coordination ring.

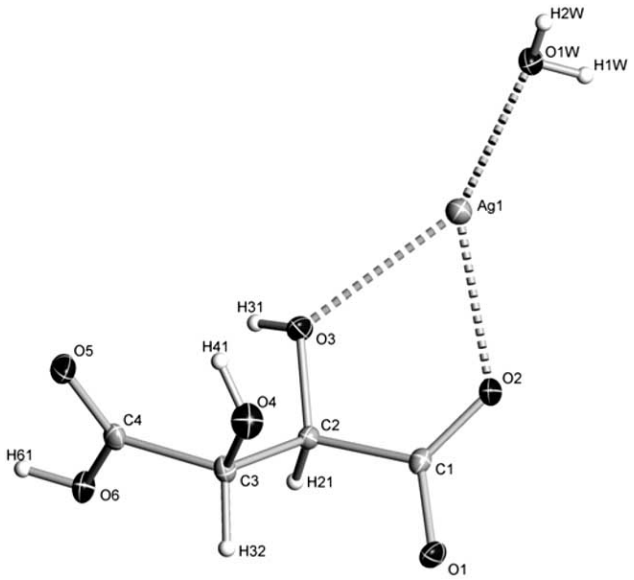

Fig. (3). Molecular structure of the title compound AAgM as determined by XRC.

7. THERMAL PROPERTIES OF AAgM

Simultaneous thermo-analytical technique that combines both differential scanning calorimetery (DSC) and thermogravimetric (TG) analyses were conducted on AAgM [29-32, 34]. Results of the DSC from 25° to 350°C at a temperature program rate of 10°C/min on crystals of AAgM depicted two endothermic bands at ~ 90-125°C with a peak at around 110°C and at ~ 170-190°C with a peak at around 180°C and one exothermic band at ~ 210-230°C with a dip at around 220°C [29-32, 34].

To further ascertain the above observations, hot-stage microscopy on AAgM is performed and the results are presented in Fig. (**4**). As can be seen in Fig. (**4a**), there were no obvious changes observed on AAgM between ~ 26-75°C. In the range of 86-157°C, particles appear more crystalline, Fig. (**4b**). Between 183-188°C, there appears to be changes in the crystalline structure of AAgM as depicted in Fig. (**4c**). At 200°C, the sample becomes blackish in color, indicating decomposition, Fig. (**4d**). As the sample is further heated to 253°C, the sample becomes more darkened and additional decomposition is observed as shown in Fig. (**4e**). It is of significance to point out here that no recrystallization was observed by lowering the temperature back to ~ 30°C.

The nature of the first low-temperature endothermic peak at around 110°C might be attributed to changes in the crystalline structure of AAgM, corroborating the results of the hot-stage microscopy Fig. (**4b**). The second endothermic peak at around 180°C might be due to dehydration of molecular water bonded to the silver atom.

A useful indication of the two endothermic peaks emanates from a comparison of the DSC and TG results or the % weight loss of the original sample as a function of temperature. Results have revealed that the % weight of the original sample does not change within the range of the first endothermic band, indicating that no phenomena characterized by

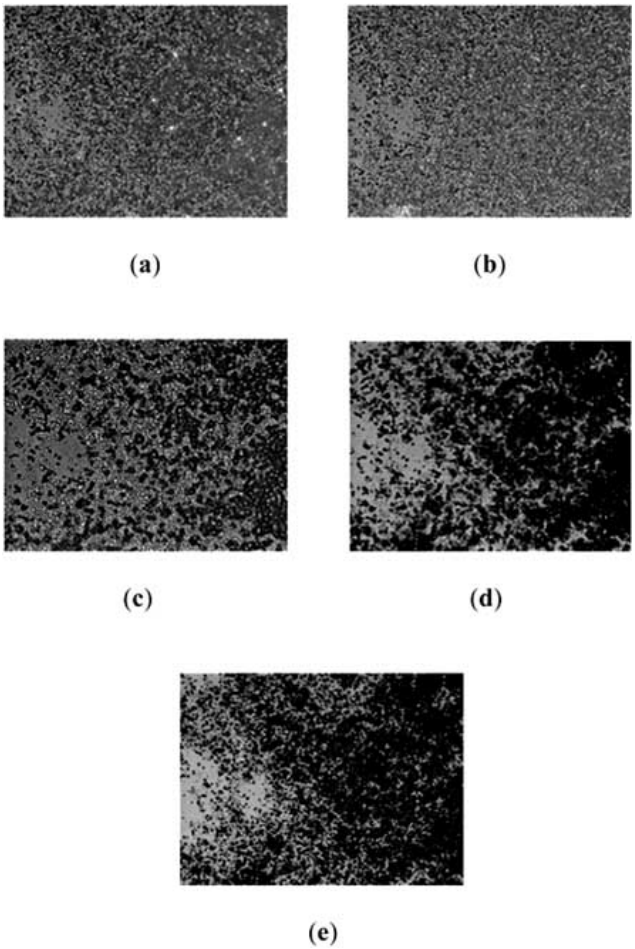

Fig. (4). Hot-stage microscopy results obtained on AAgM for temperatures (**a**) 26-75°C, (**b**) 86-157°C, (**c**) 183-188°C, (**d**) 200°C and (**e**) 253°C.

mass variation are taking place. However, at the second endothermic band, weight loss was observed. The loss of mass commenced at approximately 150°C and continued to about 200°C, and was accompanied by a weight loss of about ~ 6%. This dehydration step is also depicted in Fig. (**4c**) of the hot-stage microscopy results. The value of the weight loss of ~ 6% is equal the stoichiometric value of water present in Aliargentumycine (compare the molecular weight of AAgM of 275 with that of water, 18/275 ~ 6%). Undoubtedly, this observation is a signature of the dehydration step.

The final exothermic dip at 220°C corresponds to thermal decomposition. As the sample approaches the decomposition temperature, it suddenly decomposes, expands and makes contact inside the DSC instrument. This behavior can be substantiated by Figs. (**4d**) and (**4e**) of the hot-stage microscopy analyses. The present data suggest that AAgM is stable up to ~ 80°C, and at higher temperatures changes in the crystalline structure, dehydration and decomposition are consecutively observed.

8. CONCLUSIONS AND RECOMMENDATIONS

The antimirobial and the *in vitro* and *in vivo* antineoplastic activities, mechanism of action, structural and thermal properties of a pharmaceutically silver (I) tartaric acid chelate given the tile name Aliargentumycine (AAgM), which was synthesized by the author were summarized and reviewed here. The antimicrobial and anticancer activities of AAgM were studied *in vitro* and *in vivo* and AAgM was found to be very effective. *In vitro* results have shown that the title compound AAgM exhibits a triphasic cytotoxicity profile and its antineoplastic activities extend to the nano-concentration range. The mechanism of action of AAgM was by apoptosis. X-ray crystallography revealed that AAgM is a polymeric monohydrate distorted skew trapezoidal bipyramidal. Thermal studies have revealed that AAgM is stable to about 80°C, and at higher temperatures its crystalline structure changes, dehydrates and finally decomposes.

There are difficulties associated with metal-based cancerous compounds in that effective treatment is hampered by lack of specificity and difficulty in delivering these agents to their targets. The lack of specificity of cytotoxic drugs for tumors cells and the resulting toxicity to normal tissue hamper an additional exploitation of their cytotoxic effects. This is especially true with using cytotoxic agents on solid neoplasms since within the intercellular region of neoplasmic cells, the network of blood capillaries is far too small for such agents to be delivered [46-51]. These regions commonly exist in most major classes of solid tumors such as those associated with breast, head and neck, pancreatic, stomach, ovarian, cervical, lung, and prostate tumors. Since the present chelate has a relatively low molecular weight of 275, it can easily access solid tumors to reach its cellular target where blood capillaries are too small for large molecular drugs to be delivered.

There is a plethora of adverse side effects, some of which are irreversible, associated with cytotoxic compounds such as thrombocytopenia, nausea, peripheral and autonomic neuropathies, urticaria, cytopenia, cachexia, alopecia, angioedema, and many others related to pulmonary, reproductive and endocrine, and even cardiac arrest, especially at high doses [52, 53]. These complications are the major dose-limiting toxicity and can lead to hospitalization of the patient and analgesics for the alleviation of pain. On the other hand, silver has low toxicity compared with other heavy metals. Once absorbed, silver undergoes a first-pass effect through the liver, and is secreted into the bile, reducing the systemic distribution to tissues [54]. The only known condition, which results from chronic exposure to high levels of silver for a prolonged period of time in humans is argyria, a benign condition which results in permanent bluish-gray discoloration of the skin. The only clinical effect observed with argyria is an aesthetic effect and there are no pathological changes or inflammatory reactions resulting from silver deposition [55]. Based on patients receiving i.v. injections of silver arsphenamine, the LOAEL (lowest-observed-adverse-effect level) for argyria was determined to be 0.014 mg/kg/day [55].

Accordingly, since silver has a much safer tolerance record than most heavy metals, especially platinum and arsenic, and *in vivo* studies conducted with AAgM have shown its lack of toxicity [35], this makes silver in general and AAgM in particular very attractive for clinical implementation. The sundry of adverse side effects associated with the current systemic therapeutic methods used for the treatment of cancer such as metal-based drugs, radiation, immunother-

apy, chemotherapy, and antiangiogenic drugs might therefore be alleviated, making AAgM an excellent candidate for alternative therapy.

Based on the merits of this work, the present findings can be used for the development and the design of newly and safer silver-based antineoplastic drugs for the systemic treatment of cancer, especially those associated with solid neoplasms of epithelial and mesenchymal origins, which are not typically prone to apoptotic responses with cdk inhibitor drugs [56]. These promising findings will open new perspectives in the systemic treatment of cancer and in the formulation of novel cytotoxic silver-based drugs. The exceptionally low toxicity of AAgM and its effective antineoplastic activities make it attractive for further *in vivo* studies and future clinical trials.

ABBREVIATIONS

AAgM	=	Title name of Aliargentumycine
AgSD	=	Silver sulphadiazine
ATCC	=	American Type Culture Collection
$AUC_{t>24}$	=	Area-under-the-concentration-curve for the time period over 24 h
DSC	=	Differential scanning calorimetery
HL-60	=	Human acute myeloid leukemia cell line
HepG-2	=	Human hepatocellular carcinoma cell line
IC_{50}	=	50% inhibitory concentration
i.v.	=	Intravenously
Jurkat	=	Human T-cell lymphoblast leukemia cell line
LOAEL	=	Lowest-observed-adverse-effect level
MDS	=	Myelodysplastic syndrome
MTX	=	Methotrexate
NEG	=	Negative without treatment
S.E.	=	Standard error
s.c.	=	Subcutaneously
T-47D	=	A human ductal breast carcinoma cell line
TG	=	Thermogravimetric
TUNEL	=	Terminal deoxynucleotidyltransferase nick end labeling
XRC	=	X-ray crystallography

REFERENCES

[1] Merluzzi, V.J.; Cipriano, D.; McNeil, D. Evaluation of zinc complexes on the replication of rhinovirus 2 *in vitro*. *Res. Commun. Chem. Pathol. Pharmacol.,* **1989,** *66,* 425-40.

[2] Slawson, R.M.; Van Dyke, M.I.; Lee, H.; Trevors, T.J. Germanium and silver resistance, accumulation, and toxicity in microorganisms. *Plasmid,* **1992**, *27,* 72-9.

[3] Khurshid, H. Antimicrobial activity of iron (II) and zinc (II)-amino acid complexes. *Pak. J. Pharmacol.,* **1996**, *13*, 41-5.

[4] Klasen, H.J. A historical review of the use of silver in the treatment of burns. II. Renewed interest for silver. *Burns,* **2000**, *26*, 131-38.

[5] Dibrov, P.; Dzioba, J.; Gosnik, K.K.; Häse, C. Chemiosmotic mechanism of antimicrobial activity of Ag^+ in *Vibrio cholerae*. *Antimicrob. Agents. Chemother*., **2002**, *46*, 2668-70.

[6] Richard (III), J.W.; Spencer, B.A.; McCoy, L.F.; Carino, E.; Washington, J.; Edgar, P.; Rosenblatt, J.; Goodheart, R.; Heggers, J.P. Acticoat™ versus Silverlon® : the truth. *J. Burns and Surg. Wound Care,* **2002**, *1,* 11-18.

[7] Credé, C.S.E. Die verhürtung der augenentzündung der neugeborenen. *Archiv für Gynaekologie,* **1881***, 17*, 50-3.

[8] Heggers J.; Goodheart, R.; Washington, J.; McCoy, L.; Carino, E.; Dang, T.; Edgar, P.; Maness, C.; Chinkes, D. Therapeutic efficacy of three silver dressings in an infected animal model. *J. Burn Care Rehabil.,* **2005,** *2,* 53-6.

[9] South, J. Mild silver protein against internal and topical infections. www. smart-drugs.com/JamesSouth-silver.htm*,* **1999**.

[10] Wlodkowski, T.J.; Rosenkranz, H.S. Antifungal activity of silver sulfadiazine. *Lancet,* **1973**, *2*, 739-40.

[11] Feder, B. Scientists resurrecting use of silver as antiseptic. *The New York Times*, December **2005**.

[12] Pil, P.; Lippard, S. In *Encyclopedia of Cancer*; Bertino, J. R., Ed.; Academic Press: San Diego, **1997**; pp 392-410.

[13] Jakupec, M.; Galanski, M.; Keppler, B. Tumor-inhibiting platinum complexes-state of the art and future perspectives. *Rev. Physiol. Biochem. Pharmacol*., **2003**, *146*, 1-53.

[14] Haiduc, I.; and Silvestru, C. Rhodium, iridium, copper and gold antitumor organometallic compounds. *In Vivo,* **1989**, *3*, 285-93.

[15] Caruso, F.; Rossi, M.; Tanski, J.; Sartori, R.; Sariego, R.; Moya, S.; Diez, S.; Navarrete, E.; Cingolani, F.; Pettinari, C. Synthesis, structure, and antitumor activity of a novel tetranuclear titanium complex. *J. Med. Chem.,* **2000**, *43*, 3665-70.

[16] Caruso, F.; Rossi, M.; Tanski, J.; Pettinari, C.; Marchetti, F. Antitumor activity of the mixed phosphine gold species chlorotriphenylphosphine-1,3-bis(diphenylphosphino)propanegold(I). *J. Med. Chem.,* **2003**, *46*, 1737-42.

[17] Holler, E. In *Metal Complexes in Cancer Chemotherapy*; Keppler, B., Ed.; VCH Verlagsgesellschaft mbH: Weinheim, **1993**; p 65.

[18]. Furst, A; Schlauder, M. Inactivity of two noble metals as carcinogens. *J. Environ. Pathol. Toxicol.,* **1978**, *1*, 51-7.

[19] Pedahzur, R.; Lev, O.; Fattal, B.; Shuval, H.I. The interactions of silver ions and hydrogen peroxide in the inactivation of *E. coli*: a preliminary evaluation of a new long acting residual drinking water disinfectant. *Wat. Sci. Tech.,* **1995**, *31*, 123-9.

[20] Demerec, M.; Bertani, G.; Flint, J. A survey of chemicals for mutagenic action on *E. coli. Am. Nat*., **1951**, *85*, 119-36.

[21] Rossman, T.G.; Molina, M. The genetic toxicology of metal compounds: II. Enhancement of ultra violet light-induced mutagenesis in *Escherichia coli* WP2. *Environ. Mutagen.,* **1986**, *8*, 263-71.

[22] Nishioka, H. Mutagenic activities of metal compounds in bacteria. *Mutat. Res.,* **1975**, *31*,185-89.

[23] Stillman, M.J.; Presta, A.; Gui, Z.; Jiang, D.T. Spectroscopic studies of copper, silver, and gold metallothioneins. *Metal-Based Drugs,* **1994**, *1*, 375-94.

[24] Berners-Price, S.; Johnson, R.; Giovenella, A.; Faucette, L.; Mirabelli, C.; Sadler, P. Antimicrobial and anticancer activity of tetrahedral, chelated, diphosphine silver (I) complexes: comparison with copper and gold, *J. Inorg. Biochem.,* **1988**, *33*, 285-95.

[25] Newman, I.; Washburn, I. Ionic silver complex. US Patent No. 6, 838, 095*,* **2005**.

[26] Curran, R.; Lenehan, J.; McCann, M.; Kavanagh, K.; Devereux, M.; Egan, D.; Clifford, G.; Keane, K.; Creaven, B.; McKee, V. $[Ag_2(aca)_2]_n$ and $[Ag_4(aca)_4(NH_3)_2]$ (acaH=9-anthracenecarboxylic acid): synthesis, X-ray crystal structures, antimocrobial and anticancer activities. *Inorg. Chem. Commun.,* **2007**, *10*, 1149-53.

[27] National Cancer Institute. Cancer Screening Data. http://dtp.nci.nih.gov/dtpstandard/servlet/MeanGraphSummary?searchtype=namecontains&chemnameboolean=and&outputformat=html&searchlist=silver&Submit=Submit

[28] Batarseh, K.I. Anomaly and correlation of killing in the therapeutic properties of silver (I) chelation with glutamic and tartaric acids. *J. Antimicrob. Chemother.,* **2004**, *54*, 546-48.

[29] Batarseh, K.I. Apoptosis-inducing antineoplastic silver (I) coordination complexes. United States Patent Application No. 11/225, 565, **2006**.

[30] Batarseh, K.I. Apoptosis-inducing antineoplastic silver (I) coordination complexes. European Patent Application No. 1845997, **2007**.

[31] Batarseh, K.I. Apoptosis-inducing antineoplastic silver (I) coordination complexes. International Patent Application No. PCT/US 2006/000390, **2007**.

[32] Batarseh, K.I. Apoptosis-inducing antineoplastic silver (I) coordination complexes. Japanese Patent Application No. JP2007-550477, **2007**.

[33] Batarseh, K.I. A novel silver (I) chelate: cytotoxicity and apoptotic mechanism of action on human hematopoietic malignancies. *Leukemia* (Nature Publishing Group), **2008**, *22*, 448-50.

[34] Batarseh, K.I. Antitumor activity, structural and thermal properties of a novel silver (I)-tartaric acid chelate. Poster Session and Proffered Talk Presented at the American Association for Cancer Research (AACR) International Conference on *Advances in Cancer Research: From the Laboratory to the Clinic*, March 16-19, Dead Sea-Jordan, **2008**.

[35] Batarseh, K.I. A pharmaceutically small-novel organometallic complex: *in vivo* antineoplastic properties and kinetics on human hepatocellular carcinoma and acute myeloid leukemia. *Submitted.*

[36] Darzynkiewicz, Z.; Bedner, E.; Traganos, F.; Murakami, T. Critical aspects in the analysis of apoptosis and necrosis. *Hum. Cell,* **1998**, *1*, 3-11.

[37] Ferreira, C.; Epping, M.; Kruyt, F.; Giaccone, G. Apoptosis: target of cancer therapy. *Clin. Cancer Res.,* **2002**, *8*, 2024-34.

[38] Hengartner, M.O. The biochemistry of apoptosis. *Nature,* **2002**, *407*, 770-76.

[39] Becker, R.; Selden, G. *The Body Electric: Electromagnetism and the Foundation of Life,* William Morrow & Company: New York, **1985**.

[40] Becker, R. *Cross Current: The Promise of Electromedicine, the Perils of Electropollution*, Penguin Group: New York, **1990**.

[41] Daskalakis, M.; Nguyen, T.; Nguyen, C.; Guldberg, P.; Köhler, G.; Wijermans, P.; Jones, P.; Lűbbert, M. Demelthylation of a hypermethylated P15/INK4B gene in patients with myelodysplastic syndrome by 5-aza-2'-deoxycytidine (decitabine) treatment. *Blood,* **2002**, *100*, 2957-64.

[42] Sigalotti, L.; Altomonte, M.; Colizzi, F.; Degan, M.; Rupolo, M.; Zagonel, V.; Pinto, A.; Gattei, V.; Maio, M. 5-aza-2'-deoxycytidine (decitabine) treatment of hemotapoietic malignancies: a multimechanism therapeutic approach? *Blood,* **2003**, *101*, 4644-45.

[43] Das, P.; Singal, R. DNA methylation and cancer. *J. Clin. Oncol.,* **2004**, *22*, 4632-42.

[44] Jabbour, E.; Kantarjian, H.; Garcia-Manero, G.; Issa, J. Decitabine in myelodysplastic syndromes. *Future Drugs,* **2005**, *2*, 835-42.

[45] Karpf, A.; Lasek, A.; Ririe, T.; Hanks, A.; Grossman, D.; Jones, D. Limited gene activation in tumor and normal epithelial cells treated with the DNA methyltransferase inhibitor 5-aza-2'-deoxycytidine. *Mol. Pharmacol.,* **2004**, *65*, 18-27.

[46] Jain, R.K. Vascular and interstitial barriers to delivery of therapeutic agents in tumors. *Cancer Metastasis Rev.,* **1990**, *9*, 253-66.

[47] Forbes, N.S.; Munn, L.L.; Fukumura, D.; Jain, R. Sparse initial entrapment of systemically injected *Salmonella typhimurium* leads to heterogeneous accumulation within tumor necrosis. *Cancer Res.,* **2003**, *63*, 5188-93.

[48] Znati, C.A.; Rosenstein, M.; Mckee, T.D.; Brown, E.; Turner, D.; Bloomer, W.; Watkins, W.S.; Jain, R.; Boucher, Y. Irradiation reduces interstitial fluid transport and increases the collagen content in tumors. *Clin. Cancer Res.,* **2003**, *9*, 5508-13.

[49] Jain, R.K. Molecular regulation of vessel maturation. *Nat. Med.,* **2003**, *9*, 685-93.

[50] Jain, R.; Booth, M.F. What brings pericytes to tumor vessels? *J. Clin. Invest.,* **2003**, *112*, 1134-36.

[51] Jain, R.K. In *Clinical Oncology*, 3rd ed; Abeloff, M., Armtage, J., Niederhuber, J., Kastan, M., McKenna, W., Eds.; Elsevier: Philadelphia, **2004**; pp 153-72.

[52] Slapak, C.A.; Kufe, D.W. In *Harrison's Principles of Internal Medicine*,14th ed; Isselbacker, K. J., Ed.; McGraw-Hill: New York, **1998**; pp 523-537.

[53] Sweetman, S. C. *Martindale: The Complete Drug Reference,* 3rd Edition; Pharmaceutical Press: London-Chicago, **2002**; pp 525-27.

[54] ATSDR. Toxicological Profile for Silver. http://www.atsdr.cdc.gov/toxprofiles/tp146-p.pdf, **1990**.

[55] IRIS. Silver. http://www.epa.gov/iris/subst/0099.htm, **1987**.

[56] Shapiro, G.; Harper, W. Anticancer drug targets: cell cycle and checkpoint control. *J. Clin. Invest.,* **1999**, *104*, 1645-53.

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