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Research Article

Synthesis, Structure, and Anticancerous Properties of Silver Complexes

Shraddha Shukla and A. P. Mishra

Bio-Coordination Laboratory, Department of Chemistry, Dr. H. S. Gour University, Sagar 470 003, India

Correspondence should be addressed to Shraddha Shukla; shuklashraddha25@gmail.com

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Although the "war on cancer" is now in its fourth decade and much progress has been made in categorizing the environmental causes and cellular and molecular biological basis for this dreaded disease, we still do not have a precise understanding of the differences between a cancer cell and its normal counterpart. The completion of the human genome sequence and its subsequent improvements in the sequence data are important steps to fully comprehend cancer cell biology. Ag(I) is being used as an anticancer agent in several human cancers. The anticancer activity of these complexes against *Ehrlich's ascites* tumor cells (EACs) has been reported. The aim of the present study is to synthesize some new water soluble Ag(I) mixed ligand complexes containing nitrogen and sulfur base and to evaluate their biomedicinal properties. Mixed ligand complexes of Ag(I) metal derived from L-lysine and thiouracil with 2,2′-bipyridyl; 2-aminopyridine with thiouracil have been synthesized. The composition of the prepared complexes was discussed on the basis of microanalysis, FAB-mass, and FT-ir, measurements. Molecular weight was confirmed by FAB-mass spectra. The elemental analysis data suggest the stoichiometry to be $M : L : L' (1 : 1 : 1)$ ratio. These Ag(I) mixed ligand complexes showed excellent anticancer activity against *Ehrlich's ascites* tumor cells (EACs).

1. Introduction

Cancer is a disorder of the genetic makeup of somatic cells which results in a clone of cells with an abnormal pattern of growth control. The term cancer refers to any disease characterized by an accumulation of cells. It can result from either increased proliferation or failure of cells to undergo apoptosis in response to appropriate stimuli [1]. There is a variety of malignancies, but the most common types are cancers of lung, breast, colon and rectum, stomach, prostate, liver, cervix, and oesophagus. In a 2002 report from the GLOBOCAN database, cancer resulted to be second among the three leading causes of death in the developing countries. In the year 2002, it killed 6.7 million people around the world, more than the deaths caused by HIV/AIDS, tuberculosis, and malaria put together [2]. The strategies adopted to avoid this disease are prevention, early detection/screening, healthy diet, active lifestyle, and appropriate and effective treatment. Several treatment methods are used to treat cancer: surgery, radiotherapy, hormonal therapy, biotherapy, and chemotherapy. The radiotherapy is the simplest approach: it consists in the exposure of the cancer cells to an emission of radiations that will alter the composition of the genetic information of these dangerous cells. The hormone therapy exploits the hormones to suppress the hormonal activity that keep up the proliferation of some neoplasias. The employment of natural resources that have been modified, reinforced, or diverted from their usual role is the basis of biotherapy. Chemotherapy is the use of synthetic chemical substances or drugs extracted from plants: these compounds are toxic against cancer cells (cytotoxic), inhibiting their reproduction and division.

Conventional chemotherapeutic drugs are distributed nonspecifically in the body where they affect both cancerous and healthy cells, resulting in dose-related side effects and inadequate drug concentrations reaching the tumor. Nonspecific drug delivery leads to significant complications that represent a serious obstacle to effective anticancer therapy. In addition, the occurrence of resistance phenomena reduces

the efficacy of cancer treatment. To overcome the lack of specificity of conventional chemotherapeutic drugs, several ligand-targeted therapeutic strategies, including immune toxins, radio-immune therapeutics, and drug immune conjugates, are being developed. Nitrogen-sulphur-containing ligands such as Schiff bases and their metal complexes played an important role in the development of coordination chemistry, resulting in enormous publications, ranging from pure synthetic work to physiochemical [1] and biochemically relevant studies of metal complexes [2–6]. Other kinds of nitrogen-containing ligands are well-known pyrimidine systems such as purine analogues that exhibit a wide range of biological activities. Fused pyrimidine compounds are valued not only for their rich and varied chemistry, but also for many important biological properties. Among them, the fluropyrimidine ring system, because of a formal isoelectronic relationship with purine, is of special biological interest. It has numerous pharmacological and agrochemical applications, namely, antimalarials, antifolates, and antivirus, as well as potential radiation protection agents [7–13]. It has long been known that metal complex involves bonding to the heteroatoms of the heterocyclic residues of biological molecules, that is, proteins, enzymes, nucleic acids, and so on [14]. Late transition metals like silver have been used for years as anti-microbial agents. Silver has low toxicity as compared to other transition metals. One of the most commonly used compounds of silver is silver (I) sulfazine; it is used to treat severe burns to prevent them from bacterial infections [15]. Chlorhexidine, silver sulfadiazine is an anti-infective metal complex against catheter infections *in vivo* [16]. It is well established that only silver in its ionic or complexed forms is antimicrobially active, while the elemental silver, even in the so-called "nanocrystalline" state, is not [17]. Silver-containing compounds are attractive because of the fact that in the range of the applicable concentrations, silver ions do not exhibit toxicity and carcinogenic activities [18]. There is an increased interest in the potential use of silver (I) as a therapeutic agent for different antimicrobial applications.

From these points of view, it is interesting to study a different type of Ag (I) mixed ligand complexes of these biomedicinally active ligands. In this paper, the synthesis, characterization, and anticancerous properties of Ag (I) mixed ligand complexes are being reported.

2. Experimental

2.1. Materials and Methods. All syntheses were performed under dark condition and chemicals by Sigma Aldrich. Solvents were obtained from Merck (India); chemicals used were of analytical grade. Elemental analysis and FAB-mass spectra were recorded at SAIF-CDRI, Lucknow. FT-IR (in KBr) was recorded at Dr. H. S. Gour University Sagar (M.P.) India.

2.2. Synthesis of Complexes. A general synthesis route was used for Ag (I) mixed ligand complexes.

(i) $[Ag(bipy)(methi)]NO_3$: **(V)** silver nitrate in water was added to 2,2′-bipyridyl in methanol, this resulted in a colourless solution, to which aqueous solution of *d-l*-methionine was added. The reaction mixture was stirred in dark for 4-5 hours to produce grayish cream solid. It was filtered off, washed with little water, methanol, and diethyl ether, and then dried in vacuum. M.w.: 473, m.p.: 168°C, and yield: 98%. Elemental analysis found (calc.): C = 25.02 (25.27); H = 2.54 (2.54); N = 8.84 (8.80); S = 6.75 (6.67); Ag = 22.69 (22.42). FT-ir (KBr): Ag–N (485.9), Ag–O (522.0), C–S–C (618.4), C–N (1275.5), NO_3 (1383.7), C=N (1514.8), and C–H (2916.4).

(ii) $[Ag(bipy)(thioura)]NO_3$: **(W)** silver nitrate in water was added to 2,2′-bipyridyl in methanol; this resulted in a colourless solution, to which aqueous solution of thiouracil was added. The reaction mixture was stirred in dark for 5-6 hours to produce light orange solid. It was filtered off, washed with little water, methanol, and diethyl ether, and then dried in vacuum. M.w.: 450, m.p.: 150°C, yield: 94%. Elemental analysis found (calc.): C = 23.80 (23.27); H = 1.77 (1.54); N = 12.35 (12.30); S = 7.06 (7.03); Ag = 23.69 (23.75). FT-ir (KBr): Ag–S (456.0), Ag–N (525.7), C–N (1275.0), NO_3 (1382.8), C=N (1516.8), C=O (1686.9), C–H (2924), N–H (3461.0).

(iii) $[Ag(aminopy)(thioura)]NO_3$: **(X)** silver nitrate in water was added to 2-aminopyridine in water; this resulted in a colourless solution, to which aqueous solution of thiouracil was added. The reaction mixture was stirred in dark for 3-4 hours to produce light pink solid. It was filtered off, washed with little water, methanol, and diethyl ether, and then dried in vacuum. M.w.: 390, m.p.: 180°C, yield: 94%. Elemental analysis found (calc.): C = 27.57 (27.27); H = 2.06 (2.4); N = 14.29 (14.30); S = 8.17 (8.13); Ag = 27.61 (27.75). FT-ir (KBr): Ag–S (460.8), Ag–N (553.7), C–N (1276.9), NO_3 (1382.1), C=N (1555.2), C=O (1669.2), C–H (2929.1), and N–H (3429.6).

(iv) $[Ag(bipy)(lys)]NO_3$: **(Y)** silver nitrate in water was added to 2,2′-bipyridyl in methanol; this resulted in a colourless solution, to which aqueous solution of *l*-lysine was added. The reaction mixture was stirred in dark for 3-4 hours to produce light pink solid. It was filtered off, washed with little water, methanol, and diethyl ether, and then dried in vacuum. M.w.: 509, m.p.: 206°C, and yield: 92%. Elemental analysis found (calc.): C = 25.97 (25.95); H = 3.76 (3.70); N = 11.01 (11.04); Ag = 21.20 (21.05). FT-ir (KBr): Ag–O (526.5), Ag–N (546.8), C–N (1278.4), NO_3 (1383.8), C=N (1508.4), C=O (1629.6), and C–H (2987.5).

(v) $[Ag(bipy)(phena)]NO_3$: **(Z)** silver nitrate in water was added to 2,2′-bipyridyl in methanol; this resulted in a colourless solution, to which aqueous solution of 1,10-phenathroline was added. The reaction mixture was stirred in dark for 6-7 hours to produce light pink solid. It was filtered off, washed with little water, methanol, and diethyl ether, and then dried in vacuum. M.w.: 525, m.p.: 200°C, and yield: 90%. Elemental analysis found (calc.): C = 38.95 (38.79); H = 2.30 (2.41); N = 10.68 (10.54); Ag = 20.57 (20.58). FT-ir (KBr): Ag–N (523.7), C–N (1250.5), NO_3 (1383.8), C=N (1507.6), and C–H (2979.3).

2.3. Animals. Adult male Swiss albino mice (weight 20–25 gm) were obtained from experimental animal center,

Dr. H. S. Gour University, Sagar (M.P.) India. The animals were maintained on 12 hours light-dark cycle at room temperature (25 ± 2°C) and fed on a standard laboratory pellet diet and with water supplied *ad libitum*. Institute Animal Ethical Committee (IAEC), approved the study, and all the experiments were carried out by following the guidelines of CPCSEA, India.

2.4. Anticancer Activity against Ehrlich's ascites Carcinoma in Mice. All the compounds were screened for their anticancer activity by dissolving samples in minimum amount of water (complexes) and diluting with phosphate-buffered saline (PBS; pH = 7.2). The anticancer studies using *Ehrlich's ascites* tumor cells (EACs) were carried out by incubating 0.2 mL of cells IP. All the treatments started 24 hours after inoculation for 45 days. The tumor-bearing mice were divided into three groups. Group (1) is the standard one that received the 5-florouracil [19] (5-fu; 0.04 mg/mice/day) for comparison. Group (2) received **V, W, X, Y,**and **Z** Ag (I) complexes (0.01 mg/mice/day). Group (3) is the control one that received physiological saline (0.9% sodium chloride).

2.5. Determination of Tumor Volume. The mice were dissected, and the EACs fluid was collected from the peritoneal cavity. The volume was measured by taking it in a graduated centrifuge tube and packed cell volume determined by centrifuging at 1000 g for 5 min.

2.6. Determination of Tumor Cell Count. The EACs fluid was taken in RBCs pipette and diluted 1000 times. Then a drop of the diluted cell suspension was placed on the Neubauer counting chamber, and the number of cells in 64 small squares was counted.

2.7. Estimation of Viable Tumor Cell Count. The cells were then stained with Trypan blue (0.4% in normal saline) dye. The cells that did not take up the dye were viable, and those that took the stain were nonviable. These viable and nonviable cells were counted:

$$\text{Cell count} = \frac{(\text{No. of cells} \times \text{Dilution})}{(\text{area} \times \text{Thickness of liquid film})}. \quad (1)$$

2.8. Percentage Increase in Lifespan. Recording the mortality monitored, the effects of the Ag (I) complexes and 5-fu on tumor growth and percentage increase in lifespan (ILS%) were calculated:

$$\text{ILS}\,(\%) = \left[\left(\frac{\text{Mean survival of treated group}}{\text{Mean survival of control group}}\right) - 1\right] \times 100,$$

$$\text{Mean survival time} = \frac{[\text{1st Death} + \text{Last Death}]}{2}. \quad (2)$$

2.9. Hematological Studies. In order to detect the influence of complexes on the hematological status of EACs-bearing mice, comparison was made amongst groups of mice for each complex on the 40th day after transplantation.

The effect of the silver (I) complexes and 5-fu on peripheral blood was investigated. RBCs, WBCs counts, and estimation of hemoglobin were done by standard procedures from freely flowing tail vein blood.

3. Result and Discussion

3.1. Silver-Mixed Ligand Complexes. The reaction of silver nitrate salt with the different ligands resulted in the formation of silver (I) mixed ligand complexes. All complexes are stable and can be stored without any appreciable change. All complexes were characterized by several techniques using elemental analysis (C, H, N, and S), FT-IR, molar conductance, and FAB-mass spectra. The elemental analysis data suggest the stoichiometry to be 1 : 1 : 1 [L : M : L′] ratio. The molar conductance data measurements reveal the presence of 1 : 1 electrolytic nature of complexes. They are the least soluble in common organic solvents, such as methanol, ethanol, chloroform, or acetone; however, they are soluble in water and DMSO. Their structures were characterized by elemental and spectral data (Figure 1).

3.2. Anticancerous Activity. The reliable criteria for judging the efficacy of any anticancer drugs are prolongation of life span, improving the clinical, hematological, and biochemical profile, as well as reduction in viable tumor cell count in the host [20, 21]. We have observed that mixed ligand complexes of Ag (I) in water or DMSO exhibit potent cytotoxic activity against *Ehrlich's ascites* tumor cells (EACs). It is known that the anticancer available drugs affect the hematological and biochemical parameters (hemoglobin (Hb), red blood cells count (RBCs), and white blood cells count (WBCs); blood picture). The ultimate goal of this project is to develop mixed ligand complexes containing nitrogen bases effective against cancer without side effects on the hematological and biochemical parameters.

In order to detect the influence of **V, W, X, Y,** and **Z** on the hematological status of EACs-bearing mice, a comparison study was made among three groups of mice (each group contains ten mice) from the second day of inoculation. Group (I) is tumor-bearing mice treated with 5-fu, group (II) is tumor-bearing mice treated with Ag (I) complexes, and group (III) is the control tumor-bearing mice.

The anticancer activity of Ag (I) complexes shows remarkable efficacy manifested by survival and activity, as well as reduction of tumor size. **V, W,** and **X** Ag (I) complexes are more active in comparison to other **Y** and **Z** Ag (I) complexes because **V, W,** and **X** contain sulfur and nitrogen (Table 1 and Figure 2). The hematological parameters including Hb, RBCs, and WBCs data are reported in Table 2. It is clear that the hematological parameters of tumor-bearing mice treated with Ag (I) complexes exhibit better doses of 0.01 mg/mice/day with the standard 5-fu, market drug (~0.4 mg/mice/day).

3.3. The Side Effect and Toxicity. The side effect and toxicity of silver metal complexes have been detected. After the first

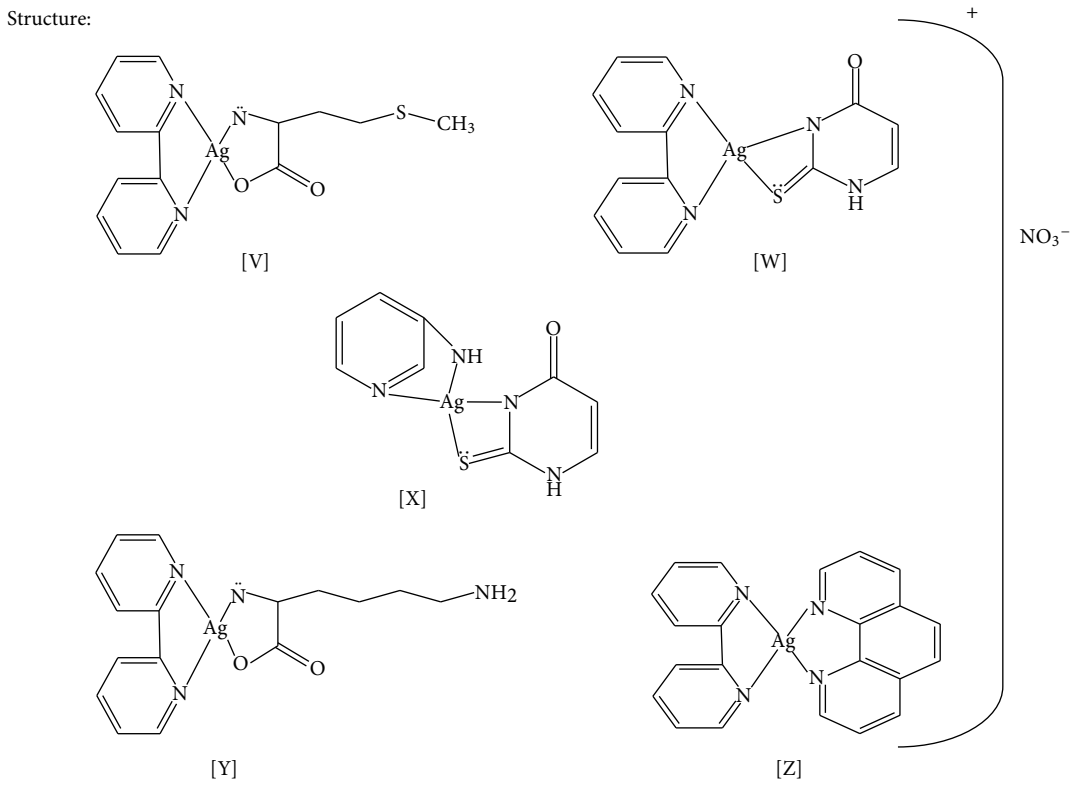

Figure 1: Proposed tentative structure of Ag (I) complexes.

Table 1: Comparative effects of treatments versus Ag (I) complexes on reduction of body weights in tumor-induced mice.

Ag (I) complex	% Decrease in weight as compared to respective control		
	15 days	30 days	45 days
V	42.52 ± 11.32	67.12 ± 07.12	91.21 ± 10.27
W	40.16 ± 10.30	60.76 ± 11.0	89.19 ± 11.03
X	45.22 ± 09.23	65.22 ± 11.23	93.22 ± 10.13
Y	36.62 ± 11.42	43.12 ± 12.12	60.28 ± 12.25
Z	33.29 ± 11.37	51.19 ± 10.07	67.09 ± 09.57

week of the treatment, the mice show flu-like attack and in the third week show spot dropping on the hair (alopecia).

The study of detailed mechanism and *in vivo* anticancer screens (phase II and III) using the studied complexes are underway.

3.4. Future Direction. Therapeutic application of silver metal complexes is an underdeveloped area of research and is full of opportunities for further progress. Basic principles to guide the synthesis and development of silver-metal-based pharmaceuticals are lacking. Development of new methodologies such as combinatorial chemistry will be helpful in the synthesis of inorganic compounds as therapeutic agents. Similarly, the action of metal complexes in the whole living organisms is expected to differ in general from the action of nonmetal containing agents and may offer unique research and diagnostic, or therapeutic opportunities. The compounds tested in our present study have shown cytotoxic activity when screened using the *in vitro* method and at the same time have shown to have good activity when tested using the *Ehrlich's ascites* carcinoma model. However, it is very difficult to conclude anything at this stage, and it can be assumed that after testing against various other cancer models and at different doses such compounds may prove to be safer drugs for future.

4. Conclusion

Some novel water soluble Ag (I) mixed ligand complexes containing nitrogen and sulphur base have been synthesized. The mixed ligand systems involved bioactive molecules L-lysine, thiouracil, 2,2′-bipyridyl, and 2-aminopyridine.

Table 2: Effect of the Ag (I) complexes on hematological parameters.

Group	Hematological parameters					
	RBCs	Hb	WBCs	Differential leucocytes count %		
				Lymphocytes	Neutrophils	Monocytes
Control	2.15 ± 0.11	10.23 ± 0.2	32.15 ± 0.9	41.18 ± 0.17	57.15 ± 0.21	1.11 ± 0.9
V	4.31 ± 0.12	17.15 ± 0.9	11.32 ± 0.11	79.05 ± 0.67	25.13 ± 0.11	1.07 ± 1.1
W	3.85 ± 0.07	15.27 ± 0.1	10.65 ± 0.7	68.34 ± 0.50	23.23 ± 0.01	1.02 ± 1.0
X	5.13 ± 0.15	19.20 ± 0.7	10.96 ± 0.10	81.23 ± 0.11	27.31 ± 0.13	1.10 ± 1.3
Y	3.10 ± 0.01	11.35 ± 0.1	21.13 ± 0.5	61.27 ± 0.09	30.45 ± 0.27	1.13 ± 1.1
Z	3.09 ± 0.16	10.85 ± 0.3	19.21 ± 0.6	60.19 ± 0.60	29.37 ± 0.10	1.17 ± 1.1
5-fu	3.05 ± 0.10	12.15 ± 0.6	10.14 ± 0.1	72.20 ± 0.19	31.20 ± 0.19	1.15 ± 1.3

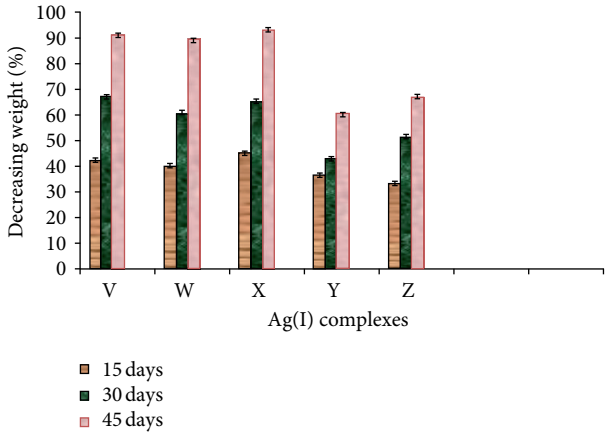

Figure 2: Comparative effects of treatments versus Ag (I) complexes on reduction of body weights in tumor-induced mice.

The synthesized complexes have been characterized to ascertain tentative structure, and the biomedical properties have been evaluated. The compounds have been designed to suit for interaction with nucleic acids and proteins. These Ag (I) mixed ligand complexes showed appreciably good anticancer activity against *Ehrlich's ascites* tumor cells. The findings reflect that these compounds might be having some active antineoplastic principle.

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