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Novel thiocyanato complexes with potent cytotoxic and antimicrobial properties

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Abstract

The aim of the present study was to assess the cytotoxic and antimicrobial properties of seven new thiocyanato complexes: Ni($C_9H_{11}N_2O$)(SCN), Cu($C_9H_{11}N_2O$)(SCN), Pd($C_9H_{11}N_2O$)(SCN), Pt($C_9H_{11}N_2O$)(SCN), K[Ti($C_9H_{11}N_2O$)$(SCN)_3$], Au($C_9H_{11}N_2O$)(SCN), and K[V(O)($C_9H_{11}N_2O$)(SCN)] (T_1–T_7, respectively). All the complexes showed toxicity against brine shrimp nauplii (*Artemia salina* L.). The titanium-based complex, T_5, exhibited potent toxicity, with a lethal concentration 50% (the concentration of test compound that kills 50% of *A. salina*) value of 1.59 μg mL^{-1}. These new complexes also exhibited promising antibacterial and antifungal properties. A macrodilution technique was used to estimate the minimum inhibitory concentrations of the seven bioactive complexes. Minimum inhibitory concentrations were found to be 8–64 μg mL^{-1} against the tested bacterial species.

Introduction

Coordination complexes of transition metals have been widely studied for their antimicrobial (Islam et al 2002a; Kamalakannan & Venkappayya 2002) and anticancer properties (Treshchalina et al 1979; Brown et al 1982; Mirabelli et al 1987; Kelland et al 1994; Amirkhanov et al 1999; Rho et al 2002). One of the most potent and effective antitumour agents was discovered serendipitously by Rosenberg et al (1965). They synthesized several simple platinum complexes, among which cisplatin (Pt(II)$(NH_3)_2Cl_2$) showed remarkable efficacy in inhibiting the growth of tumours in mice (Rosenberg et al 1969). Over the past 30 years, platinum-based drugs, notably cisplatin and carboplatin, have dominated the treatment of various cancers by chemical agents. McGowan (2001) reported the first clinical trials of cisplatin in 1971, with official approval being granted in the USA in 1978. By 1983, cisplatin was the biggest selling antitumour drug in the USA and is still one of the most widely used antitumour drugs. It is one of the most effective drugs for treating testicular, ovarian, bladder and neck cancers. Despite the success of cisplatin, however, it lacks selectivity for tumour tissue, which leads to severe side-effects, including renal impairment, neurotoxicity and ototoxicity. Various tumour cell lines are now becoming resistant to cisplatin, for example, acquired cisplatin resistance in some pre-clinical tumour models (Kelland 1993).

Researchers are now exploring other transition metal complexes as antitumour agents and considerable results have brought through the discovery of titanium-based complexes (Kurbacher et al 1994; Friedrich et al 1998) and other transition metal complexes (Mishra et al 1995; Bacchi et al 1999; Ghosh et al 2000; Joudah et al 2002; Shrivastav et al 2002; Vijayalakshmi et al 2002; Quievryn et al 2003). Among the other transition metal complexes, the titanium complex titanocenedichloride ($TiCp_2Cl_2$) is the only metallocene-based compound to have entered clinical trials for its potent and broad spectrum activity against mammalian tumours (McGowan 2001). Compared with standard antineoplastic agents such as cisplatin, doxorubicin, mitoxantrone and vinblastine, titanocenedichloride was found to exhibit greater cytotoxicity against renal cell carcinoma (Kurbacher et al 1994). It was also found to be more effective in a human ovarian cancer xenograft model than cisplatin (Friedrich et al 1998). Recently, some derivatives of titanocenedichlorides were shown to have enhanced anticancer activity (Boyles et al 2001).

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Given the considerable success of coordination complexes, researchers are keen to discover new bioactive complexes with potential antitumour and antimicrobial effects to be used against cancer and other infectious diseases, such as AIDS and SARS. We examined the cytotoxic and antimicrobial properties of some novel coordination complexes of different transition metals to assess their biological potency.

Materials and Methods

Test organisms

The bacterial species used in this experiment were *Streptococcus β-haemolyticus* (ATCC-12873), *Staphylococcus aureus* (ATCC-25933), *Pseudomonas aeruginosa* (ATCC-27853), *Escherichia coli* (ATCC-25922) and *Salmonella typhi* (ATCC-26853), all of which were obtained from the Institute of Nutrition and Food Sciences, Dhaka University, Bangladesh. The fungi, *Candida albicans* (ATCC 10231), *Aspergillus fumigatus* (ATCC 1028) and *Aspergillus niger* (CCRC 31494), were obtained from stock cultures at the Institute of Biological Sciences, Rajshahi University, Bangladesh.

Study design

Seven novel complexes were obtained from the Inorganic Research Laboratory, Department of Chemistry, Rajshahi University, Bangladesh. Chemical characterization of these bioactive complexes has been published previously (Islam et al 2002b). To determine the cytotoxicity of the complexes, we used the brine shrimp (*Artemia salina* L.) lethality bioassay and measured the lethal concentration 50% (LC50: the concentration of test compound that kills 50% of *A. salina*) values after probit transformation to assess their potency. Antibacterial activity was determined by the disc-diffusion method (Bauer et al 1966; Rios et al 1988). Minimum inhibitory concentration (MIC) values for the complexes were determined against pathogenic bacteria. We also determined the antifungal activity of the complexes against some pathogenic fungi.

Synthesis of complexes

A tridentate ligand, $C_9H_{12}N_2O$, having ONN donor sequences, was synthesized by the condensation of ethylenediamine with salicylamide. The ligand undergoes deprotonation during complexation and forms complexes of the composition: $Ni(C_9H_{11}N_2O)(SCN)$, $Cu(C_9H_{11}N_2O)(SCN)$, $Pd(C_9H_{11}N_2O)(SCN)$, $Pt(C_9H_{11}N_2O)(SCN)$ (where $C_9H_{11}N_2O$ is the deprotonated schiff base), $K[Ti(C_9H_{11}N_2O)(SCN)_3]$, $Au(C_9H_{11}N_2O)(SCN)$, and $K[V(O)(C_9H_{11}N_2O)(SCN)]$ (T_1–T_7, respectively). These complexes were characterized on the basis of elemental analysis, conductivity and magnetic measurements, nuclear magnetic resonance, infrared and electronic spectral studies (Islam et al 2002b).

Brine shrimp lethality bioassay

The brine shrimp lethality bioassay (Persoone et al 1980; Mayer et al 1982; McLaughlin & Anderson 1988; McLaughlin 1991; Jaki et al 1999) is a recent development in the assay procedure of bioactive compounds, which indicates cytotoxicity as well as a wide range of pharmacological activities (e.g. anticancer, antiviral, insecticidal, pesticidal) of the compounds. The shrimp lethality assay was proposed by Michael et al (1956) and later developed by Vanhaecke et al (1981) and Sleet & Brendel (1983). It is based on the ability to kill laboratory-cultured brine shrimp (*Artemia* nauplii). The assay is considered a useful tool for preliminary assessment of toxicity (Solis et al 1993) and it has been used for the detection of fungal toxins (Harwig & Scott 1971), plant extract toxicity (McLaughlin et al 1991), heavy metals (Martinez et al 1998), cyanobacterial toxins (Jaki et al 1999), pesticides (Barahona & Sanchez-Fortun 1999), and cytotoxicity testing of dental materials (Pelka et al 2000).

In the present study, in-vivo lethality tests were carried out using brine shrimp nauplii eggs (*A. salina* L.). Eggs were placed in one side of a small tank divided by a net containing 3.8% NaCl solution for hatching. In the other side of the tank, a light source was placed in order to attract the nauplii. After a 2-day hatching period, the nauplii were ready for the experiment. The complexes (3 mg) were accurately measured and dissolved in 0.6 mL of dimethylsulfoxide (DMSO) to give a concentration of $5\,mg\,mL^{-1}$. From the stock solutions, 1, 2, 5, 10, 20, 40 and 80 μL were placed in seven different vials and the volume was made up to 5 mL with NaCl solution. The final concentrations of the samples in the vials were 1, 2, 5, 10, 20, 40 and 80 $\mu g\,mL$, respectively.

Ten brine shrimp nauplii were then placed in each vial. For the control test of each vial, one vial containing the same volume of DMSO plus seawater up to 5 mL was used. After 24 h of incubation, the vials were observed using a magnifying glass and the number of survivors in each vial were counted. The resulting data were transformed to the probit analysis (Finney 1971) for the determination of LC50 values for the complexes.

Antibacterial screening

In-vitro antibacterial screening is generally performed by the disc-diffusion method (Bauer et al 1966; Rios et al 1988) for primary selection of compounds as therapeutic agents. The disc-diffusion method is highly effective for rapidly growing microorganisms and the activities of the test compounds are expressed by measuring the diameter of the zone of inhibition. Generally, the more susceptible the organism, the bigger the zone of inhibition. The method is essentially a qualitative or semiquantitative test, indicating sensitivity or resistance of microorganisms to the test materials as well as bacteriostatic or bactericidal activity of a compound (Reiner 1982).

The antibacterial activity of the complexes T_1–T_7 was determined at a concentration of 30 μg/disc and 200 μg/disc against two Gram-positive (*S. aureus* and *S. β-haemolyticus*)

and three Gram-negative (*S. typhi*, *P. aeruginosa* and *E. coli*) bacteria. The diameters of the zones of inhibition produced by the compounds were compared with the standard antibiotic (ciprofloxacin 30 μg/disc). The experiments were performed three times to minimize error.

Growth media and conditions

Nutrient agar was used to culture the pathogenic bacteria. Nutrient broth was used as liquid culture of all the tested bacteria and was used in the MIC determinations. In all cases of bacterial culture the temperature was maintained at 37°C. Potato dextrose agar medium was prepared in the laboratory to maintain the fungal growth. The antifungal activity of the complexes was determined on potato dextrose agar Petri dishes spread with fungal spores and kept at 28°C for about 72 h. To prepare potato dextrose agar, 20 g potato was extracted with distilled water (100 mL) at 100°C for 1 h and it was then filtered off using a cotton filter. The potato juice (100 mL) was then mixed with 2 g dextrose and 1.5 g agar, and the pH of the prepared medium was adjusted to 7.00.

MIC measurements

A current definition of the MIC is the lowest concentration that results in maintenance or reduction of inoculum viability (Carson et al 1995). Determination of the MIC involves a semiquantitative test procedure, which gives an approximation to the least concentration of an antimicrobial needed to prevent microbial growth. The method involves tubes of growth broth containing a test level of preservative, into which an inoculum of microbes is added. The end result of the test is the minimum concentration of antimicrobial (test material) that gives a clear solution (i.e. no visible growth) (Collins 1964; Davidson & Parish 1989). The serial dilution technique (Reiner 1982) was used for the determination of the MIC of the complexes. Four bacterial species were used: *S. aureus*, *S. β-haemolyticus*, *S. typhi* and *E. coli*. Replicate analyses were performed.

DMSO was used to make dilutions of the coordination complexes for MIC determinations. Bacteria were incubated on nutrient agar slants for 18 h at 30°C. MIC tests were run with the third subculture of bacteria and samples were taken during the exponential phase of bacterial growth. Bacterial inocula were prepared at 5×10^6–5×10^7 CFU mL^{-1}. The optical density was measured at an absorbance of 0.05 and a wavelength of 660 nm.

Antifungal screening

The antifungal activity of the complexes was determined by the disc-diffusion method (Bauer et al 1966; Rios et al 1988) against three pathogenic fungi, *C. albicans*, *A. niger* and *A. fumigatus*, at a concentration of 200 μg/disc for each. The medium used was potato dextrose agar. The activity was determined after 72 h of incubation at room temperature (30°C). All experiments were performed in triplicate.

Statistical analysis

Statistical analysis of the antibacterial and antifungal activities of the novel thiocyanato complexes with different concentrations of each (30 and 200 μg/disc) was performed using the Kruskal–Wallis test (Shil & Debnath 2001). Differences in individual antibacterial and antifungal activity of the tested complexes (T_1–T_7) were examined using post-hoc Nemenyi's test following the Kruskal–Wallis test. A level of $P < 0.05$ denoted significance in all cases.

Probit analysis (Finney 1971) was used to determine the LC50 values from the mortality data. The cytotoxicity of the novel thiocyanato coordination complexes was compared with the standard gallic acid and also with the anticancer agent bleomycin. Probit software was used to calculate the LC50 value from the mortality data. Determination of LC50 by probit analysis allowed the ranking of these coordination complexes with respect to their biocidal activity.

Results

Antibacterial activity

At a concentration of 30 μg/disc, the complexes T_1, T_2, T_6 and T_7 did not show remarkable biocidal activity, whereas the palladium-, platinum- and titanium-based complexes, T_3, T_4 and T_5, showed modest antibacterial activity against the tested Gram-positive and Gram-negative bacteria (Table 1). The palladium complex T_3 showed promising antibacterial activity (zones of inhibition 28–31.2 mm) at a concentration of 200 μg/disc in comparison with ciprofloxacin (zones of inhibition 26.3–29.3 mm), but it was not statistically significant. The complexes T_1, T_2, T_6 and T_7 showed promising antibacterial activity against the Gram-positive bacteria at a concentration of 200 μg/disc, but T_1, T_2 and T_7 were inactive against some Gram-negative bacteria (Table 1). At a concentration of 200 μg/disc, the palladium-, platinum- and titanium-based complexes T_3, T_4 and T_5 showed remarkable antibacterial activity against the tested bacteria in comparison with ciprofloxacin. In the present study, we found that the complexes showed comparatively greater antibacterial activity against Gram-positive bacteria compared with Gram-negative bacteria. The antibacterial activity of different transition metal complexes has been reported. Islam et al (2002a) reported the activity of Cu-, Ni- and Co-based coordination complexes and found zones of inhibition of 14–30 mm against the tested bacterial species. Sultana et al (2003) reported the activity of Cd-, Sb- and As-based coordination complexes and found zones of inhibition by the disc-diffusion method to be 8–25 mm against different pathogenic bacteria. In our previous investigation of the novel titanium-based coordination complexes (Sheikh et al 2004), we found the zones of inhibition to be 9–18 mm at 200 μg/disc. In the present study, we found the zones of inhibition for the thiocyanato complexes to be 9.3–17.3 mm at 30 μg/disc and 9.3–31.2 mm at 200 μg/disc (Table 1), which is similar to previous reports.

Table 1 In-vitro antibacterial activity of the coordination complexes T_1–T_7 (30 and 200 μg/disc) and standard ciprofloxacin by the disc-diffusion method

	Diameter of zone of inhibition (mm)														
	T_1		T_2		T_3		T_4		T_5		T_6		T_7		Ciprofloxacin
	30	200	30	200	30	200	30	200	30	200	30	200	30	200	30
Gram-positive bacteria															
Staphylococcus aureus	9.5 ± 0.5	13 ± 0.5	11.2 ± 0.6	18.5 ± 0.5	17.3 ± 0.3	29.2 ± 1.04	14.5 ± 0.5	29.3 ± 1.04	12.8 ± 0.76	23 ± 0.5	10 ± 1.0	16.2 ± 0.3	13.2 ± 1.04	20.2 ± 1.04	27 ± 1.0
Streptococcus β-haemolyticus	10.5 ± 0.5	11.6 ± 0.6	12.8 ± 0.8	18.6 ± 0.6	16.6 ± 0.6	31.2 ± 0.8	13.5 ± 0.5	28.3 ± 0.6	13.5 ± 0.5	23.7 ± 0.7	10.7 ± 0.6	18.5 ± 0.5	12.5 ± 0.5	20.8 ± 0..8	28.6 ± 1.5
Gram-negative bacteria															
Salmonella typhi	9.3 ± 0.6	11.2 ± 0.3	00 ± 00	00 ± 00	16.7 ± 0.6	29.7 ± 0.6	12.8 ± 0.3	24.7 ± 0.6	10.7 ± 0.6	18.3 ± 0.6	9.3 ± 0.6	16.2 ± 1.04	00 ± 00	00 ± 00	29.3 ± 1.5
Pseudomonas aeruginosa	00 ± 00	00 ± 00	10.5 ± 0.5	15.5 ± 0.5	13.0 ± 1.0	28.0 ± 1.0	13.8 ± 0.8	25.2 ± 1.0	12.5 ± 0.5	20.3 ± 0.6	00 ± 00	14.2 ± 0.8	00 ± 00	00 ± 00	28.3 ± 1.5
Escherichia coli	00 ± 00	9.3 ± 0.6	00 ± 00	14.3 ± 0.6	13.8 ± 0.8	29.2 ± 0.8	12.3 ± 0.6	21.2 ± 0.8	10.8 ± 0.3	15.5 ± 0.5	9.3 ± 0.6	15.1 ± 1.2	9.3 ± 0.6	14.2 ± 0.8	26.3 ± 1.5

Data are mean ± s.d.

MIC

The MIC values of the complexes against *S. aureus*, *S. β-haemolyticus*, *S. typhi* and *E. coli* are shown in Table 2. The MIC values of the complexes T_2, T_6 and T_7 against the tested bacteria were not different (32 $\mu g\,mL^{-1}$ for Gram-positive and 64 $\mu g\,mL^{-1}$ for Gram-negative), indicating that the complexes were more active against Gram-positive bacteria than Gram-negative bacteria. For T_1, the MIC values were 64, 32, 64 and 64 $\mu g\,mL^{-1}$ against the tested bacteria, respectively. The complexes T_3, T_4 and T_5 showed MIC values of 8–16 $\mu g\,mL^{-1}$, indicating more potent antibacterial activity compared with the other tested complexes.

Antifungal activity

Table 3 shows that, with the exception of T_1, the complexes were active against the tested fungi at a concentration of 200 μg/disc compared with the standard nystatin. The maximum zones of inhibition against *A. niger* and *A. fumigatus* were 21 and 22 mm, respectively, for the complex T_3, which were similar to the zones of inhibition of 23 and 24 mm of the standard nystatin. Among the other complexes, the vanadium(IV)-based complex T_7 showed a maximum zone of inhibition against *C. albicans*. This was an interesting finding as we did not find any report of antifungal activity for vanadium-based complexes. Islam et al (2002a) reported the antifungal activity of Cu-, Ni- and Co-based coordination complexes and found zones of inhibition of 8–28 mm against the tested fungi. Sultana et al (2003) reported the activity of Cd-, Sb- and As-based complexes and found zones of inhibition of 8–20 mm at 200 μg/disc against different fungi. In our previous investigation for novel titanium-based complexes, we found zones of inhibition of 9–16 mm against *C. albicans*, *A. niger* and *A. fumigatus*. (Sheikh et al 2004). The present results of zones of inhibition of 9–22.3 mm for the tested novel complexes are supported by the previous studies.

Cytotoxicity

The mortality rate of brine shrimp nauplii was found to increase with increasing concentrations of the complexes. The LC50 values of the complexes T_1, T_2, T_3, T_4, T_5, T_6 and T_7 were 6.49, 2.28, 5.56, 3.01, 1.59, 7.08, 9.80, 0.41 and 4.53 $\mu g\,mL^{-1}$ (ppm), respectively (Table 4). The standard anticancer drug bleomycin had an LC50 value of 0.41 $\mu g\,mL^{-1}$. The lowest LC50 value of 1.59 ppm was found in the case of the titanium-based complex T_5, indicating its more potent cytotoxicity compared with the other coordination complexes in this study. Titanium-based complexes have been reported previously for their potent cytotoxic properties compared with platinum-based complexes (Kurbacher et al 1994; Friedrich et al 1998). Our present findings support the previous studies as the titanium-based complex T_5 (LC50 = 1.59 ppm) was more cytotoxic than the platinum-based complex T_4 (LC50 = 3.01 ppm). The copper(II)- and platinum(II)-based complexes T_2 and T_4 showed greater cytotoxicity compared with the control DMSO and gallic acid, which was used as standard agent (Sarkar et al 1988). Here, DMSO was used as solvent only.

Table 2 In-vitro antibacterial activity of the coordination complexes T_1–T_7 and standard ciprofloxacin by the macro-dilution method

Test organism	Minimum inhibitory concentration ($\mu g\,mL^{-1}$)							
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	Ciprofloxacin
Staphylococcus aureus	64	32	8	16	8	32	32	2
Streptococcus β-haemolyticus	32	32	16	8	8	32	32	2
Salmonella typhi	64	64	16	16	16	64	64	2
Escherichia coli	64	64	16	16	16	64	64	4

Table 4 Cytotoxic effects of the complexes T_1–T_7, standard bleomycin and gallic acid

Test sample		LC50 (ppm)	95% confidence limit (ppm)	
			Lower	Upper
$Ni(C_9H_{11}N_2O)(SCN)$	T_1	6.49	4.16	10.16
$Cu(C_9H_{11}N_2O)(SCN)$	T_2	2.28	1.36	3.84
$Pd(C_9H_{11}N_2O)(SCN)$	T_3	5.56	3.52	8.79
$Pt(C_9H_{11}N_2O)(SCN)$	T_4	3.01	1.94	4.67
$K[Ti(C_9H_{11}N_2O)(SCN)_3]$	T_5	1.59	1.06	2.38
$Au(C_9H_{11}N_2O)(SCN)$	T_6	7.08	4.16	12.05
$K[V(O)(C_9H_{11}N_2O)(SCN)]$	T_7	9.80	5.66	16.99
Standard bleomycin		0.41	0.27	0.62
Gallic acid		4.53	3.33	6.15

Table 3 In-vitro antifungal activity of the complexes T_1–T_7 (200 μg/disc) and standard nystatin by the disc-diffusion method

Test organism	Diameter of zone of inhibition (mm)							
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	Nystatin
Candida albicans	00 ± 00	16.3 ± 1.2	15.8 ± 1.7	15.8 ± 1.0	18.3 ± 1.5	11.6 ± 1.52	22.3 ± 1.52	25.3 ± 1.2
Aspergillus niger	9.0 ± 00	12.0 ± 1.0	21.3 ± 1.5	17.3 ± 1.5	20.0 ± 1.0	13.3 ± 0.6	15.6 ± 0.6	23.6 ± 0.6
Aspergillus fumigatus	9.6 ± 0.6	13.6 ± 1.2	22.3 ± 1.2	21.3 ± 0.6	18.6 ± 0.6	14.3 ± 0.6	16.0 ± 1.0	24.6 ± 1.2

Data are mean ± s.d.

In the present study, the other transition metal nickel(II)-, copper(II)-, palladium(II)- and vanadium(IV)-based complexes also showed toxicity against *Artemia*. The maximum LC50 value (9.80 ppm) was shown by the vanadium(IV)-based complex T_7.

Discussion

Compared with standard antineoplastic agents such as cisplatin, doxorubicin, mitoxantrone and vinblastine, titanocenedichloride (titanium complex) was found to exhibit greater cytotoxicity against renal cell carcinoma (Kurbacher et al 1994). The titanium-based complexes were also found to be more effective than cisplatin in a mammalian cancer model (Friedrich et al 1998). Therefore, it was of interest to explore some novel transition-metal-based complexes as potentially potent cytotoxic agents.

In the present study, we found a novel titanium(III)-based complex T_5 with potent cytotoxicity (LC50 = 1.59 ppm). Among the six other novel thiocyanate complexes, only T_2 (LC50 = 2.28 ppm) and T_4 (LC50 = 3.01 ppm) showed promising cytotoxic effects compared with the reference standard gallic acid (LC50 = 4.53 ppm). The cytotoxic and antitumour properties of coordination complexes have been previously reported by others (Treshchalina et al 1979; Brown et al 1982; Mirabelli et al 1987; Berners-Price et al 1990; Kelland et al 1994; Amirkhanov et al 1999; Carotti et al 2000; Coronnello et al 2000; Rho et al 2002). The present findings also indicate the cytotoxicity of the newly synthesized thiocyanato complexes against *A. salina*. The different LC50 values of the thiocyanato complexes indicate their different cytotoxic potencies and possibly different mechanisms of action. Further investigation is required to explore the exact mechanism of their cytotoxic properties.

The newly synthesized complexes displayed poor antibacterial activity at the lower concentration of 30 μg/disc (zones of inhibition of 9.3–17.3 mm), but had promising antibacterial activity at the higher concentration of 200 μg/disc (zones of inhibition up to 31.2 mm). The MIC values (8–16 $\mu g\,mL^{-1}$) of the palladium-, platinum- and titanium-based complexes (T_3, T_4 and T_5) against the tested bacteria indicated their considerable antibacterial potency compared with ciprofloxacin (2–4 $\mu g\,mL^{-1}$). Vijayalakshmi et al (2002) and Joudah et al (2002) reported that the mechanism of biocidal activity of some metal coordination complexes involved oxidative DNA damage. The cytotoxic nature of the novel thiocyanato complexes may also be due to oxidative DNA damage, but further investigations are required to confirm this activity.

The nickel(II)-based complex (T_1) showed poor antifungal activity but the palladium(II)-, platinum(II)-, titanium(III)- and vanadium(IV)-based complexes (T_3, T_4, T_5 and T_7) showed considerable antifungal activity, indicating their potential as antifungal agents. Different ligands can modify the antifungal activity of metal-based complexes, so proper ligand selection is an important consideration. The present findings may also prompt a new search for the palladium-, platinum- and titanium-based complexes for use against fungal diseases.

In conclusion, the novel complexes described here show promising cytotoxic and antimicrobial activities. Further studies on mammalian cancer cell lines are necessary to explore their cytotoxicity and potential as anticancer agents in clinical trials.

References

Amirkhanov, V. M., Bundya, E. A., Trush, V. A., Ovchynnikov, V. A., Zaitsev, V. N. (1999) Coordination compounds of Co(II), Ni(II), Mn(II), and Zn(II) with new representative of carbacylamidophosphates – potential anticancer drugs. 5th International Symposium on Applied Bioinorganic Chemistry. Corfu, Greece

Bacchi, A., Carcelli, M., Pelagatti, P., Pelizzi, C., Pelizzi, G., Zani, F. (1999) Antimicrobial and mutagenic activity of some carbono- and thiocarbonohydrazone ligands and their copper(II), iron(II) and zinc(II) complexes. *J. Inorg. Biochem.* **15**: 123–133

Barahona, M. V., Sanchez-Fortun, S. (1999) Toxicity of carbamates to the brine shrimp *Artemia salina* and the effect of atropine, BW284c51, iso-OMPA and 2-PAM on carbaryl toxicity. *Environ. Pollut.* **104**: 469–476

Bauer, A. W., Kirby, W. M., Sherries, J. C., Turck, M. (1966) Antibiotic susceptibility testing by standardised single disc method. *Am. J. Clin. Pathol.* **44**: 493–496

Berners-Price, S. J., Girard, G. R., Hill, D. T., Sutton, B. M., Jarrett, P. S., Faucette, L. F., Johnson, R. K., Mirabelli, C. K., Sadler, P. J. (1990) Cytotoxicity and antitumor activity of some tetrahedral bis(diphosphino)gold(I) chelates. *J. Med. Chem.* **33**: 1386–1392

Boyles, J. R., Bair, M. C., Campling, B. G., Jain, N. (2001) Enhanced anti-cancer activities of some derivatives of titanocene dicholoride. *J. Inorg. Biochem.* **84**: 159–162

Brown, D. B., Khokhar, A. R., Hacker, M. P., Lokys, L., Burchenal, J. H., Newman, R. A., McCormack, J. J., Frost, D. (1982) Synthesis and antitumor activity of new platinum complexes. *J. Med. Chem.* **25**: 952–956

Carotti, S., Marcon, G., Marussich, M., Mazzei, T., Messori, L., Mini, E., Orioli, P. (2000) Cytotoxicity and DNA binding properties of a chloro glycylhistidinate gold(III) complex (GHAu). *Chem. Biol. Interact.* **15**: 29–38

Carson, C. F., Hammer, K. A., Riley, T. V. (1995) Broth microdilution method for determining the susceptibility of *Escherichia coli* and *Staphylococcus aureus* to the essential oil of *Melaleuca alternifolia* (tea tree oil). *Microbios* **82**: 181–185

Collins, C. H. (1964) Antibiotics and antibacterial substances. In: *Microbiological methods*. Butterworths, London, pp 296–305

Coronnello, M., Marcon, G., Carotti, S., Caciagli, B., Mini, E., Mazzei, T., Orioli, P., Messori, L. (2000) Cytotoxicity, DNA damage, and cell cycle perturbations induced by two representative gold(III) complexes in human leukemic cells with different cisplatin sensitivity. *Oncol. Res.* **12**: 361–370

Davidson, P. M., Parish, M. E. (1989) Methods for testing the efficacy of food antimicrobials. *Food Technol.* **43**: 148–155

Finney, D. J. (1971) *Probit analysis*, 3rd edn. Cambridge University Press, Cambridge

Friedrich, M., Villena-Heinsen, C., Farnhammer, C., Schmidt, W. (1998) Effects of vinorelbine and titanocene dichloride on human tumor xenografts in nude mice. *Eur. J. Gynaecol. Oncol.* **19**: 333–337

Ghosh, P., D'Cruz, O. J., Narla, R. K., Uckun, F. M. (2000) Apoptosis-inducing vanadocene compounds against human testicular cancer. *Clin. Cancer Res.* **6**: 1536–1545

Harwig, J., Scott, P. (1971) Brine shrimp (*Artemia salina* L.) larvae as a screening system for fungal toxins. *Appl. Microbiol.* **21**: 1011–1016

Islam, M. S., Farooque, M. A., Bodruddoza, M. A. K., Mosaddik, M. A., Alam, M. S. (2002a) Antimicrobial and toxicological studies of mixed ligand transition metal complexes of schiff bases. *J. Biol. Sci.* **2**: 797–799

Islam, M. S., Farooque, M. A., Bodruddoza, M. A. K. (2002b) Synthesis and characterization of some thiocyanato complexes of Ni(II), Cu(II), Pd(II), Pt(II), Au(III), Ti(III), and V(IV) containing a tridentate Schiff base ligand. *Syn. React. Inorg. Met. Chem.* **32**: 1811–1823

Jaki, B., Orjala, J., Bürji, H. R., Sticher, O. (1999) Biological screening of cyanobacteria for antimicrobial and molluscicidal activity, brine shrimp lethality, and cytotoxicity. *Pharm. Biol.* **37**: 138–143

Joudah, L., Moghaddas, S., Bose, R. N. (2002) DNA oxidation by peroxo-chromium(v) species: oxidation of guanosine to guanidinohydantoin. *Chem. Commun.* **21**: 1742–1743

Kamalakannan, P., Venkappayya, D. (2002) Synthesis and characterization of cobalt and nickel chelates of 5-dimethylaminomethyl-2-thiouracil and their evaluation as antimicrobial and anticancer agents. *J. Inorg. Biochem.* **21**: 22–37

Kelland, L. R. (1993) New platinum antitumor complexes. *Crit. Rev. Oncol. Hematol.* **15**: 191–219

Kelland, L. R., Barnard, C. F., Mellish, K. J., Jones, M., Goddard, P. M., Valenti, M., Bryant, A., Murrer, B. A., Harrap, K. R. (1994) A novel trans-platinum coordination complex possessing *in vitro* and *in vivo* antitumor activity. *Cancer Res.* **54**: 5618–5622

Kurbacher, C. M., Nagel, W., Mallmann, P., Kurbacher, J. A., Sass, G., Hubner, H., Andreotti, P. E., Krebs, D. (1994) *In vitro* activity of titanocenedicholoride in human renal cell carcinoma compared to conventional antineoplastic agents. *Anticancer Res.* **14**: 1529–1533

Martinez, M., Del-ramo, J., Torreblanca, A., Diaz-Mayans, J. (1998) Effect of cadmium exposure on zinc levels in the brine shrimp *Artemia* partenogenética. *Aquaculture* **172**: 315–325

Mayer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. E., McLaughlin, J. L. (1982) Brine shrimp: a convenient bioassay for active plant constituents. *Plant Medica* **45**: 31–34

McLaughlin, J. L. (1991) Bench-top bioassays for the discovery of bioactive compounds in higher plants. *Brenesia* **34**: 1–14

McGowan, D. P. C. (2001) RSC Education and Professional Development. Cancer Chemotherapy Gets Heavy. School of Chemistry, University of Leeds. Available at: http://www.rsc.org/lap/educatio/eic/2001/mcgowan_sep01.htm

McLaughlin, J. L., Anderson, J. E. (1988) Brine shrimp and crown gall tumors: simple bioassay for the discovery of plant antitumour agents. Proceeding of the NIH Workshop. Bioassay for Discovery of Antitumour and Antiviral Agents from Natural Sources. Bethesda, MD

McLaughlin, J. L., Chang, C. J., Smith, D. L. (1991) Bench-top bioassay for the discovery of bioactive natural products: an update. In: Rahman, A. U. (ed.) *Studies in natural products chemistry*. Elsevier, Amsterdam, pp 383–409

Michael, A. S., Thompson, C. G., Abramovitz, M. (1956) *Artemia salina* as a test organism for a bioassay. *Science* **123**: 464

Mirabelli, C. K., Hill, D. T., Faucette, L. F., McCabe, F. L., Girard, G. R., Bryan, D. B., Sutton, B. M., Bartus, J. O., Crooke, S. T., Johnson, R. K. (1987) Antitumor activity of bis(diphenylphosphino)-alkanes, their gold(I) coordination complexes, and related compounds. *J. Med. Chem.* **30**: 2181–2190

Mishra, L., Said, M. K., Itokawa, H., Takeya, K. (1995) Antitumor and antimicrobial activities of Fe(II)/Fe(III) complexes derived from some heterocyclic compounds. *Bioorg. Med. Chem.* **3**: 1241–1245

Pelka, M., Danzl, C., Distler, W., Petschelt, A. (2000) A new screening test: toxicity testing of dental materials. *J. Dent.* **28**: 341–345

Persoone, G., Sorgeloos, P., Roels, O., Jaspers, E. (1980) Proceeding of the International Symposium on Brine Shrimp *Artemia saline*. Ecology, Culturing, Use in Aquaculture. Vol. III. Universal Press, Wetteren, Belgium

Quievryn, G., Peterson, E., Messer, J., Zhitkovich, A. (2003) Genotoxicity and mutagenicity of chromium(VI)/ascorbate-generated DNA adducts in human and bacterial cells. *Biochemistry* **42**: 1062–1070

Reiner, R. (1982) Detection of antibiotic activity. In: *Antibiotics an introduction*. Roche Scientific Services, Switzerland, pp. 21–25

Rho, Y. S., Kim, S. A., Jung, J. C., Shin, C. C., Chang, S. G. (2002) Anticancer cytotoxicity and nephro-toxicity of the new platinum (II) complexes containing diaminocyclohexane and glycolic acid. *Int. J. Oncol.* **20**: 929–935

Rios, J. J., Reico, M. C., Villar, A. (1988) Antimicrobial screening of natural products. *J. Ethno. Pharmacol.* **23**: 127–149

Rosenberg, B., Van Camp, L., Krigas, T. (1965) Inhibition of cell division in *Escherichia coli* by electrolysis products from a platinum electrode. *Nature* **205**: 698–699

Rosenberg, B., Van Camp, L., Trosko, J. E., Mansour, V. H. (1969) Platinum compounds: a new class of potent antitumour agents. *Nature* **22**: 385–386

Sarkar, M. K., Ergil, D., Tamir, A. U., Sahin, N. (1988) Antimicrobial activity and cytotoxicity of *Ables nordmannlana* subsp. equi-trojani extract. *Fitoterapia* **69**: 457–460

Sheikh, C., Hossain, S. H., Easmin, S., Islam, M. S., Rashid, M. (2004) Evaluation of *in vitro* antimicrobial and *in vivo* cytotoxic properties of some novel titanium based coordination complexes. *Biol. Pharm. Bull.* **27**: 710–713

Shil, R. N., Debnath, S. C. (2001) *An Introduction to the theory of statistics*. Jahangir Press, Dhaka, Bangladesh

Shrivastav, A., Singh, N. K., Srivastava, G. (2002) Synthesis, characterization and antitumor studies of transition metal complexes of o-hydroxydithiobenzoate. *Bioorg. Med. Chem.* **10**: 2693–2704

Sleet, R. B., Brendel, K. (1983) Improved methods for harvesting and counting synchronous populations of *Artemia* nauplii for use in developmental toxicology. *Ecotoxicol. Environ. Saf.* **7**: 435–446

Solis, P. N., Wright, C. W., Anderson, M. M., Gupta, M. P., Phillipson, J. D. (1993) A microwell cytotoxicity assay using *Artemia salina*. *Planta Med.* **59**: 250–252

Sultana, C., Rahman, M. A. A., Al-Bari, M. A. A., Banu, M. L. A., Islam, M. S., Khatune, M. A., Sadik, G. (2003) In vitro antimicrobial screening of three cadmium coordination complexes and two addition compounds of antimony and arsenic. *Pak. J. Biol. Sci.* **6**: 525–527

Treshchalina, E. M., Konovalova, A. L., Presnov, M. A., Chapurina, L. F., Belichuk, N. I. (1979) Antitumor properties of mixed coordination compounds of copper (II) and alpha-amino acids. *Dokl. Akad. Nauk.* **248**: 1273–1276

Vanhaecke, P., Persoone, G., Claus, C., Sorgeloos, P. (1981) Proposal for a short-term toxicity test with Artemia nauplii. *Ecotoxicol. Environ. Saf.* **5**: 382–387

Vijayalakshmi, R., Subramanian, V., Nair, B. U. (2002) A study of the interaction of Cr(III) complexes and their selective binding with B-DNA: a molecular modeling approach. *J. Biomol. Struct. Dyn.* **19**: 1063–1071