BioMetals **16:** 471–477, 2003.

Synthesis, characterization and antitumor studies of N-aroyl-N′-thioaroylhydrazines and their Co(II), Ni(II), Cu(II) and Zn(II) complexes

Nand Kishore Singh[1,*], Saty Brat Singh[1], Nagendra Singh[1] & Anuraag Shriviastav[2]
[1]*Department of Chemistry, Banaras Hindu University, Varanasi - 221 005 India;* [2]*Department of Pathology & Saskaton Cancer Centre, College of Medicine, University of Saskatchewan, 20 Campus Drive S7N 4H4 Saskatoon SK Canada;* [*]*Author for correspondence: (Tel: +91-542-307320; E-mail: nksingh@banaras.ernet.in)*

Received 18 July 2002; accepted 23 September 2002; Published online February 2003

Key words: antitumor activity, N-aroyl-N′-thioaroylhydrazines, 3d-metal complexes

Abstract

N-Salicyloyl-N′-p-hydroxythiobenzohydrazide (H_2STPH) and N-benzoyl-N′-thiobenzohydrazide (H_2BTBH) and their Co(II), Ni(II), Cu(II) and Zn(II) complexes were prepared and characterized by physicochemical studies. IR and NMR spectral studies imply dibasic tetradentate behaviour of the ligands bonding through 'thiolato' sulfur, enolic oxygen and the two hydrazinic nitrogens in a polymeric fashion. The electronic spectra indicate [Ni(STPH)(H_2O)$_2$], [Co(STPH)(H_2O)$_2$] to be distorted octahedral while [Cu(BTBH)] has a square-planar geometry. *In vitro* antitumor results of the ligand and the complexes on P-815 (murine mastocytoma) and L-929 (murine fibroblast) indicate that these compounds show significant inhibition of ^{3}H-thymidine and ^{3}H-uridine incorporation in DNA and RNA, respectively, in these tumor cells at dose levels of 1, 2.5 and 5 μg cm^{-3}. Antitumor studies suggest that [Cu(BTBH)] has significant dose dependent inhibitory effect on DNA synthesis. *In vivo* administration of [Cu(BTBH)] and [Ni(STPH)(H_2O)$_2$] resulted into prolongation of life span of Dalton's Lymphoma (DL) bearing mice.

Introduction

The effectiveness of quite a number of metal complexes as antitumor agents has now been definitely established (Livingstone 1980). Several α-N-heterocyclic carboxaldehyde thiosemicarbazones and their iron and copper complexes were shown to have cytotoxic effect and inhibitory activity against DNA synthesis under controlled metal condition (Saryan *et al.* 1979). A number of derivatives of thiosemicarbazones such as 3-ethoxy-2-oxobutyraldehyde *bis*(thiosemicabazonato) copper(II) complexes have been found to exhibit antitumor activity (Vangiessen *et al.*1973). It has been shown that these complexes bind to DNA (Mikelens *et al.* 1976). Thiosemicarbazones of 1-formylisoquinoline and 2-formylpyridine and their derivatives were also effective against animal tumors (Blanz *et al.* 1970, 1974) at the molecular level and inhibit the enzyme ribonucleoside diphosphate reductase and effect the synthesis of DNA (Moore *et al.* 1971).

Many chemotherapeutic agents have also been reported to possess immunomodulatory properties (Kilenerman *et al.* 1980; Lichtenstein *et al.* 1986). We have shown that DL bearing mice administered with Mn(II), Cu(II) and Ni(II) complexes of N-salicyloyl-N′-*o*-hydroxythiobenzhydrazide(H_2Sotbh) and *o*-hydroxydithiobenzoic acid(*o*-HOdtb) showed reversal of tumor growth associated induction of apoptosis in lymphocytes. We have also reported the possible mechanisms and therapeutic implication of the H_2Sotbh and its metal complexes in tumor regression and tumor growth associated immunosuppression (Shrivastav *et al.* 2002) Although, thiohydrazides are structurally quite similar to thiosemicarbazides, scarcity of work on the antineoplastic activity of transition metal complexes of thiohydrazides, has

prompted us to study the antitumor activity of Co(II), Ni(II), Cu(II) and Zn(II) complexes of substituted thiohydrazides.

The present paper also reports the *in vitro* antitumor activities of H_2STPH, H_2BTBH Figure 1(a), (b) and their metal(II) complexes against P-815 (murine mastocytoma) and L-929 (murine fibroblast) and DL (a spontaneous murine T cell lymphoma) tumor cells.

Materials and methods

Physical measurements

The complexes were analysed for their metal content employing standard procedures after decomposition with a mixture of HNO_3 and HCl followed by H_2SO_4. Sulfur was estimated as $BaSO_4$. Magnetic susceptibilities, and electronic and IR spectra were obtained as described earlier (Singh *et al.* 1982). ^{1}H and ^{13}C spectra of H_2BTBH were recorded in $CDCl_3$ and those of H_2STPH, [Zn(STPH)] and [Zn(BTBH)] in DMSO-d_6 on a JEOL 90 Q FT spectrometer.

Antitumor studies

These were carried out as described elsewhere (Shrivastav *et al.* 2002).

% inhibition of ^{3}H-thymidine and ^{3}H-uridine: The P-815/L-929 cell suspension was prepared in complete medium (RPMI 1640 medium supplemented with antibiotics, penicillin, streptomycin and 10% heat-inactivated fetal calf serum) at a concentration of 10^6 cells cm^{-3} following the literature method (Sodhi *et al.* 1986). 2×10^5 cells/well were added to duplicate wells of a 96 well cultured plate. The cells were treated with test compounds at various doses (1, 2.5 and 5 μg cm^{-3}) and incubated for 24 h at 37 °C in a 5% CO_2 incubator. In control sets no treatment was given. After 24 h of incubation, the cells were washed thrice with RPMI 1640 culture medium (without serum) by centrifugation ($400 \times g$ for 10 min). The cell pellets were resuspended in 0.2 cm^{-3} complete medium containing 1 μCi cm^{-3} ^{3}H-thymidine or ^{3}H-uridine and pulse labelled for 4 h for thymidine and 2 h for uridine. The cells were then washed thrice with phosphate buffered saline (PBS). The cells were lysed with 1% sodium dodecyl sulfate (SDS) and the lysate was counted for radioactivity in LKB/β-liquid scintillation counter. The percentage inhibition of incorporation was calculated as follows:

$$\%\ \text{inhibition} = 1 - \frac{\text{CPM in treated tumor cells}}{\text{CPM in untreated tumor cells}} \times 100$$

CPM = count per minute

Preparation of H_2BTBH and H_2STPH

All chemicals used were of analytical reagent, or equivalent grade. PhC(O)NHNHC(S)Ph[H_2BTBH] was prepared by the reaction of carboxymethyldithiobenzoate (Singh *et al.* 1996) and benzoic acid hydrazide in equimolar amounts, each dissolved separately in one equivalent of an aqueous solution of NaOH (2N). Ph(OH)C(O)NHNHC(S)Ph(OH) [H_2STPH] was prepared by reacting equimolar amounts of salicylic acid hydrazide and carboxymethyl *p*-hydroxybezodithiocarboxylate (Singh *et al.* 1997), each dissolved separately in two and one equivalent of NaOH (2N), respectively. The above solutions were allowed to stand at room temperature for 2 h and dilute acetic acid was added dropwise to precipitate the products; these were then filtered off, washed with H_2O and recrystallized from hot EtOH.

Synthesis of the complexes

[Co(STPH)(H_2O)$_2$], [Ni(STPH)(H_2O)$_2$], [Zn(STPH)] and [Cu(BTBH)] were prepared by mixing MeOH solutions of the respective metal (II) acetate and the hydrazide in a 1:1 molar ratio in the presence of NaOAc (ca. 2 M ratio) and heating the reaction mixture for ca. 15 min in a boiling water bath. The complexes thus obtained were filtered off, washed several times with H_2O and hot ethanol and dried *in vacuo*.

Results and discussion

Chemistry

Only 1:1 deprotonated complexes were isolated, formed by loss of two protons from each ligand to give a conjugated system. The complexes show high melting points (> 300 °C) and are insoluble in water and common organic solvents but are slightly soluble in coordinating solvent such as DMSO and pyridine.

The magnetic moment of 4.98 and 3.2 BM (Table 1) for the Co(II) and Ni(II) complexes, respectively, are normal and suggest their high spin octahedral geometries. The UV-Vis. spectrum of the cobalt(II) complex displays bands at 9580 and 17700 cm^{-1} assigned to the $^4T_{1g}(F) \rightarrow {}^4T_{2g}(\nu_1), {}^4A_{2g}$

Table 1. Analytical data and physical properties of ligands and their metal complexes.

Compound	Colour	m.p. (°C)	Yield (%)	Found (Calcd)% M	S	C	H	N	μ_{eff} (B.M.)
H_2STPH	Off white	158–160	60	– (11.1)	10.7 (58.3)	58.7 (4.1)	4.5 (9.7)	9.4	–
[Co(STPH)(H_2O)$_2$]	Dark brown	>300	65	14.9 (15.4)	7.9 (8.4)	43.8 (44.1)	3.2 (3.6)	7.7 (7.3)	4.98
[Ni(STPH)(H_2O)$_2$]	Light brown	>300	70	14.8 (15.4)	7.8 (8.4)	44.1 (44.1)	3.4 (3.6)	7.8 (7.3)	3.20
[Zn(STPH)]	Light yellow	>300	68	18.1 (18.6)	9.3 (9.1)	48.1 (47.8)	2.5 (2.8)	8.1 (7.9)	–
H_2BTBH	Off white	122–124	62	– (12.5)	12.1 (65.6)	65.4 (4.6)	4.4 (10.9)	11.1	–
[Cu(BTBH)]	Black	>300	65	20.2 (19.9)	10.5 (10.0)	52.4 (52.9)	2.9 (3.1)	8.2 (8.8)	1.85

Table 2. Percentage inhibition of ^{3}H-thymidine and ^{3}H-uridine incorporation in tumor cells.

Compounds	% Inhibition of ^{3}H-thymidine incorporation						% Inhibition of ^{3}H-uridine incorporation					
	1 μg cm^{-3}		2.5 μg cm^{-3}		5 μg cm^{-3}		1 μg cm^{-3}		2.5 μg cm^{-3}		5 μg cm^{-3}	
	I	II	I	II	I	II	I	II	I	II	I	II
H_2STPH	9.91,	5.35	11.01,	6.10	22.86,	8.94	17.64,	20.62	28.20,	25.98	40.36,	30.34
[Co(STPH)(H_2O)$_2$]	30.30,	17.22	7.71,	7.35	5.78,	3.59	29.63,	30.22	15.81,	21.32	9.63,	10.11
[Ni(STPH)(H_2O)$_2$]	13.77,	14.29	61.00,	52.75	54.22,	42.09	45.28,	42.01	28.09,	29.25	23.92,	29.23
[Zn(STPH)]	46.00,	40.38	39.25,	28.09	19.14,	16.22	57.87,	62.02	34.31,	38.19	30.28,	39.46
H_2BTBH	13.08,	10.28	25.34,	18.33	36.50,	33.27	33.29,	20.25	34.34,	29.19	36.91,	39.46
[Cu(BTBH)]	60.05,	70.06	48.34,	51.83	38.84,	40.46	74.42,	78.1	51.23,	60.32	48.56,	50.32
Control (CPM)	726, 1196						3820, 5585					

I – P-815 (Murine mastocytoma); II – L-929 (Murine fibroblast); CPM – count per minute

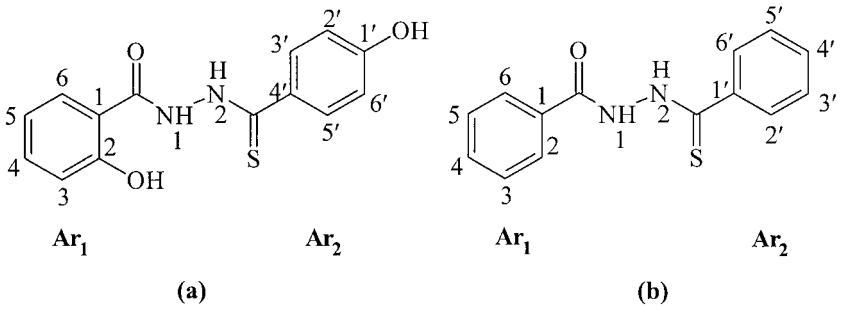


Fig. 1. (a) H_2STPH (b) H_2BTBH.

(ν_2) transitions, respectively. Ni(II) complex displays bands at 15500 and 27500 cm^{-1} assigned to $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)(\nu_2)$, $^3T_{1g}(P)(\nu_3)$ transitions, respectively, characteristics of distorted octahedral geometry (Lever 1984). The μ_{eff} value of 1.85 B.M. for [Cu(BTBH)] corresponds to the presence of one unpaired electron. The electronic spectrum of [Cu(BTBH)] shows a broad band at 17860 cm^{-1} assigned to the envelope of $^2B_{1g} \rightarrow {}^2A_{1g}, {}^2B_{2g}, {}^2E_g$ transitions, suggesting a square planar geometry around Cu(II) (Lever 1984).

In the IR spectra of the complexes of H_2STPH and H_2BTBH, two new bands appear at ca. 1510–1540 and 700 cm^{-1} assigned to ν(CN) of NCO and ν(C-S) modes, respectively, suggesting the removal of both -NH– protons via enolization and thioenolization and bonding of the resulting thiolato sulfur and enolic oxygen with the metal ion. The bands at 1500, 1330 and 1020 cm^{-1}, in the spectrum of the ligand, due to thioamide I [β(NH)+ν(CN)], thiomide II [ν(CN)+β(NH)] and ν(N-N), respectively, undergo a positive shift in the spectra of the complexes (Burns 1968). The magnitude of the positive shifts in these modes supports the bonding sites indicated above and also suggests the involvement of both the hydrazinic nitrogens in coordination (Figure 2).

The ^{1}H NMR spectrum of H_2STPH and H_2BTBH exhibits two signals in the range of δ10.1- 10.5 and δ11.2- 11.6 ppm for the two -NH-NH- protons adjacent to >C=S and >C=O groups, respectively. The signals at δ3.40 and 3.46 ppm are attributed to the -OH protons of p-hydroxybenzthioyl and salicyloyl parts, respectively. The NH- and -OH proton signals disappear on deuteration of the ligand. In the spectrum of the diamagnetic complex, [Zn(STPH)], the absence of NH protons further supports the loss of both hydrazinic protons via enolization and thioenolization. The presence of OH signals in [Zn(STPH)] shows the non-involvement of the phenolic protons in bonding.

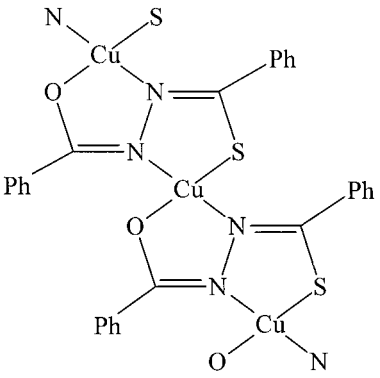
The ^{13}C spectrum of H_2STPH shows eleven signals. Their assignments have been made by taking into account the chemical shift values of p-hydroxyphenylthiocarboxyhydrazide (C=S 183.5, C1 159.7, C4 129.7, C3,5 129.2, C2,6 114.80) and salicyloyl hydrazone (Domiano *et al.* 1984). The signals at δ190.32 and 163.51 ppm are due to >C=S and >C=O carbons. [Zn(STPH)] shows an upfield shift of 13.68 ppm for -C(S) carbon as compared to H_2STPH which indicates that the metal is bonded to the sulfur in the deprotonated form. The enhanced shielding in -C(-S) carbon may be due to the change in environment around C(-S) carbon from -HN-C=S to -N=C-S, after the removal of proton upon thioenolization. Similarly, enolization of -HN-C=O group of the ligand and subsequent bonding of enolic oxygen is indicated by an upfield shift of 1.74 ppm in -C(O) carbon.

In the ^{13}C spectrum of H_2BTBH two signals are observed at 192.32 and 160.63 ppm for >C=S and >C=O carbons. The signals due to aromatic carbons are observed in the range of 116–135 ppm. The ^{1}H and ^{13}C NMR spectra of [Cu(BTBH)] could not be recorded due to its low solubility and paramagnetic nature.

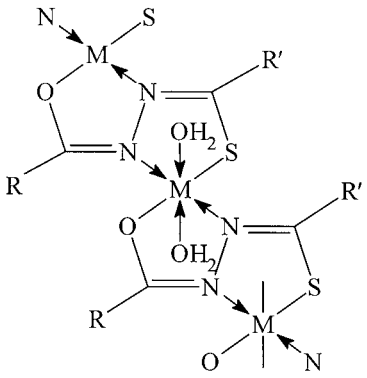
The ESR spectrum of [Cu(BTBH)] in DMSO at liquid nitrogen temperature shows a high field transition at 3200 G, from which <g> has been calculated to be 2.183.

Antitumor studies

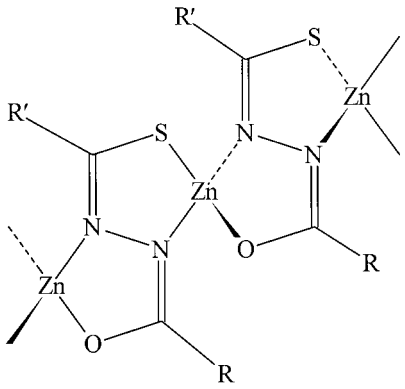
As the dose of H_2STPH and H_2BTBH increases there is an increase in percentage inhibition of ^{3}H-thymidine and ^{3}H-uridine incorporation (Table 2) in both tumor cells, but in case of [Cu(BTBH)] and [Zn(STPH)], the percentage inhibition of incorporation decreases. The compounds [Cu(BTBH)] and [Zn(STPH)] show significant percentage inhibition of ^{3}H-thymidine and ^{3}H-uridine incorporation in both P-815 and L-929 tumor cells at lower dose level. The reason for the observed inhibition of DNA blastogenesis by the metal complexes is unclear however, several possibilities could be considered. The inhibition in tumor cell blastogenesis could be a direct result of the interaction of the metal complexes with DNA, thus interfering with the normal process of its replication. Indeed, DNA binding of metal complexes of sulfur donor ligands has been documented (Steinkopf *et al.* 1995). Furthermore, % inhibition of uridine incorporation suggest inhibition/ activation of various enzymes directly/indirectly involved in DNA replication. Interaction of metal complexes with protein components of viable cells has been reported (Watt *et al.* 1999; Krynetskaya *et al.* 1998). Binding of metal complexes with protein may cause alterations in the domain organization of protein probably in a similar manner as observed in the case of allosteric enzymes leading to their malfunctioning. However, more studies will be needed to confirm the existence of one or more of such possibilities in our system. One of the additional supporting evidence comes from the result of experiments in which tumor cells, incubated in the presence or absence of metal com-



[Cu(BTBH)]



[M(STPH)$(H_2O)_2$](M = Co, Ni)



[Zn(STPH)]
R = o-OH(C_6H_4); R′ = p-OH(C_6H_4)

Fig. 2. Proposed structures of the complexes.

plexes were checked for their effect on viability by MTT assay in which 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (MTT) is metabolized to an insoluble coloured formazan salt by mitochondrial enzyme activity of succinate dehydrogenase in living cells (Buttke *et al.* 1994). The growth inhibitory effects of these complexes in terms of ID_{50} values were studied (Table 3) against murine tumor cell line. The Cu(II) and Ni(II) complexes display significant inhibitory effect on proliferation of tumor cell *in vitro*. However, the ligand alone failed to cause significant inhibitory action on tumor cell proliferation as is evident from high ID_{50} value.

In the next part of the investigation we studied the effect of the metal complexes on DL cell killing to indentify the mode of cell death. The results suggest

Table 3. Effect of ligands and their metal complexes on tumor cell growth *in vitro* (ID_{50} values in μg/mL) and responses of DL inoculated mice to a single treatment with ligands and their transition metal complexes.

Compounds	ID_{50}	% T/C
STPH	15.2	112
BTBH	17.3	105
[Cu(BTBH)]	1.2	210
[Ni(STPH)(H_2O)]	1.9	189
[Zn(STPH)]	7.8	120

ID_{50} = Average drug concentration (μg/mL) for 50% inhibition of Daltons Lymphoma growth. Values are mean ± SD of three experiments. $\rho < 0.05$ with respect to values of ID_{50} of ligand alone. % T/C = $\frac{\text{Mean life of treated mice}}{\text{mean life span of control mice}} \times 100$

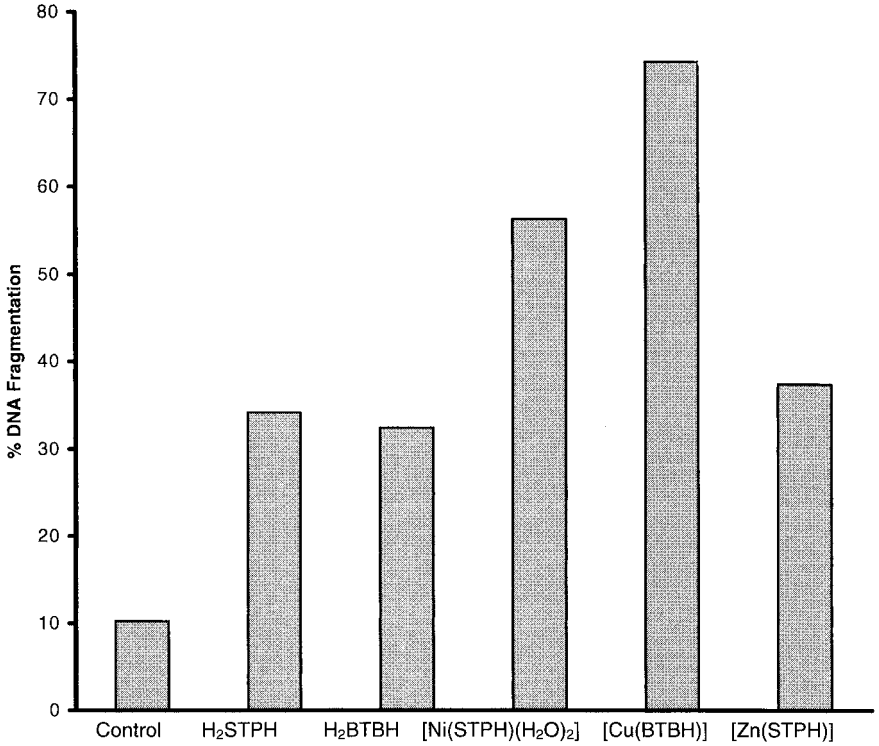


Fig. 3. Effect of H_2BTBH and H_2STPH and their metal complexes on % DNA fragmentation of tumor cells. DL cells were incubated in medium alone or containing ligand or their metal complexes (10 μg/mL) for 24 h and the % DNA fragmentation was evaluated. Values are mean of three experiments. $\rho < 0.05$ with respect to values of ID_{50} of ligand alone. % DNA fragmentation = (T/T+B)100, where T = absorbance of fragmented DNA, T+B = absorbance of total DNA.

that apoptosis was induced in tumor cells treated with [Cu(BTBH)] and [Ni(STPH)$(H_2O)_2$] were found to be most effective in the induction of tumor cell apoptosis. The mechanism of the induction of apoptosis remains poorly understood and is thought to be dependent on multiple mechanism(s) ultimately culminating in the activation of DNA cleaving endonucleases (Ranjan *et al.* 1998). Indeed results presented in Figure 3 show that [Cu(BTBH)] and [Ni(STPH)$(H_2O)_2$] cause an increase in the percentage of specific DNA fragmentation, a hallmark feature of apoptosis, indicating that these metal complexes may induce apoptosis culminating in the activation of endonucleases causing DNA fragmentation.

In the next part of the investigation we checked the life prolonging effect in DL bearing mice administered with PBS (phosphate buffer saline) alone or containing the ligand or its metal complexes. As shown in Table 3, minimal % T/C was observed in mice administered with ligand alone as compared to that of mice administered with metal complexes. Maximum % T/C was found for [Cu(BTBH)] and [Ni(STPH)$(H_2O)_2$] as compared to ligands. Increase in the value of % T/C indicates a prolongation of the life of tumor bearing mice and suggests that such effect could either result from the direct cytotoxic/cytostatic action of the complexes on tumor cells or due to the activation of certain host derived antitumor defense mechanism(s).

References

Blanz EJ, French FA, Doamaral JR, French DA. 1970 Carcinostatic activity of thiosemicarbazones of formylheteroaromatic compounds VII: 2-formylpyridine derivatives bearing additional ring substitutents. *J Med Chem* **13**, 1124–1130.

Burns GR. 1968 Metal complexes of thiohydrazide. *Inorg Chem* **7**, 277–283.

Buttke TM, Sandstrom PA. 1994 Oxidative stress as a mediator of apoptosis. *Immunol Today* **15**, 7–10.

Domiano P, Pelizzi C, Predieri G, Vignali C, Palla G. 1984 Aroylhydrazones as chelating agents: d^{10}-metal complexes of pyridine-2-carbaldehyde salicyloylhydrazone and X-ray structure of the zinc derivative. *Polyhedron* **3**, 281–286.

French FA, Blanz EJ, Shaddix SC, Brockman RN. 1974 Alpha-N-formylheteroaromatic thiosemicarbazones. Inhibition of tumor derived ribonucleoside diphosphate reductase and correlation with *in vivo* antitumor activity. *J Med Chem* **17**, 172–181.

Kleinerman ES, Zwelling LA, Muchmore AV. 1980 Enhancement of naturally occuring human spontaneous monocyte mediated cytotoxicity by cis-diamminedichloroplatinum (II). *Cancer Res* **40**, 3099–3102.

Krynetskaya NF, Kubareva EA, Timchenko MA, Belkov VM, Shabarova ZA. 1998 Transition-metal complexes as inhibitors of proteins recognizing double-standard fragments of nucleic acids. *Biochemistry(Mosc)* **63**, 1068–1073.

Lever ABP. 1984 Inorganic Electronic Spectroscopy, 2nd Edn. Elsevier Amsterdam.

Lichtenstein AK, Pende D. 1986 Enhancement of natural killer cytotoxicity by cis-diaminedichloroplatinum (II) *in vivo* and *in vitro*. *Cancer Res* **46**, 639–644.

Livingstone SE. 1980 Metal chelates as anticancer agents. *Coord Chem* **20**, 141–148.

Mikelens PE, Woodson BA, Levinson WE. 1976 Association of nucleic acids with complexes of N-methylisotinbetathiosemicarbazone and copper. *Biochem Pharmacol* **25**, 821–827.

Moore EC, Booth BA, Sartorelli AC. 1971 Inhibition of deoxyribonucleotide synthesis by pyridine carboxaldehyde thiosemicarbazones. *Cancer Res* **31**, 235–238.

Ranjan P, Sodhi A, Singh SM. 1998. Murine peritonal macrophages treated with cisplatin and interferron-gamma undergo No-mediated apoptosis via activition of endonucleasis. *Anticancer Drugs* **9**, 333–341.

Saryan LA, Ankel E, Krishnamurti C, Petering DH.1979 Comparative cytoxic and biochemical effects of ligands and metal complexes of α-N-heterocyclic carboxaldehyde thiosemicarbazones. *J Med Chem* **22**, 1218–1221.

Shrivastav A, Singh NK, Singh SM. 2002 Synthesis, characterization and antitumor studies of Mn(II), Fe(III), Co(II), Ni(II), Cu(II) and Zn(II) complexes of N-salicyloyl-N′-*o*-hydroxythiobenzhydrazide. *Bioorg Med Chem* **10**, 887–895.

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

Singh NK, Agrawal S, Aggarwal RC. 1982 Synthesis and structural studies of oxovanadium (IV), Cr(III), Mn(II), Co(II & III), Ni(II), Cu(II) and Zn(II) complexes of 2-furanthiocarboxyhydrazide. *Indian J Chem* **21**, 973–976.

Singh NK, Singh N, Prasad GC, Sodhi A, Shrivastava A. 1997 Antitumor activity studies of newly synthesized N-salicylodyl-N′-(p-hydroxybenzthioyl) hydrazine and its copper(II) complexes both *in vivo* and *in vitro*. *Bioorg Med Chem* **5**, 245–251.

Singh NK, Singh N, Prasad GC. 1996 Complexes of N-aroyl-N′-thiobenzhydrazide with Co(II), Ni(II) and Zn(II). *Transition Met Chem* **21**, 296–299.

Sodhi A, Singh SM. 1986 Release of cytolytic factor(s) by murine macrophases *in vitro* on treatment with cis-platin. *Int J Immunopharmacol* **8**, 701–707.

Steinkopf S, Garoufis A, Nerdal W, Sletten E. 1995 Sequence-selective metal ion binding to DNA oligomers. *Acta Chem Scand* **49**, 495–502.

Van Giessen GJ, Crim JA, Petering DH, Petering HG. 1973 Effect of heavy metals on the *in vitro* cyctoxicity of 3-ethoxy-2-oxobutyraldehyde bis (thiosemicarbazone) and related compounds. *Natl Cancer Inst* **51**, 139–146.

Watt RK, Ludden PW. 1999 Nickel-binding proteins. *Cell Mol Life Sci* **56**, 604–625.