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Synthesis, characterization of novel Copper(II), Nickel(II) complexes of N-substituted thiosemicarbazides: Evaluation of anti-bacterial, anti-fungal and anti-cancer activities

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ABSTRACT

Copper(II) and Nickel(II) complexes of N-substituted Thiosemicarbazide ligands derived from condensation of two thiosemicarbazide and a dicarboxylic acid(malonic/ succinic/phthalic acid) have been synthesized and characterized by elemental and thermal analysis, IR, UV-Vis and EPR spectral studies, and magnetic susceptibility studies. Anti-bacterial, Anti-fungal and Anti-cancer activities have also been carried out on these complexes. Most of the complexes have good antibacterial and antifungal activity and some of them show excellent anticancer activity too.

Keywords: Copper(II) / Nickel(II) complexes – malonyl / succinyl / pthalyl bis N-substituted Thiosemicarbazide, Anti-bacterial, Anti-fungal and Anti-cancer studies

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INTRODUCTION

Thiosemicarbazide is a versatile multidonor ligand and acts bidentate coordinating through N and S either as neutral ligand or in the anionic form[1-3]. They are of special interest to researchers in different fields such as catalysis, pharmaceutical chemistry and electrochemistry due to their wide application as catalysts in redox reactions, antibacterial, antifungal, antitumour, anticancer and antimalarial properties and use as electrochemical sensors[4-14]. They are unique in stabilizing uncommon oxidation states through redox reactions and generating complexes with different coordination geometries[15-19].Copper(II) complexes with semicarbazide and thiosemicarbazide have received much attention due to their application as antiviral agents, exhibiting superior activity compared to that of uncomplexed ligands. They also have been used in the treatment of tumours, including Hodgkin's disease[20]. Most of the complexes involving thiosemicarbazide are Schiff bases (thiosemicarbazones) and the formation of amide from thiosemicarbazide is very rare. In our present study, we have attempted at insitu synthesis of amides through condensation of thiosemicarbazide and dicarboxylic acids (malonic/succinic/phthalic acid) and complexation with metal ions such as copper(II) and Nickel(II). They are characterized by elemental, thermal analysis, IR, UV, EPR spectral studies and magnetic susceptibility studies.

EXPERIMENTAL SECTION

All reagents and solvents used were of analytical grade and used without purification. The copper and Nickel salts used for the preparations are AR Copper(II) chloride and Nickel(II) chloride. The complexes were prepared by the following method:

Synthesis of complexes:
Thiosemicarbazide (0.02 mol in 50ml hot methanol) with dicarboxylic acid(malonic/succinic/phthalicacid)(0.01 mol in 50ml methanol)in the presence of Nickel(II) chloride/Copper(II) chloride (0.01 mol in 20 ml methanol) and few drops of con.HCl was refluxed for 6-8 h with constant magnetic stirring. Green colour Nickel(II) complexes/brown

copper (II) complexes separated from the mixture. The complexes were washed with methanol, filtered and dried in air. The copper(II) and Nickel(II) in the complexes were determined by optical emission spectroscopy using ICP-OES Perkin Elmer optima 5300 DV Spectrometer. Nitrogen and Sulphur were estimated by kjelhdhal's and Barium sulphate method. Chlorine in the complexes were estimated by standard vohlhard's method. TG/DTA were recorded in nitrogen atmosphere using NETZSCH STA 409 C/CD thermal analyzer with a heating rate of 10°C/min. Magnetic susceptibility studies were carried out using Vibrating magnetometer EG and GPARC model 155. UV – Visible absorption spectra were done using Varian Cary Spectrophotometer 5E – UV-Vis-NIR. The IR spectra were recorded as KBr disc using Schimadzu IR spectrometer. Antibacterial and antifungal activities of Copper(II) and Nickel(II) complexes were studied using a minimum modification of the disc diffusion method originally described by Bauer [21]. The invitro cytotoxicity of the prepared coordination complexes was determined by MTT-based assay with colon cancer cell line (HT 29)[22]. In parallel the activity was tested on monkey kidney cells (normal cell line VERO).

RESULTS AND DISCUSSION

A series of bivalent Copper and Nickel complexes have been synthesized by template condensation of dicarboxylic acids(malonic/succinic/phthalic acid) with thiosemicarbazide in methanol. The Nickel(II) complexes were green while copper(II) were brown in colour. Brown colored copper(II) complexes are common with thiosemicarbazide ligand due to sulfur-to-metal charge-transfer transition, which dominates the visible spectra of the complexes[23].Table 1 gives elemental analysis data and uv-visible absorption bands when recorded in aqueous medium. The elemental analysis data confirm the proposed composition. All the complexes are readily soluble in water and partially soluble in organic solvents such as dmso. The molar conductance of the complexes range from 5-10 $ohm^{-1}cm^2$per mole, also it was found that the complexes did not precipitate silver chloride in the presence of silver nitrate confirming coordination of the two chloro groups.

Table 1- Elemental analysis data on the complexes

Complexes	%N (the) exp	%S(the) exp	%Cl (the) exp	%M (the) exp	Λmax (nm)
$C_5H_{10}N_6O_2S_2Cu.Cl_2$	(21.8)20.8	(8.32)8.03	(18.4)17.8	(16.3)17.2	355,559
$C_6H_{12}N_6O_2S_2Cu.Cl_2$	(19.7)19.0	(7.51)6.95	(16.6)16.0	(15.9)16.7	355,323,560
$C10H_{12}N_6O_2S_2Cu.Cl_2$	(18.8)18.5	(7.19)6.31	15.7)15.1	(14.2)15.0	355,562
$C_5H_{10}N_6O_2S_2Ni.Cl_2$	(22.1)21.5	(8.41)7.85	(18.6)17.9	(15.6)16.5	362, 601
$C6H_{12}N_6O_2S_2Ni.Cl_2$	(21.3)20.8	(8.12)7.63	(17.9)16.9	(15.0)15.8	373,363,605
$C10H_{12}N_6O_2S_2Ni.Cl_2$	(19.1)18.5	(7.27)7.01	(16.1)15.7	(13.4)14.0	372, 610

The IR spectral studies given in Table 2 indicate the absence of any characteristic band around 1700 cm^{-1} corresponding to carboxylic acid group which confirms condensation involving the same. The presence of intense strong band in the region 1635-1660 cm^{-1} provides evidence for the formation of amide group in the complexes. The band around 3300 and 3200 cm^{-1} indicates the presence of NH_2 group and the ones appearing in the region 680-702 cm^{-1} suggest the involvement of thione sulphur in the bonding. The presence of band in the region 430-460 cm^{-1} confirms the M-N bonding in the complexes.

Table 2-IR Spectral data on the complexes (cm^{-1})

Complexes	υ_{N-H2}	υ_{N-H}	$\upsilon_{C=o}+\upsilon_{N-H}$	$\upsilon_{C=s}$	υ_{M-N}
$C_5H_{10}N_6O_2S_2Cu.Cl_2$	3340, 3248	3126	1633, 1602	1373, 696	428
$C_6H_{12}N_6O_2S_2Cu.Cl_2$	3302, 3170	3101	1653, 1575	1323, 694	460
$C10H_{12}N_6O_2S_2Cu.Cl_2$	3336, 3240	3120	1631, 1595	1365, 686	430
$C_5H_{10}N_6O_2S_2Ni.Cl_2$	3348, 3251	3149	1639, 1604	1381, 702	435
$C6H_{12}N_6O_2S_2Ni.Cl_2$	3344, 3250	3130	1637, 1604	1379, 696	430
$C10H_{12}N_6O_2S_2Ni.Cl_2$	3346, 3240	3128	1637, 1602	1377, 698	428

Table 3-TGA and DTA data on the complexes

Complexes	%Residue from TGA (the) exp	DTA peaks	
		Exothermic	Endothermic
$C_5H_{10}N_6O_2S_2Cu.Cl_2$	24.6(23.5)	197.4, 267.5, 641.1	700
$C_6H_{12}N_6O_2S_2Cu.Cl_2$	23.9(23.4)	801	632.9,260
$C10H_{12}N_6O_2S_2Cu.Cl_2$	21.4(22.1)	-	244
$C_5H_{10}N_6O_2S_2Ni.Cl_2$	23.7(22.9)	323.8	120
$C6H_{12}N_6O_2S_2Ni.Cl_2$	23.1(22.8)	320.6	550
$C10H_{12}N_6O_2S_2Ni.Cl_2$	20.6(20.9)	318,721.5, 845.1	-

The Thermal analysis data are furnished in Table 3. Generally, Very less information is known about the thermal analysis of transition metal complexes of thiosemicarbazides [24].The thermograms were run up to 1000^{o}C, and the final residue corresponds to copper and nickel sulphides. The theoretical values were in good agreement with the experimental values, confirming the proposed composition .

The Cu(II) complexes exhibit a low intense band in the region 550-562nm corresponding to $^{2}Eg \rightarrow {}^{2}T_{2}g$ d-d transition[25].The copper complexes also show a high intense band around 350 nm corresponding to sulphur to metal charge transfer [26]. Therefore, it may be suggested that Cu(II) has a distorted octahedral geometry around it [27]. The Nickel(II) complexes show bands in the region 360-370nm and around 600nm corresponding to $^{3}A_{2g}(F) \rightarrow {}^{3}T_{1g}(F)$ and $^{3}A_{2g}(F) \rightarrow {}^{3}T_{1g}(P)$ suggesting distorted octahedral geometry for the complexes[28]. The absence of any band below 700 nm eliminates the possibility of tetrahedral environment for the Nickel complexes[29]. The EPR spectrum of succinyl copper complex given in fig 1 shows two signals corresponding to $g_{//}$=2.122 and $g_{\perp}$ = 2.03 confirming anisotropic environment and paramagnetic nature of the brown Copper(II) complex. The EPR spectrum of malonyl Nickel(II) complex shows less intense g// signal showing coupling with nickel. There is very little evidence of fine structure due to ^{61}Ni (1.19% natural abundance I=3/2)[30]. Ni(II) being a non-kramer's ion, Epr spectra is generally observable at low temperature. However, very few reports are available in the literature about this ion at room temperature. The $g_{\perp}$ signal shows hyperfine interaction with four nitrogen atoms(I=1).The VSM plot given in fig 3&4 indicate that the complexes exhibit room temperature ferromagnetism. The complexes thus may be tentatively assigned a distorted octahedral geometry, with coordination through two amide nitrogens, two thione sulphur and two chloride ions.

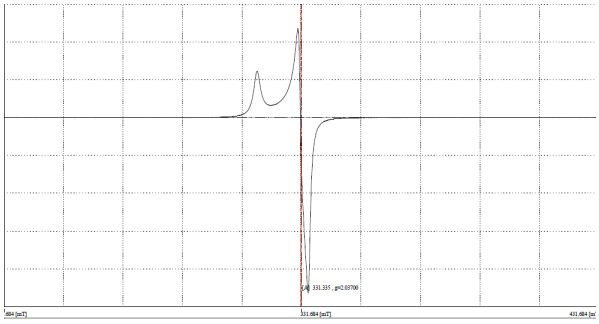

Fig 1 Epr spectrum -succinylcopper complex

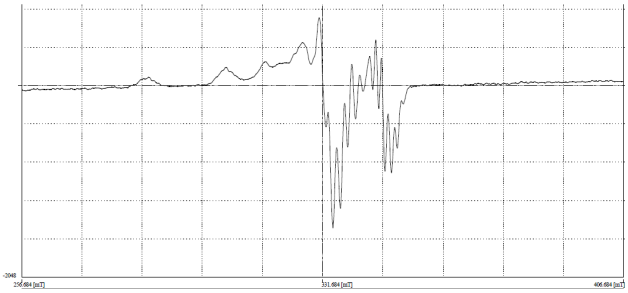

Fig 2 Epr spectrum- malonyl nickel complex

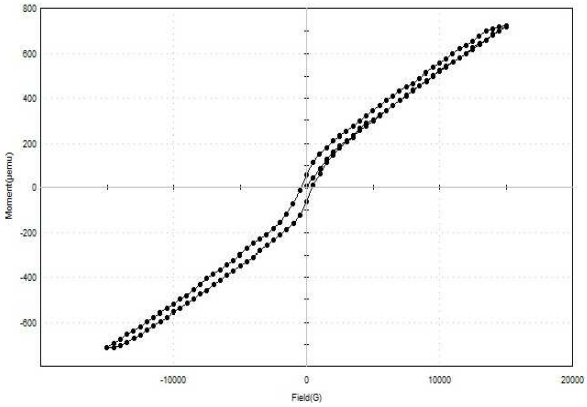

Fig 3- vsm plot-succinyl copper complex

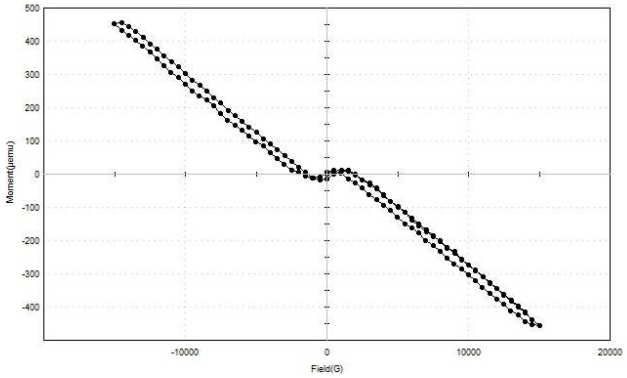

Fig 4- vsm plot-malonyl nickel complex

Table 4a-Antibacterial activities on the Copper complexes

Complexes	Organism	Zone of Inhibition(mm) Concentration(µg/ml)			Antibiotic 1mg/ml
		1000	750	500	
$C_5H_{10}N_6O_2S_2Cu.Cl_2$	*E.coli*	9	7	6	12
	Aeromonas spp.	8		-	13
	Staphylococcus aureus	9	8	7	12
	Vibrio parahemolyticus	-	-	-	14
	Bacillus subtilis	8	7	-	15
$C_6H_{12}N_6O_2S_2Cu.Cl_2$	*E.coli*	9	8	7	15
	Aeromonas spp.	10	8	6	13
	Staphylococcus aureus	10	9	8	17
	Vibrio parahemolyticus	10	9	7	18
	Bacillus subtilis	8	7	6	17
$C_{10}H_{12}N_6O_2S_2Cu.Cl_2$	*E.coli*	8	-	-	12
	Aeromonas spp.	-	-	-	13
	Staphylococcus aureus	9	8	7	12
	Vibrio parahemolyticus	-	-	-	14
	Bacillus subtilis	8	7	-	15

Table 4b-Antibacterial activities on the Nickel complexes

Complexes	Organism	Zone of Inhibition(mm) Concentration(µg/ml)			Antibiotic 1mg/ml
		1000	750	500	
$C_5H_{10}N_6O_2S_2Ni.Cl_2$	*E.coli*	-	-	-	12
	Aeromonas spp.	8	7	-	13
	Staphylococcus aureus	-	-	-	17
	Vibrio parahemolyticus	-	-	-	12
	Bacillus subtilis	10	7	6	18
$C_6H_{12}N_6O_2S_2Ni.Cl_2$	*E.coli*	8	7	6	11
	Aeromonas spp.	9	8	6	12
	Staphylococcus aureus	10	8	7	17
	Vibrio parahemolyticus	6	-	-	16
	Bacillus subtilis	7	6	-	15
$C_{10}H_{12}N_6O_2S_2Ni.Cl_2$	*E.coli*	9	7	6	12
	Aeromonas spp.	8	-	-	13
	Staphylococcus aureus	9	8	7	12
	Vibrio parahemolyticus	-	-	-	14
	Bacillus subtilis	8	7	-	15

Biological Studies:

Antibacterial Studies:

All the six complexes were screened for their antibacterial activity by disc diffusion method against five different bacteria's namely *E. Coli, Aeromonas spp. Staphylococcus aureus, Vibrio parahemolyticus, Bacillus subtilis*. The diameter of the inhibitory zone from anti-bacterial studies are presented in Table 4a and 4b. The results are compared to that of standard ampicillin. As the concentration of the complex increases, the diameter of the inhibitory zone also increases, indicating that the complexes are active. The succinyl copper complex, showed a good activity for all the bacteria, even at low concentration. In particular, it proved nearly equivalent activity with that of standard drug for Aero monas spp. The malonyl copper complex showed a better activity for the three bacteria namely *E. Coli, Aeromonas spp. Staphylococcus aureus*, and half of the activity for Bacillus subtilis, it was

not active against *Vibrio parahemolyticus*. Phthalyl copper complex showed the activity similar to succinyl copper and it was mostly active only at higher concentration. Among the nickel complexes, the succinyl complex was found to be active against almost all the bacteria only at higher concentration compared to malonyl and phthalyl complexes. The malonyl and phthalyl nickel complexes showed nearly similar activity and these were not active against Vibrio parahemolyticus.

Table 5-Antifungal studies on the complexes

Complexes	ORGANISM	Zone of Inhibition(mm) Concentration(µg/ml)			Antibiotic 1mg/ml
		1000	750	500	
$C_5H_{10}N_6O_2S_2Cu.Cl_2$	*Candida albicans*	10	8	7	12
	Trichoder maviridi	13	10	9	15
	Aspergillus niger	-	-	-	8
$C_6H_{12}N_6O_2S_2Cu.Cl_2$	*Candida albicans*	-	-	-	8
	Trichoder maviridi	11	10	8	14
	Aspergillus niger	-	-	-	7
$C_{10}H_{12}N_6O_2S_2Cu.Cl_2$	*Candida albicans*	10	8	7	12
	Trichoder maviridi	12	9	8	14
	Aspergillus niger	6	-	-	7
$C_5H_{10}N_6O_2S_2Ni.Cl_2$	*Candida albicans*	10	7	6	12
	Trichodermaviridi	9	8	6	11
	Aspergillus niger	-	-	-	8
$C_6H_{12}N_6O_2S_2Ni.Cl_2$	*Candida albicans*	-	-	-	9
	Trichoder maviridi	9	7	-	12
	Aspergillus niger	8	6	-	9
$C_{10}H_{12}N_6O_2S_2Ni.Cl_2$	*Candida albicans*	10	7	-	13
	Trichoder maviridi	10	-	-	12
	Aspergillus niger	-	-	-	7

Anti-fungal studies

All the six complexes were screened for their anti-fungal activity against three different fungi namely *Candida albicans*, *Trichoder maviridi*, *Aspergillus niger*. The diameter of the inhibitory zone from anti-bacterial studies were presented in Table 5. All the Complexes, (except succinyl and phthayl copper) showed nearly equivalent activity against the fungi namely *Candida albicans*, compared to the standard penicillin. Phthalyl copper complex showed better activity against *Aspergillus niger* than all other complexes. Most of the complexes showed moderate activity against *Trichoder maviridi* at higher concentration.

Table 6a-Anticancer activity on the Copper complexes

Complexes	Concentration (µg/ml)	Absorbance (O.D)	Cell viability (%)
$C_5H_{10}N_6O_2S_2Cu.Cl_2$	1000	0.02	3.57
	500	0.06	10.71
	250	0.09	16.07
	125	0.11	19.64
	62.5	0.14	25
	31.2	0.17	30.35
	15.6	0.2	35.71
	7.8	0.24	42.85
	Cell control	0.56	100
$C_{10}H_{12}N_6O_2S_2Cu.Cl_2$	1000	0.03	5.35
	500	0.07	12.5
	250	0.1	17.85
	125	0.13	23.21
	62.5	0.15	26.78
	31.2	0.19	33.92
	15.6	0.2	35.71
	7.8	0.23	41.07
	Cell control	0.56	100
$C_{10}H_{12}N_6O_2S_2Cu.Cl_2$	1000	0.05	8.92
	500	0.07	12.5
	250	0.08	14.28
	125	0.09	16.07
	62.5	0.12	21.42
	31.2	0.16	28.57
	15.6	0.19	33.92
	7.8	0.21	37.5
	Cell control	0.56	100

Table 6b-Anticancer activity on the Nickel complexes

Complexes	Concentration (µg/ml)	Absorbance (O.D)	Cell viability (%)
$C_5H_{10}N_6O_2S_2Ni.Cl_2$	1000	0.04	7.14
	500	0.06	10.71
	250	0.07	12.5
	125	0.09	16.07
	62.5	0.11	19.64
	31.2	0.14	25
	15.6	0.18	32.14
	7.8	0.22	39.28
	Cell control	0.56	100
$C_6H_{12}N_6O_2S_2Ni.Cl_2$	1000	0.03	5.35
	500	0.05	8.92
	250	0.08	14.28
	125	0.12	21.42
	62.5	0.17	30.35
	31.2	0.2	35.71
	15.6	0.21	37.5
	7.8	0.23	41.07
	Cell control	0.56	100
$C_{10}H_{12}N_6O_2S_2Ni.Cl_2$	1000	0.04	7.14
	500	0.08	14.28
	250	0.12	21.42
	125	0.14	25
	62.5	0.19	33.92
	31.2	0.22	39.28
	15.6	0.26	46.42
	7.8	0.27	48.21
	Cell control	0.56	100

Anti-cancer studies:

The *invitro* cytotoxicity data on all the six complexes is presented in Table 6a and 6b.The complexes showed excellent anticancer activity against the colon cancer cell line. Infact, 97 % of the cancer cell was destroyed by using succinyl copper complex. More than that, all the complexes destroyed more than 90% of the cancer cell in 1mg concentration itself and 60% of the cancer cell were destroyed in very minimum concentration. The malonyl copper complexes showed higher activity than the other two complexes and the same trend is observed for nickel complexes also. In parallel the activity was also tested on monkey kidney cells (normal cell line VERO). At minimum concentration, the activity is good against normal cell line, but when the concentration increases, the normal cell line is also destroyed to some extent. It is common that the anticancer drugs given to patients are said to bring about nephrotoxicity.

CONCLUSION

All the amide complexes are found to exhibit excellent antibacterial, antifungal and anticancer activities. The fact that normal cells are not affected much, suggests the significance of the complexes reported here. A very narrow hysteresis loop suggest that the complexes are also potential ferromagnetic materials.

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