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Copper (II) Complexes of Hard and Soft Donor Ligands - N, O and S: Synthesis, Characterization and Study of Antimicrobial and Anticancer Activities.

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ABSTRACT

Mixed ligand copper(II) complexes of thiosemicarbazoneof acetophenone/ benzophenone/benzaldehyde/vanillin together with chloroethanol and ethylenediamine (en) as other ligands, $[Cu(R_2C=N-N=C(S^-)NH_2)\ (ClCH_2CH_2O)(en)]$ were synthesized and characterized by elemental and thermal analysis, IR , UV –Visible and Electron spin resonance spectral studiesand magnetic susceptibility measurements. These complexes were subjected to antimicrobial studiesagainst the bacteria, *E.coli, Salmonella typhi, Bacillus subtilis*, *staphylococcus auerus, and Pseudomonasaeruginosa and against the fungi, candida albicans,Trichoderma viridi and Aspergillus Niger*. The complexes were found to be good antibacterial and antifungal agents. MTT assay of cytotoxicity ofthe complexes against HT 29-cancer cell line (colon cancer cells) and Vero cell line (Normal kidney cells) indicated that the complexes exhibited excellent anticancer activity with very low IC_{50} values together with significantly higher selectivity index.

Keywords: Copper (II) mixed ligand complexes-acetophenone, benzophenone, benzaldehyde and vanillin thiosemicarbazone, Chloroethanolantimicrobial, anti-cancer studies.

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INTRODUCTION

The research , development and discovery of new antimicrobial and anticancer agent is vital for the scientists, since many people in the world are affected with contagious diseases caused by multi resistant bacteria and other infections.[1,2] Complexes of several Nitrogen and sulphur donor ligands dithiocarbamates [3-10] and Schiff bases [11] have been reported with excellent pharmacological applications. Thiosemicarbazide is anand interesting class of ligand with variable binding modedue tostructural diversityand also exhibits a wide range of biological applications .[12-20] It existsin two tautomeric forms either asthione or thiol. Thiosemicarbazone and its complexes are extensively used even in catalytic field.[21,22] In addition, use of these complexes in radiopharmaceuticals is the best application in today's suphur chemistry.[23]In the previous work, we have reported some copper and nickel complexes based on thiosemicarbazide ligand and the copper complexes have been shown to have an enhanced activity.[24]Therefore, we attempted at the synthesis of copper complexes with different Thiosemicarbazones derived from the condensation of thiosemicarbazide with acetophenone / benzophenone /benzaldehyde /vanillin in the presence of chloroethanol and ethylene diamine as other ligands .The complexes isolated are $[Cu(R_2C{=}N\text{-}N{=}C(S^-)NH_2)(ClCH_2CH_2O)(en)]$ and the abbreviations aptsc, bptsc, bztsc and vantsc represent deprotonated, thiosemicarbazone of acetophenone, benzophenone, benzaldehyde and vanillin respectively

EXPERIMENTAL

The chemicals employed for the synthesis are of Analar grade and used without further purification. Copper (II) chloride, thiosemicarbazide, acetophenone, benzophenone, benzaldehyde, vanillin, chloroethanol, and ethylene diamine are pure chemicalsfrom Merck. Thiosemicarbazide (0.01m) in hot methanol(50ml) was taken to which acetophenone/benzophenone/benzaldehyde/vanillin (0.01m) in 20 ml methanol was added and the reaction mixture was refluxed for2 hrs. Then copper (II) chloride (0.01m) in 20 ml methanol, ethylenediamine (0.01m) and chloroethanol (0.01m) weresimultaneously added and the reaction mixture was further stirred for half an hour. Green colouredcomplex isolated out which was washed, filtered and air dried.The metal content in the complexes was estimatedby ICP-OES (Inductively coupled plasma - optical emission Spectroscopy). The nitrogen and sulphur content were estimated by Kjelhdhal's method and barium sulphate method respectively. The Chloride in the complexes was estimated by standard Vohlhard's method.TG/DSC were recorded in NETZSCH STA 449F3 thermal analyzer with a heating rate of 10°/min. Magnetic susceptibility studies were carried out using Vibrating sample magnetometer Lakeshore VSM 7410. UV-Visible absorption spectra of the complexes in chloroform were recorded using a Shimadzu UV 1600 model spectrometer. The IR spectra of the complexes were recorded as KBr disc using SCHIMADZU Infra-red Spectrometer. The EPR spectra of the complexes were recorded using JES-FA200 electron spin resonance spectrometer in the region from 1000-8000 gauss. The antibacterial and antifungal activities of the complexes were studied by agar disc diffusion method. The anti-cancer activities were studied by the MTT assay.

RESULTS AND DISCUSSION

All the complexes were green in colour and found to be soluble in DMSO and DMF. They were stable at room temperature. The elemental analysis data are furnished in Table-1 whichconfirms the proposed composition for the complexes. The molar conductances of the complexes were found to be 3.8 to4.7ohm^{-1} cm^2 mol^{-1} indicating the non-electrolytic nature of the complexes.The thermal analysis and UV-Vis spectral data are given in Table-2.The thermo grams were run up to 1000^o c and the residual mass percentage obtained corresponds to metal sulphides in acetophenone and benzaldehyde semicarbazonecomplexes and oxides in benzophenone and vanillin semicarbazone complexes. [25] The electronic spectra of all the copper(II) complexes showed high intense band in the region 271-290, 320-350 which may be due to Sulphur-→M charge transfer transition or intra ligand charge transfer transition. In addition one low intense band observed in the region 595-610 nm, is assigned to ^2E_g-$^2T_{2g}$ transition as it is unresolved and this is consistent with distorted octahedral arrangement around Cu(II).[26-28] The IR spectral data of the complexes are furnished in Table-3. The presence of two bands, one in the region 3300-3464cm^{-1} and other in the region 3300 -3387 cm^{-1}are characteristic ofethylene diamine in the complexes. .In addition, the complexes show peaks in the region around 3350-3140cm^{-1} corresponding to υ_{NH2} of thiosemicarbazide. The absence of band around 1700cm^{-1} and the presence of sharp band in the region 1593-1602cm^{-1} ($\upsilon_{C=N}$) confirms the condensation of aldehyde or ketone with thiosemicarbazide and coordination of the imine Nitrogen.[29] The bands in the range 744-771cm^{-1} and 681-697 cm^{-1}are due to $\upsilon_{C\text{-}Cl}$and $\upsilon_{C\text{-}S}$ of chloroethanol and coordinatedmono

ionicthiosemicarbazone[30] respectively. The presence of band in the region 410-532 cm^{-1} confirms the Cu-N coordination in the complexes[31].The EPR spectra of the complexes show only one signal with g values 2.052, 2.165, 2.094, 2.064 for [Cu(aptsc)($ClCH_2CH_2O$)(en)], [Cu(bptsc)($ClCH_2CH_2O$)(en)], [Cu(bztsc)($ClCH_2CH_2O$)(en)] and [Cu(vantsc)($ClCH_2CH_2O$) (en)] which is characteristic of octahedral geometry.[32] The VSM plots show hysteresis loops indicating that the complexes are ferromagnetic in nature.

Table 1: Elemental analysis data on the complexes

Complexes	%N (theo) exp	%Cl (theo)exp	% S (theo) exp	% Cu (theo) exp
[Cu(aptsc)($ClCH_2CH_2O$)(en)]	(17.7)16.7	(9.0)8.1	(8.1)8.9	(16.0)14.8
[Cu(bptsc)($ClCH_2CH_2O$)(en)]	(15.3)16.1	(7.8) 7.9	(7.0)7.8	(13.9)12.9
[Cu(bztsc)($ClCH_2CH_2O$)(en)]	(17.9)20.0	(9.3)8.6	(8.4)8.5	(16.7)17.3
[Cu(vantsc)($ClCH_2CH_2O$)(en)]	(16.4)16.1	(8.2)8.4	(7.5)7.8	(14.9)15.2

Table 2: Electronic spectral and Thermal decomposition data

Complexes	L-→M (nm)	E_g-→T_{2g}(nm)	Final Residual mass % TGA data
[Cu(aptsc) ($ClCH_2CH_2O$) (en)]	290,320	595	(24.2)24.8
[Cu(bptsc) ($ClCH_2CH_2O$) (en)]	260, 330	610	(17.4)18.1
[Cu(bztsc) ($ClCH_2CH_2O$) (en)]	288,350	610	(25.0)25.8
[Cu(vantsc) ($ClCH_2CH_2O$) (en)]	271	605	(18.6)17.7

Table 3: IR spectral data on the complexes

Complexes	υ_{NH2} (en)	υ_{NH2} (tsc)	$\upsilon_{C=N}$	υ_{C-S}	υ_{C-O}	υ_{C-Cl}	υ_{M-N}
[Cu(aptsc)($ClCH_2CH_2O$) (en)]	3464 3305	3213 3180	1602	689	1096	761	528 425
[Cu(bptsc)($ClCH_2CH_2O$) (en)]	3416 3341	3341 3249	1604	697	1089	771	532 471
[Cu(bztsc)($ClCH_2CH_2O$) (en)]	3420 3387	3258 3149	1601	692	1058	757	507 428
[Cu(vantsc)($ClCH_2CH_2O$)(en)]	3429 3300	3300 3231	1593	681	1037	744	530 410

Applications

Antibacterial Activities

The antibacterial activities of the complexes were tested against five different bacteria namely, E.coli, Pseudomonas aeruginosa, Salmonella typhi, Bacillus subtilis and Staphylococcusaureus by the Agar disc diffusion method[33] and compared against standard Ampicillin. The diameter of the inhibitory zone at three different concentrations are given in Table-4. As the concentrationincreases, the diameter of the inhibitory zone also increases indicating that the complexesare active. Among the complexes, the benzophenone complex showednearly the same activityagainst E.coliasthe standard ampicillin. The benzaldehydeanalogue showedvery good activity against Salmonella typhi while the vanillin complexshowed its best activity against*Bacillus subtilis* .The vanillin complex showed least activity against pseudomonasaeruginosa and was found to be inactive even at 1000µg/ml. The acetophenone analogue is found to be highly active against E.coli and least active against staphylococcus aureus compared to standard. All the other copper complexes showreasonable activity against all the bacteria.

Antifungal Activities

The Complexes were tested against three different fungi namely Candida albicans, Trichoderma viridi, and Aspergillus Niger and the data are tabulated in Table-5. As the concentration increases, the activity of all the complexes increases indicating that the complexes are active. The benzophenone and vanillin complexes showed excellent activity equivalent to the standard against Aspergillus niger while the

acetophenonecomplex showed activity equivalent to the standard against Trichoderma viridi. Thebenzaldehyde complex exhibited activityin par with the standard against Candida albicans. Activity of all the complexes against all the fungi are good with only exception of the benzophenone complex showing moderate activity against Candida albicans.

Table 4: Antibacterial studies

Complexes	Bacteria	Inhibition Zone(mm) Concentration(µg/ml)			Standard (1µg/ml)
		1000	750	500	
[Cu(aptsc)(ClCH$_2$CH$_2$O)(en)]	*E .coli*	15	10	6	29
	Pseudomonas aeruginosa	18	13	10	35
	Salmonella typhi	16	14	9	22
	Bacillus subtilis	15	11	7	23
	Staphylococcus aureus	12	11	7	29
[Cu(bptsc)(ClCH$_2$CH$_2$O)(en)]	*E .coli*	26	11	10	29
	Pseudomonas aeruginosa	20	15	13	35
	Salmonella typhi	15	13	11	22
	Bacillus subtilis	12	10	8	23
	Staphylococcus aureus	14	11	8	29
[Cu(bztsc)(ClCH$_2$CH$_2$O)(en)]	*E .coli*	20	20	14	29
	Pseudomonas aeruginosa	15	13	7	35
	Salmonella typhi	24	22	14	22
	Bacillus subtilis	8	6	4	23
	Staphylococcus aureus	19	16	10	29
[Cu(vantsc)(ClCH$_2$CH$_2$O)(en)]	*E .coli*	18	11	8	29
	Pseudomonas aeruginosa	-	-	-	35
	Salmonella typhi	18	17	11	22
	Bacillus subtilis	21	20	13	23
	Staphylococcus aureus	16	14	9	29

Table 5: Antifungal studies

Complexes	Fungi	Zoneof Inhibition(mm)			Antibiotic (1mg/ml)
		1000	750	500(µg/ml)	
[Cu(aptsc)(ClCH$_2$CH$_2$O) (en)]	*Candida albicans*	20	15	8	25
	Trichoderma viridi	26	18	15	28
	Aspergillus niger	19	15	10	23
[Cu(bptsc)(ClCH$_2$CH$_2$O) (en)]	*Candida albicans*	6	-	-	11
	Trichoderma viridi	22	18	16	29
	Aspergillus niger	10	8	8	10
[Cu(bztsc)(ClCH$_2$CH$_2$O) (en)]	*Candida albicans*	9	8	7	9
	Trichoderma viridi	9	8	7	12
	Aspergillus niger	10	9	9	12
[Cu(vantsc)(ClCH$_2$CH$_2$O) (en)]	*Candida albicans*	12	8	6	20
	Trichoderma viridi	16	12	10	22
	Aspergillus niger	11	10	9	12

Anticancer Activities

The anticancer activities of the complexes were studied by MTT assay [34] on HT 29 cell line. In parallel the activity was tested against Vero cell line(Normal). %Cell viability data on Normal and Cancer cell lines are presented in Table 6.100 minus %Cell viability gives percentage cell toxicity. IC 50 refers to the concentration require to cause 50% cell death. The IC50 values for the cancer cell line are 7.8, 31.2, 31.2 and 125 µg/ml for the complexes[Cu(aptsc)(ClCH$_2$CH$_2$O)(en)], [Cu(bptsc)(ClCH$_2$CH$_2$O)(en)],[Cu(bztsc) (ClCH$_2$CH$_2$O)(en)] and [Cu(vantsc)(ClCH$_2$CH$_2$O) (en)] respectively. As observed, the Complexes show excellent

activity even at lower concentrations. The data clearly reveals that, acetophenone complex has greater anticancer activities followed by benzophenone and benzaldehyde complexes. The vanillin complex has least activity compared to other analogues. However, it can be seen that the complexes, at 1000µg/ml concentration cause only 50.0, 52.78, 33.34 and 44.45% cell death with respect to normal cells, for the acetophenone, benzophenone, benzaldehyde and vanillin complexes respectively. This fact indicates that, not only the complexes possess good anticancer actitvity but are also less toxic to normal cells.

Table 6: Anticancer studies- % cell viability data on normal and cancer cell lines

Conc. (µg/ml)	Acetophenone semicarbazone complex		Benzophenone semicarbazone complex		Benzaldehyde semicarbazone complex		Vanillin semicarbazone complex	
	VERO	HT-29	VERO	HT-29	VERO	HT-29	VERO	HT-29
1000	50.00	4.16	47.22	11.11	66.66	5.55	55.55	6.94
500	55.55	6.94	61.11	15.27	75.00	9.72	66.66	16.66
250	63.88	9.72	66.66	23.61	77.77	19.44	75.00	34.72
125	75.00	16.66	77.77	34.72	80.55	29.16	80.55	45.83
62.5	83.33	26.38	83.33	47.22	83.33	41.66	88.88	56.94
31.2	88.88	33.33	88.88	55.55	83.33	50.00	91.66	65.27
15.6	91.66	40.27	97.22	61.11	83.33	58.33	94.44	72.22
7.8	97.22	51.38	100	68.05	86.11	70.83	94.44	77.77
Cellcontrol	100	100	100	100	100	100	100	100

CONCLUSIONS

The antibacterial, antifungal studies on the complexes indicate excellent activity and most of the complexes are active even at lower concentration. The anticancer activities of the complexes with low IC_{50} values, as low as 7.8 µg, in addition to being very less harmful to normal cells, is an indication thatthese complexes have promising future in the medicinal field as anticancer agents.

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