IOSR Journal of Applied Chemistry (IOSR-JAC)
e-ISSN: 2278-5736.Volume 9, Issue 6 Ver. I (Jun. 2016), PP 72-76
www.iosrjournals.org

Synthesis, Characterization and Study of Biological Applications of Piperazine Dithiocarbamate Bridged Homo Binuclear Mixed Ligand Complexes of Co (II) with Amino Acids

Bharathi Krishnan, R. Vijayanthimala*
*Formerly Associate Professor, Department of Chemistry, Ethiraj College for Women, Chennai-8, Tamilnadu

Abstract: *Binuclear Cobalt(II) complexes of the type* ***$[Co_2(pipdtc)(aa)_2(H_2O)_4]$,*** *containing bridging Piperazine Dithiocarbamate (pipdtc) and amino acids(aaH) such as alanine(alaH), Phenyl alanine(phealaH), Tyrosine(tyrH), Methionine(metH) and Glycine(glyH) as the deprotonated chelated anion , have been prepared and Characterized by elemental and thermal analysis, IR, UV-Vis , ESR spectral studies, and magnetic susceptibility studies. Antibacterial, and Antifungal activities have also been carried out on these complexes which indicate promising results. The anticancer and antioxidant properties along with the DNA Cleavage studies have also been done which confirms the complexes are biologically significant..*

Keywords:*Binuclear Cobalt complexes, Amino acids, piperazine Dithiocarbamate, Antioxidant property and DNA cleavage studies.*

I. Introduction

Numerous complexes containing mixed ligands have been reported frequently in the literature due to their wide range of applications (1).Particularly the complexes containing dithiocarbamate ligand are largely focused due to the ease in synthesis and versatility in binding. They have a wide range of applications due to their well demonstrated properties like lubrication, and usage as accelerators in Vulcanization, Antimicrobial, Anti- cancer, Anti HIV, Anti-oxidant characteristics and so on , as good ligands for gold surfaces, either in the coating of the nanoparticles or in forming monolayers on Au(1 1 1) surfaces. (2-6). Piperazine dithiocarbamate (pipdtc) has been reported in literature for many decades now and its complexes have been studied for their biological applications (7-10). Amino acids have special importance compared to other chemical compounds in the sense that they are regarded as the foundation stones of living organisms. Hence complexes containing amino acids as ligands are considered to exhibit better biological applications (11-13). Guttierrez et al have reported the synthesis of Au (I) complexes containing amino acids and dithiolates (14) and these have been reported to have good antitumor activity. With the proven advantages of amino acids and dithiocarbamates, we have attempted the Synthesis of binuclear Co (II) complexes containing these ligands and tested their anti-oxidant, anti-cancer, antibacterial and antifungal activities. Further evidence for their anti-cancer activity has been supported with DNA cleavage studies.

II. Experimental Section

The chemicals employed for the preparation are of Analar grade and hence used without further purification. The Cobalt (II) chloride, piperazine, glycine, alanine, phenyl alanine, tyrosine and methionine used for the synthesis are of analytical grade. Carbon disulphide is pure grade chemical from Merck chemicals. The alcohol used as solvent in all our studies is distilled by standard procedures**.**

One equivalent of piperazine was weighed accurately and dissolved in 20ml of ethanol and stirred for 10 min. To this added two equivalents of Carbon di sulphide and stirred continuously at room temperature followed by the addition of potassium hydroxide when the potassium salt of piperazine dithiocarbamate separated out. To one equivalent of the piperazine dithiocarbamate synthesized, added two equivalence of Cobalt amino acid complex prepared by the addition of two equivalents of the Cobalt chloride dissolved in water to two equivalents of amino acid dissolved in aqueous solution of sodium hydroxide with a constant stirring for about 20 mins. This mixture is continuously stirred, when the complexes separated out. This solid is washed repeatedly with a mixture of alcohol and water.The complexes were filtered and dried over vacuum.

The metal content present in the complexes were estimated using ICP-OES (Inductively coupled plasma - optical emission Spectroscopy). The nitrogen and sulphur content were estimated by Kjelhdhal's method and barium sulphate method respectively.TG/DSC were recorded in NETZSCH STA 449F3 thermal analyzer with a heating rate of 10^{o}/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 Spectrometer. The EPR spectra of the complexes were recorded using JES-FA200 electron spin resonance spectrometer in the region from 1000-8000 gauss.The SEM image was recorded using Quanta 200 FEG scanning electron microscope. The bactericidal and fungicidal activities of the complexes were studied by agar disc diffusion method (15). The anti-cancer activities were studied using the MTT assay (16). The DNA binding studies were done by UV spectrophotometric method on genomic DNA (17). The antioxidant properties of the Complexes were studied using two methods viz: DPPH free radical scavenging Assay and FRAP (Ferric ion reduction potential) method (18).

III. Results and Discussions

All the complexes were green in colour ,found to be fairly soluble in chloroform and DMSO and insoluble in alcohol, DMF and water The Elemental and Thermal analysis data on the complexes confirmed the composition of the complexes as $[Co_2(\text{deprotonated amino acid})_2(\text{pipdtc})(H_2O)_4]$ and the data are furnished in TABLE I. The final residue corresponded to metal sulphides in all the complexes .The molar conductance of the complexes were found to be in the range of 1-2 $Ohm^{-1}cm^2mol^{-1}$ indicating the non-electrolytic nature of the complexes (19).The IR spectral data of the complexes are furnished in TABLE II. The O-H stretching of coordinated water appeared around $3400cm^{-1}$ in all the complexes. The strong band around $1000cm^{-1}$ proved the presence of bidentate, mono ionic binding of dithiocarbamate in the complexes. The lowering in the asymmetric stretching frequency of COO^- group of amino acid has been reported as a result of more single bond character as a consequence of coordination. The electronic spectra of the complexes were recorded in chloroformic solution and the results are tabulated in TABLE I. The peak around 640nm has been assigned to $4T_{1g} \rightarrow {}^4T_{2g}$ (F) transition while the peaks around 420nm and 325nm have been assigned to ${}^4T_{1g} \rightarrow {}^4A_{2g}$ (F) and ${}^4T_{1g} \rightarrow {}^4T_{1g}$ (P) respectively (20). This confirmed the presence of an octahedral environment around the central metal ion. The ESR spectra of the alanine and glycine complexes gave a broad signal instead of the expected three lined spectra due to fast spin lattice relaxation, while the methionine and tyrosine complexes showed a three lined pattern. An eight lined ESR spectrum obtained for the phenyl alanine complex could be attributed to the presence of hyperfine splitting with the Co nucleus with I=7/2. The magnetic susceptibility studies of these complexes show an increase in mass in the presence of the magnetic field and hysteresis loop, indicative of Ferro magnetism. Observation of loop with a negligible height and the coercivity suggested that these complexes have particles of significantly small size and hence are magnetically significant too. The surface morphology of the complex obtained from the SEM images revealed the spherical shape of the complexes. The agglomeration is evident from the picture and particle size of roughly less than 500 nm could also be seen.

IV. Biological Studies

Anti-Bacterial Studies:

The bactericidal activities of the complexes were tested against the following bacteria viz: *Staphylococcus aureus, E.coli, Pseudomonas aeruginosa, Aeromonashydrophila and Vibrio spp.* by the Agar disc diffusion method and compared against standard Ampicillin. The anti-bacterial activity data of the complexes are tabulated in TABLE III. To get a clear picture, a comparative ratio of the activities of the complex to the standard at 1000µg had been calculated as diameter of inhibitory zone of complex at 1000 µg x 10 / diameter of inhibitory zone of standard at 1000 µg and been plotted. The plots are given in Figure I .It can be clearly noted from the plots that the complexes showed similar activity towards *E. Coli and Vibrio spp*. and so are the behaviour of the complexes towards *Pseudomonas aeruginosa* and *Aeromonas hydrophillia*. The behaviour of all the complexes towards the only Gram Positive bacteria, *Staphylococcus* followed a distinct pattern.

Anti -Fungal Activities:

All the complexes showed moderate activity towards the fungi tested, viz: *Candida albicans, Aspergillus niger and Rhizopus spp*. The results have been tabulated in TABLE IV. The activity was compared against standard Amphotericin B. Except the methionine analogue, all the other complexes exhibited excellent activity towards *Candida Albicans*. Among all the complexes studied, tyrosine analogue has been found to display outstanding results at 1000µg.

Anti- Cancer and DNA Cleavage studies:

These studies were carried out for the alanine and Phenyl alanine complexes. The cell viability data of the complexes on the VERO cell line and MCF-7 cell line have been tabulated in TABLE V. The alanine and phenyl alanine complexes exhibited a selectivity index of 32 and 64 respectively, where Selectivity index = IC_{50} for normal cell line / IC_{50} for cancerous cell line and IC_{50}: Inhibitory concentrations for 50% cell death in any cell line. The anticancer study is generally inferred due to DNA binding of the metal complexes followed by cleavage. The DNA cleavage study for the above said complexes were performed at two temperatures viz; 300

and 310K on genomic DNA and the results are presented in TABLE VI. A Plot of concentration Vs absorbance for the alanine and phenyl alanine complexes at 300 and 310 K is given in Figure II

DNA Cleavage Studies of Phenyl Alanine Complex at 300 and 310K:

A decrease in absorbance was observed with a rise in concentration. A similar behaviour has been reported by Hassan Mansouri – Torshizi et al in case of Pt dithiocarbamate complexes (18).The probable reason for such a behaviour cited by the group are i) Straightening of DNA double helix due to the interaction with the metal complex causing a conformational change thereby leading to denaturation, ii) A more stacked structure appeared after denaturation, iii) decrease in absorbance due to the suppression of hydrophobic bases by the metal complexes after penetrating the double helix.

Antioxidant properties:

The antioxidant properties of the complexes have been studied by DPPH and FRAP assay and the results are tabulated in TABLE VII. Among the complexes studied, the glycine complex followed by the tyrosine complex showed best results in the DPPH assay. The results obtained from the FRAP assay reiterated the same results. Presence of electron releasing groups in Alanine, phenyl alanine and methionine perhaps make them less promising antioxidants compared to Glycine and tyrosine complexes.

V. Conclusion

From the spectral studies, it is concluded that the piperazine dithiocarbamate acts as a bridging ligand coordinating to two Co^{2+} ions. Thus two of the octahedral sites around Co are satisfied by the chelated dithiocarbamate moiety. A chelated amino acid satisfies two more coordination sites around each Cobalt. The fifth and the sixth coordinating sites are satisfied by aquo ligands. The anticancer activity of the alanine and phenyl alanine complexes showed promising results further supported by the DNA studies. The antioxidant properties of the complexes show the gradation due to presence of different amino acids and hence it could be proved that the presence of a second ligand affects the properties of the complex. The Selectivity index as high as 32 and 64 indicates the fact that these compounds could be used as good anticancer agents.

TABLE 1: Elemental, Thermal decomposition and Electronic spectral data.

Complexes	%N (theo) exp	%S (theo) exp	%Co (theo) exp	%Residual mass (Theo) exp	Λ_{max}(nm)
$[Co_2(pipdtc)(tyr)_2(H_2O)_4]$	(7.12) 6.52	(16.28) 16.57	(14.98) 13.99	(23.13) 22.07	637, 492, 378
$[Co_2(pipdtc)(ala)_2(H_2O)_4]$	(9.30) 8.76	(21.25) 22.03	(19.57) 19.84	(30.20) 31.55	632, 424,333
$[Co_2(pipdtc)(phenala)_2(H_2O)_4]$	(7.42) 8.11	(16.96) 16.23	(15.62) 16.35	(24.10) 25.08	640,419, 324
$[Co_2(pipdtc)(met)_2(H_2O)_4]$	(7.75) 7.47	(26.57) 26.66	(16.31) 15.89	(25.17) 25.88	638,440,327
$[Co_2(pipdtc)(gly)_2(H_2O)_4]$	(9.75) 8.66	(22.29) 22.12	(20.53) 20.86	(31.67) 32.36	609,424,333

TABLE II: IR Spectral data of the Complexes

Complexes	ν_{O-H} H_2O	ν_{N-H}	ν_{C-H} (pip)	$\nu_{C=S}$	ν_{N-C}	ν_{C-S}	ν_{C-O}	ν_{COO} amino acid
$[Co_2(pipdtc)(tyr)_2(H_2O)_4]$	3441	3220	2938	1275	1357	997	1127	1619
$[Co_2(pipdtc)(ala)_2(H_2O)_4]$	3541	3250	2988, 2947	1272	1357	993	1100	1648
$[Co_2(pipdtc)(phenala)_2(H_2O)_4]$	3420	3210	2920	1274	1360	994	1099	1626
$[Co_2(pipdtc)(met)_2(H_2O)_4]$	3445	3278	2971, 2948	1275	1358	997	1092	1644
$[Co_2(pipdtc)(gly)_2(H_2O)_4]$	3364	3248	2987, 2939	1274	1357	995	1096	1613

TABLE III: Anti- Bacterial Studies:

Complex	Bacteria	Zone of Inhibition (mm) Concentration(µg/ml)			Standard (1mg/ml)
		1000µg	750µg	500µg	
$[Co_2(pipdtc)(tyr)_2(H_2O)_4]$	*Staphylococcus aureus*	8	6	2	9
	E.coli	5	4	2	7
	Pseudomonas aeruginosa	4	3	1	7
	Aeromonashydrophila	6	5	-	8
	Vibrio spp.	5	2	-	7
$[Co_2(pipdtc)(met)_2(H_2O)_4]$	*Staphylococcus aureus*	7	6	4	8
	E.col	6	5	4	8
	Pseudomonas aeruginosa	8	6	5	7
	Aeromonashydrophila	5	3	-	8
	Vibrio spp.	4	2	-	6
$[Co_2(pipdtc)(ala)_2(H_2O)_4]$	*Staphylococcus aureus*	3	2	-	5
	E.coli	3	1	-	6
	Pseudomonas aeruginosa	5	4	1	5
	Aeromonashydrophila	6	5	4	5
	Vibrio spp.	4	2	-	5
$[Co_2(pipdtc)(phenala)_2(H_2O)_4]$	*Staphylococcus aureus*	8	7	4	9
	E.coli	8	5	4	8
	Pseudomonas aeruginosa	7	3	2	9
	Aeromonashydrophila	7	2	-	9
	Vibrio spp.	5	-	-	6
$[Co_2(pipdtc)(gly)_2(H_2O)_4]$	*Staphylococcus aureus*	5	4	2	9
	E.coli	8	6	5	5
	Pseudomonas aeruginosa	4	2	-	6
	Aeromonashydrophila	6	5	2	6
	Vibrio spp.	8	5	1	8

TABLE IV: Anti-fungal studies:

Complexes	Fungi	Zone of Inhibition (mm)			Standard (1mg/ml)
		1000µg	750 µg	500 µg	
$[Co_2(pipdtc)(Tyr)_2(H2O)_4]$	*Candida albicans*	10	9	7	8
	Aspergillus niger	9	7	6	7
	Rhizopus spp.	9	5	4	8
$[Co_2(pipdtc)(Met)_2(H2O)_4]$	*Candida albicans*	5	3	-	8
	Aspergillus niger	6	5	4	7
	Rhizopus spp.	4	2	-	7
$[Co_2(pipdtc)(Ala)_2(H2O)_4]$	*Candida albicans*	7	5	4	7
	Aspergillus niger	5	3	2	6
	Rhizopus spp.	4	2	-	6
$[Co_2(pipdtc)(Phenala)_2(H2O)_4]$	*Candida albicans*	5	1	-	5
	Aspergillus niger	7	6	4	5
	Rhizopus spp.	4	2	-	6
$[Co_2(pipdtc)(Gly)_2(H2O)_4]$	*Candida albicans*	8	7	5	7
	Aspergillus niger	4	3	1	5
	Rhizopus spp.	7	6	4	8

TABLE V: Cytotoxicity and anticancer activity of alanine and Phenyl alanine complexes on VERO and MCF-7 Cell line respectively

S.No	Conc-Entration	Dilution	Absorbance				% Cell viability			
			VERO Cell line		MCF-7 Cell line		VERO Cell line		MCF-7 Cell line	
			Ala	Phen ala	Ala	Phen ala	Ala	Phen ala	Ala	Phen Ala
1	1000	Neat	0.12	0.18	0.17	0.14	33.04	35.04	21.22	22.22
2	500	1:1	0.17	0.22	0.19	0.16	49.93	50.93	27.39	25.39
3	250	1:2	0.24	0.26	0.22	0.19	60.70	62.77	30.15	30.15
4	125	1:4	0.30	0.27	0.27	0.23	67.88	68.18	32.50	33.50
5	62.5	1:8	0.32	0.30	0.30	0.25	78.79	73.59	39.68	36.68
6	31.2	1:16	0.38	0.34	0.33	0.28	80.62	78.61	44.44	39.44
7	15.6	1:32	0.43	0.38	0.35	0.31	85.40	87.13	49.20	4420
8	7.8	1:64	0.45	0.40	0.40	0.34	90.63	92.36	54.96	49.96
9	Cell control	-	0.47	0.45	0.63	0.63	100	100	100	100

TABLE VI: The change of absorbance of DNA at constant temperature of 300 amd 310K of the alanine and Phenyl alanine complexes with concentration

S.No	CONCENTRATION	Optical density at 260nm Temperature = 300K		Optical density at 260nm Temperature = 310K	
		Alanine	Phenyl Alanine	Alanine	Phenyl Alanine
1	0.02	0.99	0.85	1.12	0.93
2	0.04	0.91	0.79	0.97	0.84
3	0.06	0.86	0.72	0.81	0.77
4	0.08	0.74	0.67	0.75	0.61
5	0.1	0.63	0.59	0.67	0.52
6	0.12	0.52	0.45	0.58	0.41
7	0.14	0.33	0.37	0.47	0.32
8	0.16	0.26	0.22	0.30	0.24
9	0.18	0.11	0.13	0.19	0.10

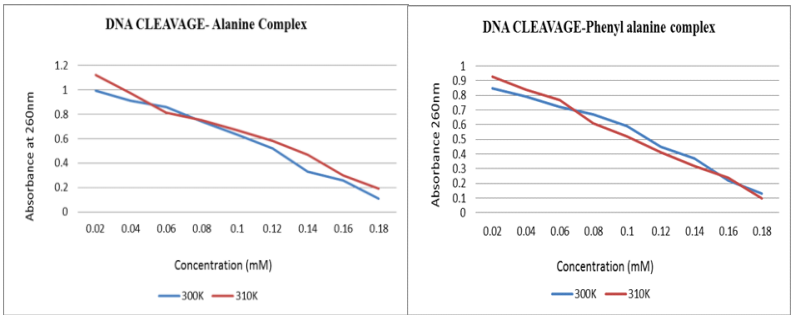

FIGURE IIDNA Cleavage Studies of Alanine and phenyl alanine Complexes at 300 and 310K respectively

TABLE VII: ANTIOXIDANT STUDIES

S.No	COMPLEX	DPPH ACTIVITY (%)	FRAP ACTIVITY
1	$[Co_2(pipdtc)(tyr)_2(H_2O)_4]$	58.7	228.7
2	$[Co_2(pipdtc)(gly)_2(H_2O)_4]$	51.1	218.4
3	$[Co_2(pipdtc)(phenala)_2(H_2O)_4]$	48.4	217.2
4	$[Co_2(pipdtc)(met)_2(H_2O)_4]$	46.2	211.3
5	$[Co_2(pipdtc)(ala)_2(H_2O)_4]$	43.5	196.9
6.	STANDARD BHT	99.9	-

References

[1]. AAM Aly; MS El-Meligy; ASA Zidan. Anionic complexes of some transition metals containing mixedβ-diketonato and alkylxanthato ligands; Transition Met. Chem., 14, 1989, 366–368

[2]. Bond AM and Martin RL. Electrochemistry and redox behaviour of transition metal dithiocarbamates. Coord. Chem. Rev. 1984;54,23.

[3]. Joris SJ. On the mechanism of decomposition of dithiocarbamates. J. Phys. Chem. 1970; 74:860.

[4]. Onwudiwe DC and Ajibade PA. Synthesis, Characterization and Thermal Studies of Zn(II), Cd(II) and Hg(II) Complexes of NMethyl- N-Phenyldithiocarbamate: The Single Crystal Structure of [(C6H5)(CH3)NCS2]4Hg2. Int.J.Mol.Sci. 2011; 12: 1964-1978.

[5]. Beer PD. Self assembled dithiocarbamatecopper (ii) macrocycls for electrochemical anion recognition. Chem. Commun. 2001; 4: 199-200.

[6]. Morf P and Raimondi. Dithiocarbamates: functional and versatile linkers for theformation of self-assembled monolayers.Langmuir. 2006; 22: 658-663.

[7]. R.S.Upadhayaya, N.Sinha, S.Jain, N.Kishore, R.Chandra and S.K.Arora, Bioorganic and Medicinal Chemistry, 12(9), 2225-2238, 2004.

[8]. P.Chaudhary, R.Kumar and A.K.Verma, Bioorganic and Medicinal Chemistry, 14(6), 1819-1826, 2006.

[9]. L.Mallesha and K.N.Mohana, European Journal of Chemistry, 2,193-199, 2011.

[10]. K.Rossen, S.A.Wiessman, J.Sagar, Tetrahedron Letters, 36, 6419-6422, 1995.

[11]. H. Zhao, H. Thurkauf, K. Hodgetts, X. Zhang, Bioorg. Med. Chem. Lett., 10, 3105, 2002.

[12]. A. Foster, H. Wu, W. Chen, W. Williams, W. D. Bowen and R. R. Matsumoto, Bioorg. Med. Chem. Lett., 2003, 13, 749-751.

[13]. S. Younes, Y. Labssita, G. Baziard-Mouysset and M. Payard, Eur. J. Med. Chem.,2000, 35, 107.

[14]. Gutierrez A, GraciaFleta L, Marzo I, CativielaC,Laguna A, Gimeno MC ,Dalton Trans.,2014;43(45):17054-66.

[15]. A.W.Bauer, W M MKirby,J C Sherris and M Turck, J. Am , ClinPathol, 36,493,(1966).

[16]. T.Mossman, J.Immunol Methods, 65(1-2), 55, (1983).

[17]. Hasan – MansouriTorshizi, MaryanSaeidifar, FatemehKhosravi, AdelehDivsalar, Al Akbar Saboury, and FatemehHassani, Bioinorganic Chemistry and Applications, article ID:394506, online version, (2011). NazninAra and HasanNur.,Research journal of medicine and medical agencies,4(1),107,2009.

[18]. NazninAra and HasanNur.,Research journal of medicine and medical agencies,4(1),107,2009.

[19]. Geary WJ. The use of conductivity measurements in organic solvents for the characterisation of coordination compounds. ,Coord.Chem. Rev. 1971; (7): 81.

[20]. Serrano J. New dithiocarbamate and xanthate complexes of nickel(II) with iminophosphines. Inorg.Chem.Acta. 2003;355: 33-40.