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Piperazine Dithiocarbamate Bridged Homo Binuclear Mixed Ligand Complexes ofMn (II) With Amino Acids – Synthesis, Spectral Characterization and Biological Studies

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Abstract: *Piperazinedithiocarbamate (pipdtc) bridged homobinuclear mixed ligand complexes of Mn(II) withchelated amino acidato ligands such as glycine, alanine, phenyl alanine, tyrosine, methionine and cystine have been synthesized and characterized by elemental and thermal analysis ,UV-Vis, infra-red, ESR Spectral analysis, magnetic susceptibility ,powder X-ray diffraction and scanning electron microscopicstudies. The complexeswere screened for antimicrobial activity againstBacillussubtilis, Staphylococcus aureus, E. coli, SalmonellaSpp and PseudomonasSppand three fungus namely Candida albicans, Trichodermaviridi and Aspergillusniger by agar disc diffusion method which showed promising results.The complexes were also evaluated for their toxicity towards human breast cancer line MCF-7 and the complexes exhibited low IC50 valuesand high selectivity index values. The antioxidant studies based on DPPH and FRAP assaywere also promising.DNAbinding studies have also been carried out on two of the complexes by spectrophotometric method.*

Keywords*:Mn(II) amino acidPiperazine dithiocarbamate complex,Antibacterial, antifungal, anticancer,antioxidant,DNA bindingstudies.*

I. Introduction

Piperazine, having a six membered ring with two nitrogen atoms at 1 and 4 positions of the ring, exits in chair and boat conformations, with the chair form preferred,due to greater stability (1). The boat form can however be stabilized by binding to a metal (2). The presence of nitrogen in the piperazine ring plays an important role in selectivity and sensitivity towards the biological systems. The piperazine derivatives and their complexes actas important pharmacological agents in various therapeutic areas such as antimicrobial, antifungal, antioxidant,antipsychotic, antimalarial and anti HIV protease (3-6). Amino acidbased drugs are gaining increasing popularity in diagnosing, preventing and treating diseases and maintaining or restoring the normal body conditions (7). Due to low toxicity, biocompatibility, in vivo stability, selectivity, high cell permeability and favoured interaction of aminoacids with the biological system, they have been in the current research, in biomedical field (8-12). Therefore, the functionalization of amino acids in the presence of heterocycles such as piperazine is expected to result in the formation of products with biological activity. Piperazinedithiocarbamates have been known for years due to their wide range of applications. Tandon and Kachru (13) have used piperazine and substituted piperazine based dithiocarbamate for lead removal in rats. Mohammad Tarique et al (14) have done an extensive study on the Physico-chemical behaviour of complexes of some 3d-series transition metals with piperazine 1, 4 dicarbodithioate. Ali Jassim Mohammad et al (15) have studied the Synthesis, characterization and antifungal activities of 3d-transition metal complexes of 1-acetylpiperazinyldithiocarbamate.K.S.Siddiq et al (16) have worked on Piperazine-bridged homo binuclear transition metal complexes in the presence of diethyl dithiocarbamate. Herein, we report the synthesis, characterization and biological studies such as antibacterial, antifungal, antioxidant, anticancer and DNA binding studies on piperazinedithiocarbamate bridged homo binuclear mixed ligand complexes of Mn(II) with amino acids.

II. Materials And Methods

The chemicals employed for the preparation are of very pure grade and used without further purification. The Manganese sulphate used for the synthesis is of analytical grade.Piperazine, carbon disulphide and sodium hydroxide, are pure grade chemicals from Merck. The amino acids, glycine, alanine, phenyl alanine, tyrosine, methionine,cystineand genomic DNA were purchased from Sigma Aldrich. The chloroform and DMSO used as solvent in all our studies are distilled by standard procedures. Nitrogen analysis was done by Kjeldhal's method,Sulphur estimated gravimetrically as barium sulphate and Manganese estimated by ICP-OES using PerkinElmer Optima 5300 spectrometer. Thermal analysis studies (TGA/DSC) were performed with a NETZSCH STA 490C/CD thermal analyser with a heating rate of 10^{o} C/minin nitrogen atmosphere.The electronic spectra were recorded on a Schimadzu UV 1600 model spectrometer in DMSO. IR spectra (4000-

550cm-1) were recorded on a FTIR Schimadzu spectrometer as KBr disc. EPR Spectra were done with a JES-FA 200 Electron spin resonance spectrometer in the region from 1000-8000 gauss. Magnetic susceptibility studies were carried out using Vibrating magnetometer Lakeshore VSM 7410 instrument.PowderXRD studies were done using a Bruker D8 Focus Advance Diffractometer equipped withLynx Eye detector with a Ni-filtered Cu Kα radiation (λ = 1.5406 Å) operating at 40 *kV* and 40 *mA* in 2θ range of 1.0 to 8.0 with a 2θ step size of 0.01° and step time of 2 *s*.The antibacterial and antifungal studies were carried out by using agar disc diffusion method originally described by Baeur (17). The invitro cytotoxicity of the prepared complexes was carried out by MTT based assay (18) with cancer cell line, MCF-7 (human cervical cell line). In parallel the activity was tested on normal cell line, VERO (monkey kidney cell line). The antioxidant studies were carried out by FRAP and DPPH assay (19, 20). The DNA binding studies were done by spectrophotometric method(21).

The complexes were prepared by one pot synthesis as follows.To one equivalence of piperazine (0.1g, 0.00116mole) added 20ml of rectified spirit and stirred for 10 minutes. Then added two equivalents of CS_2 (0.1764g, 0.0023mole) and again stirred for 20minutes at room temperature. To this, added two equivalents of potassium hydroxide (0.0023mole) resulting in the formation of potassium salt of dithiocarbamate.Thenadded aqueous solution of $MnSO_4$. $7H_2O$ (0.5 g, 0.0023mole) and aqueous solution of amino acid(0.0023mole) simultaneously and vigorously stirred for 1 hour until it afforded complete precipitation. The formed brown colouredprecipitate was filtered off and washed repeatedly (6-7times) with water-alcohol mixture and dried in vacuum.

III. Results And Discussion

The complexes are stable and non-hygroscopic solids. They are partially soluble in chloroform and DMSO.The complexes were insoluble in alcohol, water and DMF. All the complexes showed a non-electrolytic nature with a molar conductance in the range 1-2ohm^{-1} cm^{-2} mol $^{-1}$.The elemental analysis confirm the proposed composition Table-1. The abbreviations gly, ala, phenala, met, tyr and cys represent the deprotonated amino acids glycine, alanine, phenyl alanine, methionine, tyrosine and cystine respectively.The thermal analysis data from TGA for the complexes are furnished in Table-1.The thermograms were run upto 1000^o C and final residue corresponds to manganese sulphide. A higher decomposition temperature is also indicative of good thermal stability of the complexes and also supports the fact that water present is only coordinated water. The obtained experimental values of % MnS are in good agreement with the theoretical values once again confirming the proposed compositions. The electronic spectral data on the complexes are also given in Table- 1. In high spin d^5 manganese (II) configuration, the (d-d) transitions areLaporte forbidden and spin forbidden and hence low intense bands around 450 nmare assigned to octahedral Mn(II).The peaks in the region 350nm and below correspondto metal to ligand charge transfer (22). The IR spectral data on the complexes are given in Table 2. The stretching vibration of O-H of water molecules appears around 3400cm^{-1}. The tyrosine complex alone exhibita unique band at 3573cm^{-1} corresponding to the OH group attached to the phenyl ring. The ν N-H of amino acids appears in the region at 3220-3278 cm^{-1} whereas thev C-H of piperazine appears at 2900-2954 cm^{-1}. The ν COO$^-$ of coordinated amino acids shows a band in the region 1610-1650 cm^{-1} confirming bidentate chelate binding of the amino acidato group. The bands in the region 1250-1350cm^{-1} are assigned to ν N-C stretching vibration. The two bands around 870-1010cm^{-1} are assigned to ν C-S group of dithiocarbamate moiety and these confirm bidendatemonoionic nature of coordination of dithiocarbamate (23). The intense peak around 500 cm^{-1} correspond to the S-S bond of cystine (24). All the complexes give a single peak in the EPR spectrum and the g value corresponds to 2.00 for glycine, phenylalanine, methionine, tyrosine complexes and 1.99 for alanine, cystine complexes. The magnetic susceptibility studies showan increase in mass in the presence of magnetic field. The VSM plot of magnetic moment in emu vs. field shows hysteresis loop indicating ferromagnetism and negligible height of the loops and the coercivity suggest that these complexes have significantly small size**.** The powder x-ray diffraction pattern of alanine complex indicated that the data did not match with the standard JCPDS pattern and hence the complex may be amorphous in nature.The surface morphology of tyrosine complex was examined by SEM. It was found that the complex consisted of rod shaped particles.There was too much agglomeration of particles and the size was roughly about 500nm.

IV. Biological Studies

Antibacterial studies:

The antibacterial studies on the complexes were done using the agar disc diffusion method and the diameters of the inhibitory zones are tabulated in Table 3. Two gram positive bacteria (Bacillus subtilis and Staphylococcus aureus) and three gram negative bacteria (Pseudomonas Spp, Salmonella Spp and E.coli) were used for the analysis. The antimicrobial effect was quantified based on the inhibition zone measured in the disc diffusion tests conducted in plates at three different concentrations. With increasing concentration of the dithiocarbamate complexes, an increase in the diameter of the zone of inhibition was observed indicating that

the complexes are active. To make a proper comparison and bring about structure activity relationship, a standard index was calculated as the ratio of the diameter of the inhibitory zone at 1000µg of the complex to that of the standard multiplied by a factor of 10. A comparative ratio of the activities at 1000µg with respect to standard antibiotic ampicillin in terms of the diameter of the inhibitory zone is presented in Table 4andplotted in Figure 1.The observed antibacterial activity of the complexes are due to the presence of both amino acids and pipdtc moiety. The presence of hydrophobicity of amino acid side chain is expected to help the molecule to interact and penetrate well with the cell membrane of microorganisms and thereby inactivate them (25). On the other hand, the presence of two nitrogen atoms in the piperazinering and the dithiocarbamate moiety are also expected to increase the activity. From the Table 4 and Figure 1 it may be inferred that the phenyl alanine and tyrosine complexes exhibit activity almost comparable to that of the standard. The increased antibacterial activity of phenylalanine and tyrosine may be due to the presence of aromatic ring in both amino acids. Among the remaining compounds containing the aliphatic chain, the activities of glycine, alanine and methionine complexes are comparable and nearly half that of standard antibiotic. While cystine complex shows better activity than glycine, alanine and methionine complexes and lesser compared to phenyl alanine and tyrosine complexes.

Antifungal studies

The three fungi used were Candida albicans, Trichodermaviride and Aspergillusniger and data on the diameter of the inhibitory zone are tabulated in Table 5. The diameter increases with increase in the concentration of the complexes indicating that the complexes are active. All the complexes show moderate activity compared to standard Amphotericin B. A comparative ratio of the activities at 1000µg for the three fungi are calculated and presented in Table 6 and effect of complexes on various fungi tested are exemplified through simple plots given in Figure 2.The factors explained for antibacterial activity holds good for antifungal activity too. The complexes containing aromatic ring such as phenylalanine and tyrosine show increased activity towards the fungi studied. But in the case of other complexes moderate antifungal activity was only observed.

Antioxidant studies

The antioxidant property of all the complexes were determined by DPPH and FRAP method. The results of antioxidant studies are given in Table 7. In the present context, phenylalanine and alanine complexes show better activity than the other complexes but less than that of standard BHT in both DPPH and FRAP method.

Anticancer studies

The invitro cytotoxicity of alanine and phenyl alanine complex was determined by MTT assay. The results of the anticancer activity are presented in Tables 8 and 9.The complexes show good activity against the cancer cells and less toxicity towards normal cells. The IC_{50} value for the alanine and phenylalanine complexes are 31 µg/ml and 15 µg/ml .The selectivity indexvalues of alanine and phenyl alanine complexes arefound to be 16 and 32 respectively.

DNA binding studies

The DNA binding studies of the alanine and phenyl alanine complex were determined by spectrophotometric method. The complexes were interacted with Genomic DNA in TrisHCl buffer containing 20mM NaCl solution. The optical density at 260 nm was determined for various concentrations of the complexes at two different temperatures and the results are plotted in Figures 3 and 4. The plot of absorbance versus concentration showed an increase in absorbance with increase in concentration of the complexes for both temperatures suggesting direct cleavage.

Table 1 Elemental & Thermal Analysis And UV-Visible Spectral Data

Complexes	% nitrogen (theo)exp	% sulphur (theo)exp	% metal (theo)exp	% residue TGA(theo)Exp	Λmax (nm)
$[Mn_2(pipdtc)(gly)_2.4H_20]$	(9.89) 8.84	(22.61) 22.01	(19.41) 18.9	(30.84) 30.54	310,400
$[Mn_2(pipdtc)(ala)_2.4H_20]$	(9.43) 9.12	(21.55) 21.34	(18.50) 18.23	(29.3) 29.03	330,430
$[Mn_2(pipdtc)(phenala)_2.4H_20]$	(7.50) 7.89	(17.15) 16.89	(14.72) 13.22	(23.4) 23.13	300,360, 430
$[Mn_2(pipdtc)(met)_2.4H_20]$	(7.84) 6.72	(26.88) 26.21	(15.38) 15.01	(24.4) 25.08	350,430, 465
$[Mn_2(pipdtc)(tyr)_2.4H_20]$	(7.20) 6.94	(16.45) 17.82	(14.12) 13.78	(22.4) 22.07	320,410
$[Mn_2(pipdtc)(cys)_2.4H_20]$	(12.50) 11.98	(28.58) 28.21	(12.27) 11.56	(19.5) 19.58	340,360, 450

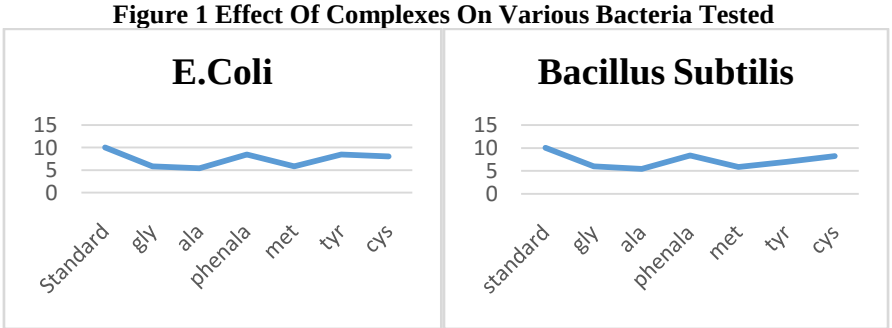
Table 2 Ir Spectral Data On The Complexes (ν Cm^{-1})

Complexes	νO-H of H_2O	νN- H	νC- H (pip)	νCOO^- (amino acid)	νN-C	ν C-S
$[Mn_2(gly)_2(pipdtc)(H_2O)_4]$	3414	3229	2921	1648	1356	920
$[Mn_2(ala)_2(pipdtc)(H_2O)_4]$	3393	3216	2919	1627	1356	910
$[Mn_2(phenala)_2(pipdtc)(H_2O)_4]$	3438	3212	2992	1621	1363	910
$[Mn_2(met)_2(pipdtc)(H_2O)_4]$	3391	3286	2918	1613	1356	914
$[Mn_2(tyr)_2(pipdtc)(H_2O)_4]$	3385	3284	2911	1616	1358	908
$[Mn_2(cys)_2(pipdtc)(H_2O)_4]$	3430	3220	2999	1651	1343	910

Table 3 Antibacterial Study On The Complexes

Complexes	Organisms	Zone of inhibition			Antibiotic (1mg/ml)
		1000 µg	**500 µg**	**250µg**	
$[Mn_2(pipdtc)(gly)_2.4H_20]$	Bacillus Subtilis	6mm	5mm	4mm	10mm
	Pseudomonas Spp	7mm	6mm	5mm	11mm
	Staphylococcus aureus	7mm	6mm	5mm	12mm
	Salmonella Spp	6mm	5mm	4mm	10mm
	E.coli	7mm	6mm	5mm	12mm
$[Mn_2(pipdtc)(ala)_2.4H_20]$	Bacillus Subtilis	6mm	5mm	4mm	11mm
	Pseudomonas Spp	7mm	6mm	5mm	11mm
	Staphylococcus aureus	6mm	5mm	4mm	13mm
	Salmonella Spp	7mm	6mm	5mm	14mm
	E.coli	7mm	6mm	4mm	13mm
$[Mn_2(pipdtc)(phenala)_2.4H_20]$	Bacillus Subtilis	10mm	9mm	8mm	12mm
	Pseudomonas Spp	9mm	8mm	7mm	11mm
	Staphylococcus aureus	11mm	10mm	9mm	13mm
	Salmonella Spp	10mm	9mm	8mm	12mm
	E.coli	11mm	10mm	9mm	13mm
$[Mn_2(pipdtc)(met)_2.4H_20]$	Bacillus Subtilis	7mm	6mm	5mm	12mm
	Pseudomonas Spp	6mm	5mm	4mm	10mm
	Staphylococcus aureus	7mm	6mm	5mm	14mm
	Salmonella Spp	6mm	5mm	4mm	11mm
	E.coli	7mm	6mm	5mm	12mm
$[Mn_2(pipdtc)(tyr)_2.4H_20]$	Bacillus Subtilis	7mm	6mm	5mm	10mm
	Pseudomonas Spp	11mm	10mm	9mm	14mm
	Staphylococcus aureus	10mm	9mm	8mm	12mm
	Salmonella Spp	10mm	9mm	8mm	12mm
	E.coli	11mm	10mm	9mm	13mm
$[Mn_2(pipdtc)(cys)_2.4H_20]$	Bacillus Subtilis	9mm	8mm	7mm	11mm
	Pseudomonas Spp	10mm	9mm	8mm	13mm
	Staphylococcus aureus	9mm	8mm	7mm	13mm
	Salmonella Spp	10mm	9mm	8mm	11mm
	E.coli	8mm	7mm	6mm	10mm

Figure 1 Effect Of Complexes On Various Bacteria Tested



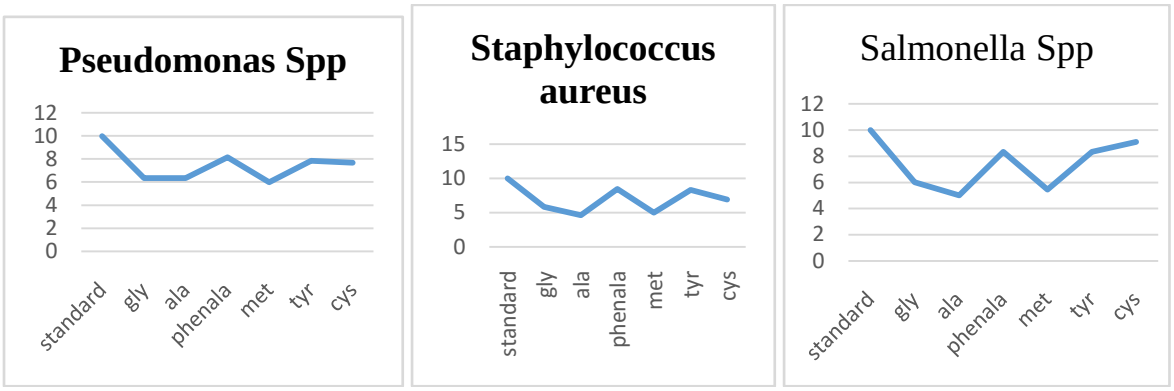


TABLE 4 COMPARATIVE RATIO OF THE ACTIVITIES AT 1000µg

Complexes	E.coli	Pseudomonas Spp	Staplylococcusaureus	Salmonella Spp	Bacillus Subtilis
$[Mn_2(gly)(_2(pipdtc)(H_2O)_4]$	5.83	6.36	5.83	6	6
$[Mn_2(ala)_2(pipdtc)(H_2O)_4]$	5.38	6.36	5.61	5	5.45
$[Mn_2(met)_2(pipdtc)(H_2O)_4]$	5.83	6	5	5.45	5.83
$[Mn_2(cys)_2(pipdtc)(H_2O)_4]$	8	7.69	6.92	9.09	8.18
$[Mn_2(phenala)_2(pipdtc)(H_2O)_4]$	8.46	8.18	8.46	8.33	8.33
$[Mn_2(tyr)_2(pipdtc)(H_2O)_4]$	8.46	7.85	8.33	8.33	7
Ampicillin (Standard)	10	10	10	10	10

Figure 2 Effect Of Complexes On Various Fungi Tested

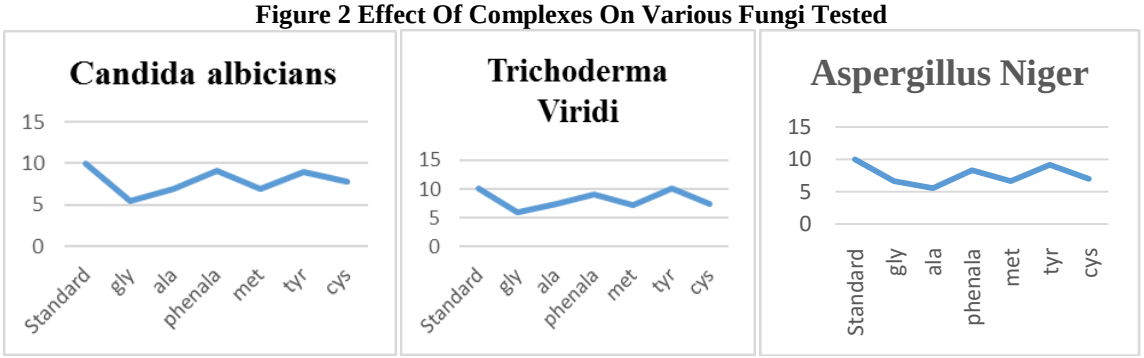


Table 5antifungal Studies On The Complexes

Complexes	Organisms	Zone of inhibition Concentration(µg/ml)			Antibiotic (1mg/ml)
		1000	750	500	
$[Mn_2(gly)_2(pipdtc)(H_2O)_4]$	Candida albicians	6mm	5mm	4mm	11mm
	TrichodermaViridi	6mm	5mm	4mm	10mm
	Aspergillusniger	6mm	5mm	4mm	9mm
$[Mn_2(ala)_2(pipdtc)(H_2O)_4]$	Candida albicians	7mm	6mm	5mm	10mm
	TrichodermaViridi	6mm	5mm	4mm	8mm
	Aspergillusniger	6mm	5mm	4mm	7mm
$[Mn_2(phenala)_2(pipdtc)(H_2O)_4]$	Candida albicians	10mm	9mm	8mm	11mm
	TrichodermaViridi	11mm	10mm	9mm	12mm
	Aspergillusniger	10mm	9mm	8mm	12mm
$[Mn_2(met)_2(pipdtc)(H_2O)_4]$	Candida albicians	7mm	6mm	5mm	8mm
	TrichodermaViridi	8mm	7mm	5mm	11mm
	Aspergillusniger	6mm	5mm	4mm	9mm
$[Mn_2(tyr)_2(pipdtc)(H_2O)_4]$	Candida albicians	9mm	8mm	7mm	10mm
	TrichodermaViridi	10mm	9mm	8mm	11mm
	Aspergillusniger	11mm	10mm	9mm	12mm
	Candida albicians	7mm	6mm	5mm	9mm
	TrichodermaViridi	6mm	5mm	4mm	8mm
$[Mn_2(cys)_2(pipdtc)(H_2O)_4]$	Aspergillusniger	7mm	6mm	5mm	10mm

TABLE 6 COMPARATIVE RATIO OF THE ACTIVITIES AT 1000µg

Complexes	Candida albicians	Trichodermaviridi	Aspergillusniger
$[Mn_2(gly)(_2(pipdtc)(H_2O)_4]$	5.45	6	6.66
$[Mn_2(ala)_2(pipdtc)(H_2O)_4]$	7	7.5	5.57
$[Mn_2(met)_2(pipdtc)(H_2O)_4]$	7	7.27	6.66
$[Mn_2(cys)_2(pipdtc)(H_2O)_4]$	7.77	7.5	7
$[Mn_2(phenala)_2(pipdtc)(H_2O)_4]$	9.09	9.16	8.33
$[Mn_2(tyr)_2(pipdtc)(H_2O)_4]$	9	10	9.16
Ampicillin (Standard)	10	10	10

Table 7 Antioxidant Activity Using DpphAnd Frap Method

COMPLEXES	DPPH Activity %	FRAP Activity %
$[Mn_2(gly)_2(pipdtc).(H_2O)_4]$	45.9	205.2
$[Mn_2(ala)_2(pipdtc).(H_2O)_4]$	63.3	259.8
$[Mn_2(phenala)_2(pipdtc).(H_2O)_4]$	70.2	270.1
$[Mn_2(met)_2(pipdtc).(H_2O)_4]$	59.8	232.6
$[Mn_2(tyr)_2(pipdtc).(H_2O)_4]$	55.5	225.5
$[Mn_2(cys)_2(pipdtc).(H_2O)_4]$	60.3	248.9
Standard BHT	99.9	299.0

Table 8 Anticancer Activity Of $[Mn_2(Ala)_2(Pipdtc)(H_2O)_4]$

Concentration µmg/ml	Dilutions	Absorbance(O.D)		Cell Viability(%)	
		Vero	MCF-7	Vero	MCF-7
1000	Neat	0.21	0.04	42.85	7.40
500	1:1	0.25	0.09	51.02	16.66
250	1:2	0.28	0.13	57.14	24.07
125	1:4	0.31	0.17	63.26	31.48
62.5	1:8	0.34	0.23	69.38	42.59
31.2	1:16	0.39	0.28	79.59	51.85
15.6	1:32	0.42	0.31	85.71	57.40
7.8	1:64	0.45	0.34	91.83	62.96
Cell Control	-	0.49	0.54	100	100

Table 9anticancer Activity Of $[Mn_2(Phenala)_2(Pipdtc)(H_2O)_4]$

Concentration µmg/ml	Dilutions	Absorbance(O.D)		Cell Viability(%)	
		Vero	MCF-7	Vero	MCF-7
1000	Neat	0.23	0.05	46.93	9.25
500	1:1	0.25	0.07	51.02	12.96
250	1:2	0.29	0.09	59.18	16.66
125	1:4	0.30	0.12	61.22	22.22
62.5	1:8	0.32	0.16	65.30	29.62
31.2	1:16	0.34	0.20	69.38	37.03
15.6	1:32	0.37	0.25	75.51	46.29
7.8	1:64	0.41	0.31	83.67	57.40
Cell Control	-	0.49	0.54	100	100

Figure 3 Absorbance of DNA at Constant Temperature at 300K and 310K $[Mn_2(ala)_2(pipdtc).(H_2O)_4]$Complex

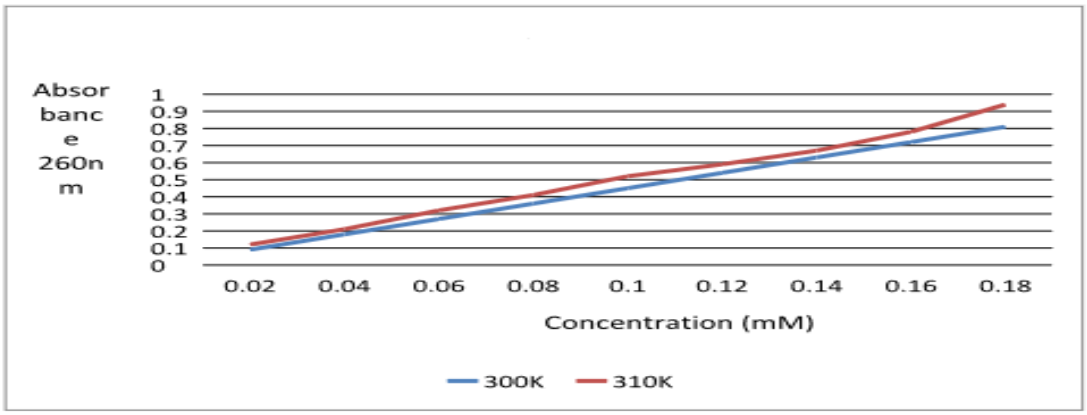
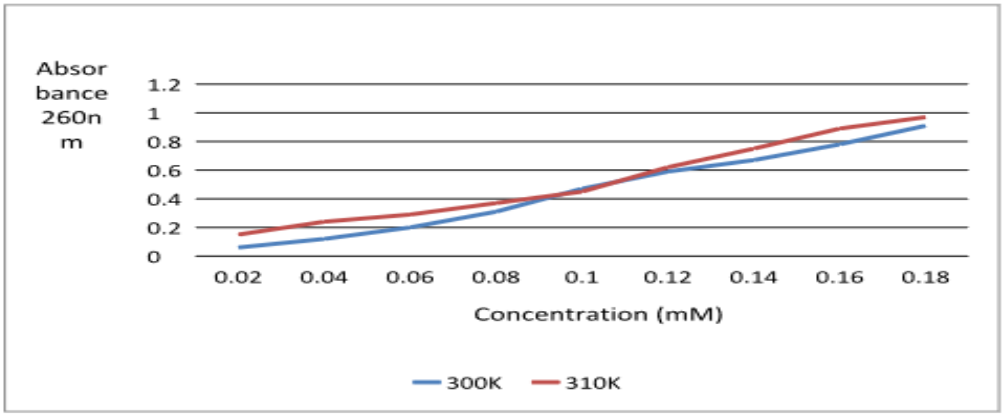


Figure 4 Absorbance of DNA at Constant Temperature at 300K and 310K For $[Mn_2(phenala)_2(pipdtc).(H_2O)_4]$Complex



V. Conclusion

From the above various spectral and analytical studies, it may be concluded that the complexes are binuclear with the bridging piperazine dithiocarbamate. Two aquo ligands and a bidentate amino acidato group satisfy the other four coordination sites around each Mn.The complexes show significant antibacterial, antifungal, antioxidant characteristics. The anticancer studies are also indicate greater significance of the studies because the selectivity index is as high as 32 which means normal cells are not affected.

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