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Spectral Investigation, Structural Assignments and Anti-Tumor Activities of Pyrimidine Based Transition Metal (Ii) Complexes

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ABSTRACT

Three novel Mannich bases 5-[(4-chlorophenyl)-(4-methylpiperazin-1-yl)-methyl]-pyrimidine-2,4,6-trione :CBzBAPz(NM), 5-[(4-nitrophenyl)-(4-methylpiperazin-1-yl)-methyl]-pyrimidine-2,4,6-trione: NBzBAPz(NM) and 5-[(phenyl)-(4-methylpiperazin-1-yl)-methyl]-pyrimidine-2,4,6-trione: BzBAPz(NM) are prepared by the condensation of N-methyl piperazine with p-chlorobenzaldehyde / p-nitro benzaldehyde / benzaldehyde and barbituric acid and characterized by physico-chemical, spectroscopic, and powder X-ray diffraction studies. Further, Cu (II), Cd (II) and Pd (II) complexes of titled ligands are synthesized and characterized. Based on the experimental results, the chelating (bidentate) behavior of the ligands is accessed. The X-band e.p.r.spectrum, electrochemical studies of the Cu (II) complex have been carried out and discussed. Three novel Mannich bases and their complexes exhibit comparable potential cytotoxicity against breast cancer cell line (MCF-7), particularly, strong cytotoxicity is observed in the case of palladium. Morphological features of cells used for the study is photographed. Use of fluorochromesacridine orange and ethidium bromide reveals apoptotic morphological features.

Keywords: barbiturates**,** N-methyl piperazine, anticancer activity, Mannich bases, MTT assay.

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INTRODUCTION

Multicomponent reactions (MCRs) has emerged as efficient and powerful tools in modern synthetic organic chemistry because, the synthesis of complex organic molecules from simple and readily available substrates can be achieved in a very fast and efficient manner without the isolation of any intermediate [1,3]. Therefore, developing new MCRs and improving known MCRs are popular areas of research in current organic synthesis.

Owing to the light-emitting[4],self-assembling[5] and complex forming[6]properties with metal ions or organic molecules, such materials are of interest in biological, chemical and materials science. 2-Aryl or 2-heteroaryl pyrimidines and their derivatives is reported as inhibitors of tubulin polymerization,1 and as activators of cell type-specific NF-kB[7].

Pharmacological and cell biological effects of barbiturates are studied including immunosuppressive and immunomodulatory actions on lymphocyte and leukocyte function. Barbiturates such as thiopental are used in patients suffering from severe traumatic brain injury to control intracranial hypertension and cerebral perfusion [8]. Prolonged infusion and high-dose administration are necessary to achieve the effect, but the regimen is associated with a loss of protective immunity and an increased incidence of infectious diseases [9].

The anti-proliferative properties of the chemical compounds were predetermined using an MTT assay. The principle is based on the reduction of a soluble tetrazolium salt, by mitochondrial dehydrogenase activity of viable tumor cells, into a soluble colored formant product that can be measured spectrophotometrically after dissolution [10]. Apoptosis is a biological phenomena that involves a process ranging from embryogenesis to ageing, from normal tissue homoeostasis to many human diseases. Apoptotic cells share a number of common features such as cell shrinkage, nuclear condensation, membrane blabbing, chromatin cleavage, and formation of pyknotic bodies of condensed chromatin [11-12].

MATERIALS AND METHOD

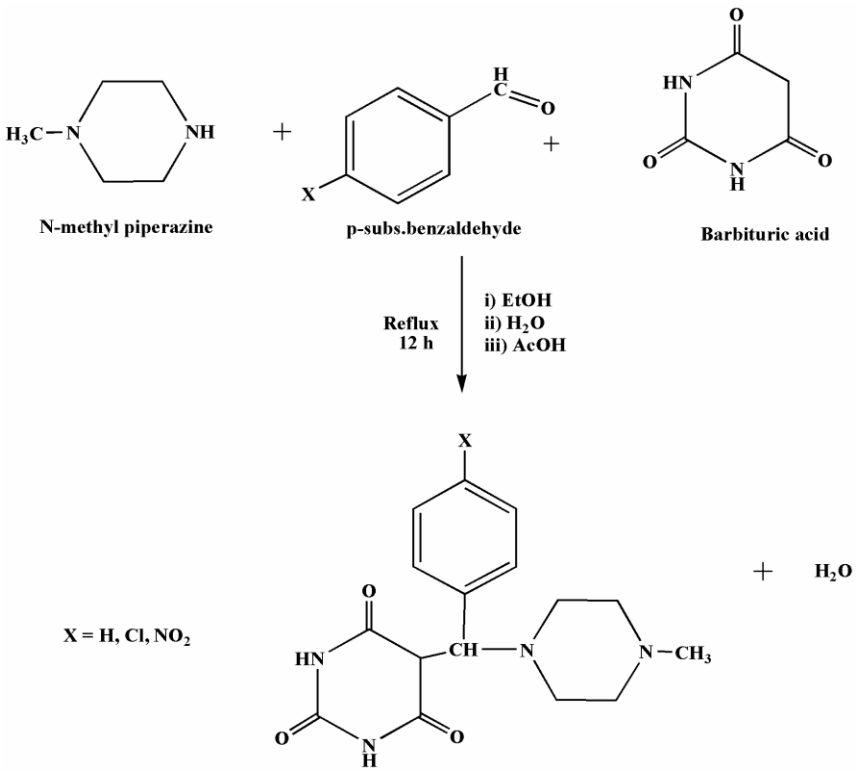
Solvents are obtained from commercial sources and used as received. Piperazine is purchased from Fluka, while N-methylpiperazine, barbituric acid, benzaldehyde, 4-chloro benzaldehyde and 4-nitro benzaldehyde for the synthesis of corresponding piperazine are obtained from Aldrich. Nuclear magnetic resonance spectroscopic measurements are made on Bruker300 MHz spectrometer (0-15δ). Deuterated organic solvents along with tetramethylsilane (TMS) as the internal standard is used. Infrared spectra for all the complexes and ligands are recorded on a JASCO FT-IR–410 (4000-400 cm^{-1}) spectrophotometer. Potassium bromide disc is employed for

sample preparation. The instrument is calibrated against polystyrene film. Electronic absorption spectral measurements are recorded in solution using JASCO V-550 UV-Vis spectrophotometer, and diode array spectrophotometer (Analytica Jena specord S 100).

The as prepared samples were characterized by X-ray powder diffraction (XRD) on a Rigaku /max-γB X-ray diffractometer with a Cu Kα radiation source (λ = 1.5418 A°) operated at 40 kV and 80 mA [13-14].

General procedure for the synthesis of barbiturate derivatives of N-methylpiperazine ligands CBzBAPz(NM), NBzBAPz(NM), BzBAPz(NM)

The titled ligands are prepared by Mannich condensation of N-methylpiperazine (1g/0.01M), barbituric acid (1.28g/0.01M) with the corresponding aldehyde, 4-chloro benzaldehyde (1.4g) and 4-nitro benzaldehyde (1.5g)), (benzaldehyde(1.4g) under refluxing for 10-12 hrs in ethanol-water (30mL) (1:1 ml) system at 70ºC. The compounds are isolated as yellow solid (powder) by keeping the reaction mixture for 2 days at room temperature. The compound is washed with ethanol, dried and recrystallized from aqueous ethanol **(Scheme 1)**.

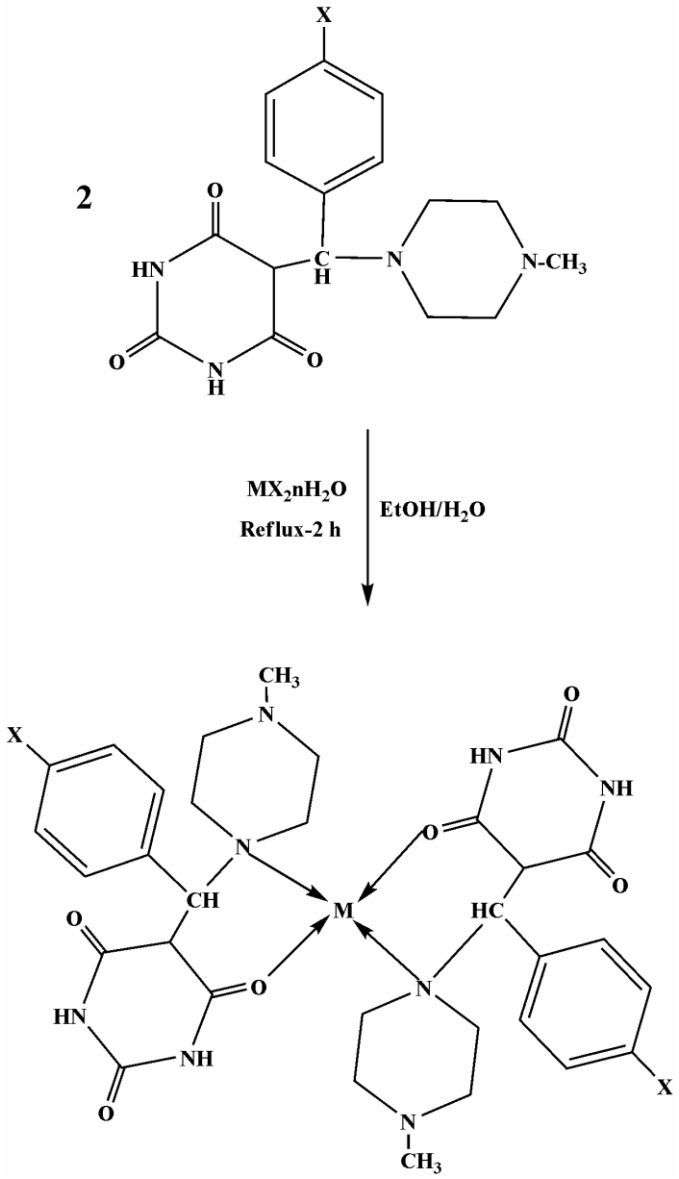


Scheme 1. Preparation of N-methylpiperazine base ligands

Synthesis of the Cu(II)/Cd(II) complexes

Copper (II) and Cadmium (II) complexes are synthesized by mixing 0.01M of a solution of transition metal chloride (copper (II) chloride dihydrate / cadmium (II) chloride hexahydrate) with

0.02M of the manic based ligand previously dissolved in aqueous ethanol solution (1:1 30mL). The reaction mixture is refluxed at 70°C for 2 hrs. Then, it is allowed to cool at room temperature. The solid complexes are filtered, washed with ethanol, and air-dried **(Scheme 2)**.



Scheme 2. Preparation of N-methylpiperazine base complexes

Synthesis of the Pd(II) complex

Palladium(II) complexes were prepared by mixing 0.01M of a solution of palladium(II)chloride tetrahydrate in ethanol containing a few drops of HCl with 0.02M of Mannich base ligand previously prepared by dissolving in hot aqueous ethanol-water (1:1) solution. The reaction mixture is refluxed at 70°C for 2 hrs and the pH of the solution is adjusted to 7 by adding a few drops of aqueous ammonia solution and kept at room temperature. The brown colored palladium

complex is separated, filtered and washed successively with cold ethanol to remove unreacted ligands and then air dried.

Cytotoxicity Tests in Cancer Cell Lines

Cytotoxicity is determined by means of a colorimetric micro culture assay [MTT assay, MTT = 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide]. For this purpose, MCF-7 cells are harvested from culture flasks by trypsinization and seeded in 200 µL aliquots in RPMI supplemented with 15% heat inactivated fetal bovine serum, 1 mM sodium pyruvate, 4 mM L-glutamine, and 1% non essential amino acids (100x) (all purchased from Sigma-Aldrich) into 96-well micro culture plates in cell densities of $5x10^3$ and $2.5x10^3$ cells per well, respectively, in order to ensure exponential growth of untreated controls throughout drug exposure. For 24 hrs, cells are allowed to settle and resume exponential growth, followed by the addition of dilutions of the test compounds in aliquots of 200 µl well-1 in the same medium. Only for the less water-soluble compounds 5t and 6t, stock solutions is prepared in a dimethyl sulfoxide (DMSO)/H_2O mixture (the stability of the compounds in a DMSO solution is proven by ^1H NMR spectroscopy). The DMSO content in the actually tested dilutions did not exceed 0.5%. After continuous exposure for 24, 48, 72 and 96 hrs, drug solutions are replaced by a 130 µl $well^{-1}$ RPMI 1640 culture medium (supplemented with 15% heat-inactivated fetal bovine serum and 4 mM L-glutamine) plus 20 µL well-1 MTT solution in phosphate-buffered saline (5 mg mL^{-1}). After incubation for 4 h, the medium/MTT mixtures are removed, and the formazan crystals formed by viable cells were dissolved in 100 µL of DMSO per well. Optical densities at 550 nm are measured with a micro plate reader (Bio-Rad), using a reference wavelength of 690 nm to correct for unspecific absorption. The quantity of vital cells are expressed in terms of [Test/Control] T/C values by comparison to untreated control micro cultures, and 50% inhibitory concentrations (IC_{50}) are calculated from concentration effect curves by interpolation. Evaluation is based on means format least three independent experiments, each comprising three replicates per concentration level.

Cell culture and drug treatment

MCF-7 cell line is maintained in RPMI 1640 medium supplemented with 0.2% penicillin streptomycin and 15% fetal bovine serum, and grown at 37ºC in a 5% CO_2 humidified environment. Cells are plated in 6-well tissue culture plates at a cell density of $2.5x10^5$ cells per well or in 96-well tissue culture plates at a density of 5000 cells per well. The medium is replaced with medium containing compounds after 24 hrs. The cells are treated with compounds

with indicated times (for each assay) and then the cells are harvested and the extent of apoptosis is assessed.

RESULTS AND DISCUSSION

In the present work, we report the synthesis, characterization and *in vitro* anticancer activity of Mannich bases of N-methylpiperazine. Cu(II), Cd(II) and Pd(II) complexes have also synthesized, characterized and subjected to cytotoxic studies. All the complexes are colored, non-hygroscopic solids, stable in air and soluble in DMSO. The elemental analyses show stoichiometry ML_2X_2, where L stands for ligand moiety, X- halide. The molar conductance values indicate the electrolytic nature of the complexes. Anal. (Calc) found for [$C_{16}H_{20}N_4O_3$ (BzBAPz(NM)); C, (60.70), 61.20; H, (6.31), 6.52; N, (17.80), 18.90); $C_{16}H_{19}ClN_4O_3$ (CBzBAPz(NM)); C, (54.71), 55.12; H, (5.40), 5.99; N, (15.90), 16.70; $C_{16}H_{19}N_5O_5$ (NBzBAPz(NM)); C, (53.11), 53.98; H, (5.31), 5.66; N, (19.39), 19.68;

The proposed chemical structures of the complexes are known to be in good agreement with the ratios concluded from the analytical data.

NMR Spectra

The ^{1}H NMR spectral results obtained for the CBzBaPz(NM), NBzBAPz(NM) and BzBAPz(NM) ligands and $(CBzBAPz(NM))_2Cd$ complex in d_6-DMSO together with assignments are given in Table 1. The proton resonance appears as two multiplets at 7.0-7.9 ppm for aromatic protons of benzaldehyde and 2.1-2.5 ppm for N-methyl piperazine. The four singlets at 2.8ppm, 10.0ppm, 3.7ppm and 5.9 ppm: the first one is assigned to methyl proton on N-methyl piperazine group, the second one for NH proton on barbituric acid, third one for CH proton on barbituric acid and the last one for CH proton of methine (Mannich) group.

In the ^{1}H NMR spectrum of $(CBzBAPz(NM))_2Cd$ complex in d_6-DMSO the signal at 10.1 ppm may be assigned to the N-H proton of the barbituric acid which confirm that the barbituric acid NH proton does not involve any coordination to metal ion. A chemical shift which belongs to proton resonance, generally observed confirms the chelation of ligands with metal ions.

Table 1.^{1}H NMR Signals of the synthesized N-methylpiperazine base- ligand

Compound	Signals (δ, ppm)	Assignment
BzBAPz(NM)	7.1- 7.9	Phenylproton≈ 5H
	3.4	C-H (barbiturate) ≈ 1H
	5.9	C-H (methine) ≈ 1H
	2.1-2.6	Piperazinering≈ 8H
	10.1	N-H ≈ 2H (barbiturate)
	2.5	N-methyl≈ 3H

CBzBAPz(NM)	7.0- 7.9	Phenylproton≈ 5H
	3.1	C-H (barbiturate) ≈ 1H
	5.2	C-H (methine) ≈ 1H
	2.1-2.5	Piperazinering≈ 8H
	10.0	N-H ≈ 2H (barbiturate)
	2.8	N-methyl≈ 3H
NBzBAPz(NM)	7.1- 8.4	Phenylproton≈ 5H
	3.3	C-H (barbiturate) ≈ 1H
	5.5	C-H (methine) ≈ 1H
	2.2-2.3	Piperazinering≈ 8H
	10.2	N-H ≈ 2H (barbiturate)
	2.5	N-methyl≈ 3H
$(CBzBAPz(NM))_2Cd$	7.2- 8.4	Phenylproton≈ 5H
	3.4	C-H (barbiturate) ≈ 1H
	5.3	C-H (methine) ≈ 1H
	2.2-2.5	Piperazinering≈ 8H
	10.1	N-H ≈ 2H (barbiturate)
	2.4	N-methyl≈ 3H

^{13}C NMR spectra

The spectrum consists of sharp signal at 46.0 ppm and 55.9 ppm attributed to N-methyl piperazine ring carbon atoms $C_{(2)}$, $C_{(6)}$ and $C_{(3)}$, $C_{(5)}$. A carbon signal of methyl attached to N of piperazine moiety is observed at 43.7 ppm, while the signals at 128.9, 151.1ppm and 172.1ppm is attributed for carbonyl and CH of barbiturate moiety. The signals at 129.8-135.8 ppm is assigned to the aromatic phenyl carbons and the sharp signal at 52.5 ppm is ascribed to the methine carbon produced from the Mannich reaction

Analysis of Mass Spectrum

Mass spectrometry is an extremely authoritative tool concerns with identification and structure determination of organic compounds [15-18]. Mass spectrum of the CBzBAPz(NM)-ligand is recorded and compared for their stoichiometric composition. It exhibits the molecular ion peak at m/z =347 and fragments ion peaks at m/z = 247($C_{11}H_8N_2O_3Cl^+$), m/z = 112 ($C_{10}H_{15}N_4O_3$), m/z = 252 ($C_{15}H_{18}N_2O_2^+$) and m/z = 187 ($C_{12}H_{17}N_2^+$). The mass spectral results not only confirm the formation of Mannich base ligands but also support the ^{1}H &^{13}C NMR spectral interpretation.

Infrared Spectra

The significant infrared spectral bands of the ligands and complexes are given in Table 2. A comparison of the characteristic infrared absorption bands of N-methylpiperazine base ligands with those of the corresponding Cu(II), Cd(II) and Pd(II) metal complexes reveals the following important features.

a). The infrared spectra of the ligands shows bands at 1661cm^{-1} and 1354cm^{-1} which may be assigned to $\nu_{C=O}$ (barbituric acid) and ν_{C-N} (piperazine) respectively. The first band is shifted towards the higher frequency region (20-40 cm^{-1}) in their metal complexes indicating the coordination of the carbonyl group of the ligand. However, the second band ν(C-N) (barbituric acid &piperazine) is found to split into two; one almost located at the original position indicating non-coordinated ν(C-N)and the other shifted to higher frequency in the region of1354-1386cm^{-1}, arising due to coordinated ν(C-N) mode.

Table 2. The main IR frequencies of the N-methyl piperazine ligands and their complexes

Compound	υ_{N-H}	υ_{C-N}	$\upsilon_{C=O}$	υ_{C-H}	$\upsilon_{C=C}$	υ_{M-N}	υ_{M-O}
BzBAPz(NM)	3452	1344	1648	3198	1413	---	---
$(BzBAPz(NM))_2Cu$	3455	1393	1707	3188	1414	451	559
$(BzBAPz(NM))_2Cd$	3453	1389	1712	3185	1417	458	554
$(BzBAPzNM))_2Pd$	3456	1383	1707	3186	1412	454	567
CBzBAPz(NM)	3454	1354	1661	3178	1420	---	---
$(CBzBAPz(NM))_2Cu$	3454	1386	1699	3179	1419	452	572
$(CBzBAPz(NM))_2Cd$	3455	1385	1701	3169	1422	463	569
$(CBzBAPz(NM))_2Pd$	3457	1382	1711	3176	1421	448	559
NBzBAPz(NM)	3452	1350	1709	3168	1418	---	---
$(NBzBAPz(NM))_2Cu$	3455	1393	1741	3165	1420	445	545
$(NBzBAPz(NM))_2Cd$	3454	1389	1742	3169	1416	452	548
$(NBzBAPz(NM))_2Pd$	3457	1388	1750	3170	1421	464	551

b). The splitting of the ν(C-N) absorption band suggests that only one of the ring nitrogen is involved in coordination and the other is free and non-coordinated. This is further confirmed by a new band at 450-470 cm^{-1} assignable to ν(M-N) in the complexes. The metal-oxygen band appears at ~500-570 cm^{-1}.

c). Absorption bands occurring at ~2980 cm^{-1} for ν(C-H), ~1415 cm^{-1} for ν(C=C), ~1680 for ν(C=O) of 2nd& 4th position and ~ 3450 cm^{-1} for ν(N-H) of 1st& 3rd position of barbituric acid in the complexes indicate the presence of the other groups in their respective position and confirm the non-coordination with metal ions.

Electronic Spectra

The electronic spectral data of the ligand and complexes in DMSO are listed in Table. 3. The ligand spectrum consists of maximum at 36,900 cm^{-1} which can be assigned to the n-π* transition of the carbonyl groups. Further, the medium intensity bands at 32,258cm^{-1} are due to π-π* transition in the ligand remains as such in the spectrum of the complexes. Tetragonal copper (II) complexes are expected to show two transitions, namely $^2B_1g \rightarrow {}^2A_1g$ and $^2B_1g \rightarrow {}^2B_2g$, but bands due to these transitions usually overlap to give a broad absorption band [19]. The broad band observed in the Cu(II) complex around 20,661 cm^{-1} indicates a square planar configuration

around Cu(II) ion. The electronic spectrum of Cu(II) complex in solution exhibits a broad band at 20,661 cm^{-1} which can be assigned to $^2B_1g \rightarrow {}^2A_1g$ transition. Therefore, the components of these transitions, observed at about 24,295 and 20,661 cm^{-1} can be denoted as N→M and O→M LMCT because, the complexation of the ligand to the metal ion occurs through oxygen and nitrogen atoms. The magnetic moment value of this complex is 1.93BM (calculated from EPR) corresponding to the presence of one unpaired electron and confirms the square planar configuration [20].

The electronic spectrum of palladium (II) in DMSO solution shows two broad bands at 24,330cm^{-1} and 20,325cm^{-1} assigned to $^1A_1g \rightarrow {}^1A_2g$ and $^1A_1g \rightarrow {}^1B_1g$ transitions. This suggests that Pd(II) complexes showed square planar geometry and diamagnetic properties. This result does not show any splitting characteristics, which indicate spin paired, diamagnetic and square planar geometry around Pd(II) ion.

Table 3. Electronic spectral data and their assignments of ligands and complexes

Ligand			**π- π* (cm^{-1})**	**n-π* (cm^{-1})**
BzBAPz(NM)	----	----	33,193	37,097
CBzBAPz(NM)	----	----	32,258	36,900
NBzBAPz(NM)	----	----	33,411	37,012
Complexes	υ_1 (cm^{-1}) $^1A_1g \rightarrow {}^1A_2g$, Eg	υ_2 (cm^{-1}) $^1A_1g \rightarrow {}^1B_1g$	π- π* (cm^{-1})	n-π* (cm^{-1})
Pd $(BzBAPz(NM))_2$	20,612	24,297	33,317	37,250
Pd $(CBzBAPz(NM))_2$	20,325	24,330	33,222	37,037
Pd$(N\ BzBAPz(NM))_2$	20,480	24,413	33,210	37,793
Complexes	υ_1 (cm^{-1}) $^2B_1g \rightarrow {}^2A_1g$	υ_2 (cm^{-1}) $^2B_1g \rightarrow {}^2B_2g$	π- π* (cm^{-1})	n-π* (cm^{-1})
Cu$(\ BzBAPz(NM))_2$	20,741	24,301	33,282	35,864
Cu$(C\ BzBAPz(NM))_2$	20,661	24,295	33,557	35,714
Cu$(N\ BzBAPz(NM))_2$	20,584	24,312	33,384	35,918

ESR Spectra

ESR spectra of the Cu(II) complex recorded using DMSO with DPPH (Biphenyl PicrylHydrazyl) as internal standard at room temperature provide information about the coordination environment around Cu(II) in complex. The ESR spectrum of the square planar Cu(II) complex shows isotropic spectrum of an axial – elongated type and Table 4. It gives two g value, g_{II}= 2.46 and $g_{\perp}$ = 2.12. The g_{av} value is calculated from the relation [21] $g_{av} = (g_{II} + 2g_{\perp}) / 3$ and equals to 2.23. The deviation of the g_{av} value from that of the free electron 2.0023 is due to the covalence property with a further support for tetragonal distorted symmetry associated with $d_{x^2-y^2}$ state rather than dz^2 state [22].

It is possible to measure the α^2, where α is the coefficient of the ground state $d_{x^2-y^2}$orbital, from the expression [23].

$$\alpha^2 = A_{II}/0.036+ (g_{II} - 2.0023) + 3/7 (g_{\perp} - 2.0023) + 0.04$$

In all the complexes $g_{II} > g_{\perp} > 2.00$ and $G = (g_{II} - 2) / (g_{\perp} - 2)$ values are < 4.4, are consistent with a $d_{x^2-y^2}$ ground state having a small exchange coupling [24-25].

Table 4. ESR-Spin Hamiltonian parameters of CBzBAPz(NM)-Cu(II) complex

Temperature (K)	g_{II}	$g_{\perp}$	H_{II}	$H_{\perp}$	A_{II}	$A_{\perp}$	g_{iso}	μ (BM)
300	2.26	2.19	2950	3220	---	---	2.19	1.90
77	2.46	2.12	2654	3080	110	100	2.23	1.93

Electrochemistry

The redox properties of complexes are investigated by cyclic voltammetry in DMSO and the potentials are reported Vs Ag /AgCl. The solution is degassed with nitrogen prior to use and kept under nitrogen atmosphere throughout the experiment. All the measurements are carried out using 1mM solution at room temperature in the potential range of +1.5 to -1.5 V with scan rate of 100mV. Examination of the experimental data shows that the reduction of copper (II) is reversible (Fig.1).ΔEp = Epa – Epc is greater than 59 (111). Ipa/Ipc is nearer to unity (0.72) indicate the chemical reversibility of the redox change. The reversibility, associated with the reduction based on the ΔEp value, probably, arises from the relaxation process involved in a stereochemical change from Copper(II) to Copper(I) [26].The Copper(I) redox processes are influenced by the coordination number, stereochemistry and the hard/soft character of the ligand donor atoms

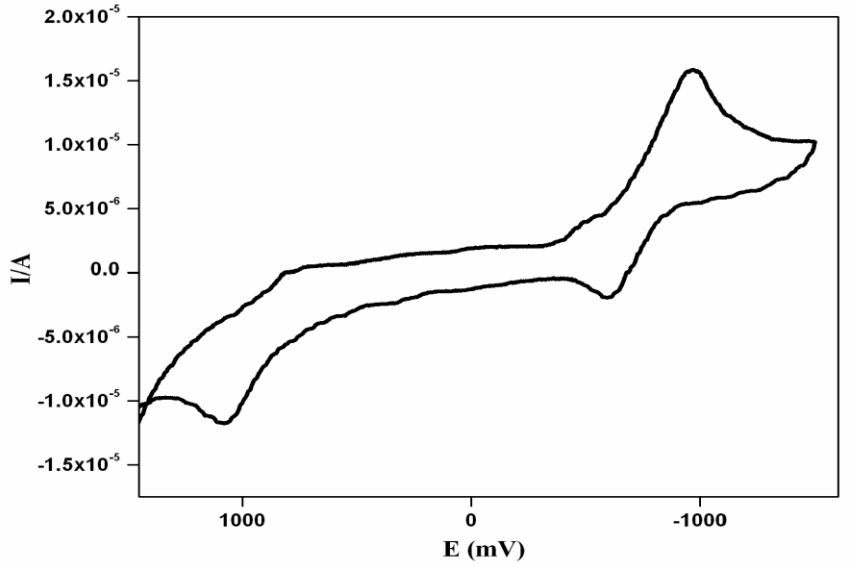


Figure. 1. Cyclic voltammogram of CBzBAPz(NM)-Cu(II) complex

Cytotoxicity of complexes in tumor cell lines

The ligand and three complexes are tested for their *in vitro* cytotoxicity with the use of the MTT assay. Breast cancer cell line MCF-7 is cultured with all test compounds at varying concentrations for 24, 48, 72, and 96 hrs(Fig.2) then cell viability is determined. In a dose dependent manner, all four compounds inhibit cell proliferation presented in Fig.3. Of these four complexes, $(CBzBAPz(NM))_2Pd$ consistently shows greater activity in breast cancer cell line.

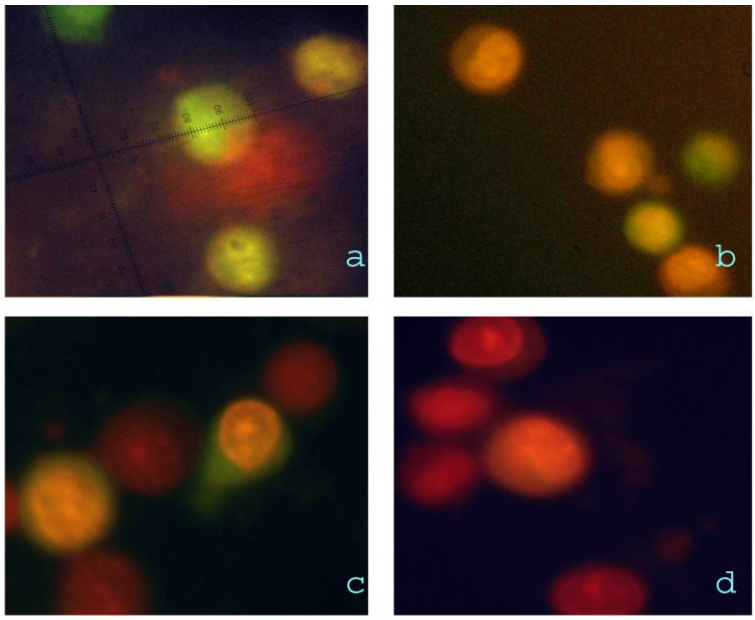


Figure.2. IC_{50} dose of (BzBAPz(NM))2Pd at 24(a), 48(b), 72(c) and 96(d) hrs respectively

Cytotoxicity of novel Cu, Cd, and Pd complexes

CBzBAPz(NM), $(CBzBAPz(NM))_2Cu$, $(CBzBAPz(NM))_2Cd$ and $(CBzBAPz(NM))_2Pd$ are tested for their cytotoxic activity against the human breast carcinoma cells (MCF-7). The ligand and three complexes exhibit remarkable growth inhibitory activities with mean IC_{50} values after different time intervals at 24, 48, 72, and 96 hrs respectively.

The inhibitory effect of CBzBAPz(NM) on cultured MCF-7 cells is appreciable because it can produce high frequency of cytotoxicity. The cytotoxicity assay of three complexes and ligand reveals that duration dependent on cell death in the cells and cell killing occurred at doses ranging from 50-70 µM/ml. Treatment CBzBAPz(NM) causes necrosis, swelling of cells, initiation of apoptotic body formation and early apoptotic cells are analysed by supravital staining (AO/EB fluorescent staining). Among the four complexes $(BBPz(NM))_2Pd$ has greater potential against MCF-7 cells by exposing cells at 50-70 µM/ml for 24, 48, 72 and 96 hrs, and the way of cell death is in duration dependent. Cells incubated with palladium complexes experienced following morphological changes i.e, increased cellular granularity, and dispersion of dilated nuclear membranes and cytoplasmic extrusions. Prominent necrosis and prominent apoptosis are also

produced. Copper produced a moderate anti proliferative effect on MCF-7 breast cancer cells with IC_{50} values for 40 µM/ml and its anti-cancerous effect is time and duration dependent. Fluorescent double staining of cells brings about cytoplasmic blabbing, extrusions, holes, early apoptosis features, fragmented DNA, nuclear marginations, apoptosis followed by apoptotic bodies formation. MCF-7 cells are highly sensitive to cadmium complexes and IC_{50} values were found to be 40-70 µM/ml in all type of exposures. The sensitivity of tumor cells exposed to palladium complexes is quite significant and the results suggest that the compound at different time exposures exhibits similar range of toxicity. Marvelous cell shrinkage, nuclear membrane blabbing, nuclear condensation, fragmentations, and pyknotic bodies of condensed chromatin are observed due to the treatment with palladium complexes.

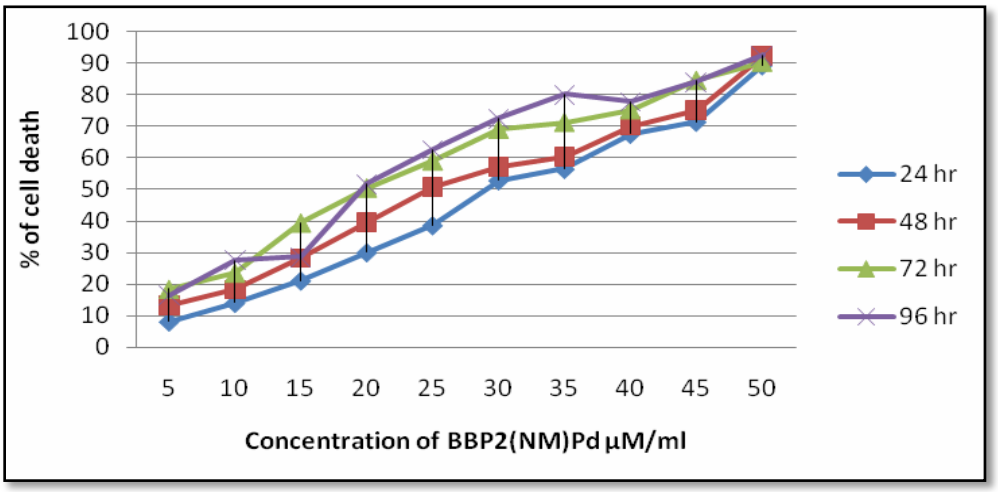


Figure. 3. Graphical representation of IC_{50} dose of $(BzBAPz(NM))_2Pd$ at 24(a), 48(b), 72(c) and 96(d) hrs respectively

CONCLUSION

A series of (BzBAPz(NM))5-[(4-methyl-piperazin-1-yl)-phenyl-methyl]-pyrimidine-2,4,6-trione derivatives and their Cu(II), Cd(II) &Pd(II) metal complexes have been synthesized through Mannich route and their structures are elucidated by spectral, analytical and X-ray diffraction techniques. All the complexes are square planar with metal ion coordinated to ligand through pyrimidine oxygen atom and N-methylpiperazine nitrogen atom. Further information about the complexes is obtained from IR, electronic spectra, ESR, magnetic moment and powder xrd and all supporting the monomeric nature of MN_2O_2 type complexes. This recent advances in medicinal inorganic chemistry demonstrate significant prospects for the utilization of metal complexes as drugs, presenting a flourishing arena for inorganic chemistry. Significant progress in Cu, Cd and Pd based anti-cancer an agent has been achieved. Application of new that combinatorial

chemistry, extensively used in organic drug discovery, will be beneficial for the development of inorganic compounds as therapeutics.

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