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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITIES OF SOME METAL CHELATES OF A NEW MANNICH BASE *N*-(DIPHENYLAMINO) METHYL] ACRYLAMIDE

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ABSTRACT

Some metal complexes of the new ligand *N*-[(Diphenylamino)methyl]acrylamide with Mn^{II}, Fe^{II}, Co^{II}, Ni^{II} and Cu^{II} were prepared and characterized by elemental analysis, molar conductance values, magnetic susceptibility measurements and various spectral studies. The spectral data revealed that the ligand acts as bidentate coordination to the metal ion through the azomethine nitrogen and carbonyl oxygen atoms. The conductance measurements indicated non-electrolytic nature of the complexes. Tetrahedral geometry was assigned to the thiocyanato complex of Ni^{II} and chloro complex of Cu^{II}. The remaining complexes were assigned distorted octahedral geometry. The ligand and the complexes were screened for their antibacterial and antifungal activities.

KEY WORDS: Mannich base, stoichiometry, polycrystalline, chelation, inhibition, deshielding

INTRODUCTION

The compounds containing the amide moiety have a strong tendency to form metal complexes [1,2] and exhibit a wide range of biological activities[3,4]. The coordination chemistry of amide group has received much attention due to its diverse coordinating behaviour and the role it plays in biological processes [5]. Metal complexes of Mannich bases have been studied extensively in the recent years due to selectivity and sensitivity of the ligands towards various metal ions[6-8]. Much work has been done so far on isolation of solid complexes of transition metals derived from formaldehyde and semicarbazones[9,10]. A new Mannich base ligand, *N*-[(Diphenylamino)methyl]acrylamide was prepared by the condensation of acrylamide, formaldehyde and diphenylamine.Its Mn^{II}, Fe^{II}, Co^{II}, Ni^{II} and Cu^{II} complexes were synthesized. The structure of the ligand synthesized in the present study, is shown in Figure 1.

Experimental

High purity reagents (Merk) and AR grade metal salts and solvents were used. Micro elemental (C, H and N) data were obtained with Carlo Erba 1108 elemental analyzer. The metal contents were estimated by usual procedures after digesting the complexes with con.HNO_3.The results were further confirmed by atomic absorption spectroscopy. Sulphate was estimated gravimetrically as $BaSO_4$ and chlorides were estimated volumetrically by Volhard's method[11]. The conductance data of the complexes were obtained in ~10^{-3} M DMF solutions at room temperature using a Systronics direct reading digital conductivity meter. IR spectra were recorded using a Spectrum-One Perkin Elmer FT-IR spectrometer by using KBr pellets. Absorbance in UV-Visible region was recorded in DMF solution using a double beam UV-Visible spectrometer of working range 1100-190 nm. The ^{1}H and ^{13}C NMR of the ligand were recorded on a Bruker instrument, by using TMS as internal reference and DMSO-d_6 as solvent at ambient temperature. The FAB mass recorded for the ligand was carried out using a Jeol GC mate mass spectrometer. The room temperature magnetic susceptibility measurements of the complexes were carried out by using a Gouy magnetic balance calibrated using mercury(II)tetrathiocyanatocobaltate(II). The biological activities of the ligand and the complexes were screened by the disc diffusion technique under prefectly sterile conditions.

Synthesis of the Ligand

N-[(Diphenylamino) methyl] acrylamide (DPAMACry) was synthesized by employing the Mannich synthetic route[12]. Acrylamide (7.2 g, 0.1 mol) in ethanol was mixed with formaldehyde (10 ml, 0.1 mol) followed by diphenylamine (8.5 g, 0.05 mol) in acetone with constant stirring in an ice bath. A paste like semisolid was observed. After 15 days a colourless solid obtained was washed with distilled water and finally with acetone. The compound was dried at 80^{o}C and recrystallised from ethanol. The yield was 83% (M.P.: 120^{o}C). It was insoluble in water but completely soluble in organic solvents.

Synthesis of the Complexes

The hot methanolic solution of each of the metal salts (Mn^{II}, Fe^{II}, Co^{II}, Ni^{II} and Cu^{II}) was added slowly with constant stirring to the hot ethanolic solution of the ligand in 2:1 mol ratio. The insoluble complexes[13] formed were filtered, washed with appropriate solvents to remove the unreacted metal and ligand. They were dried in air and then in an air oven at 80^{o}C. The divalent metal salts were used as such without dehydrating them.

Antibacterial and Antifungal Screening

Antibacterial and antifungal activities of the ligand and their metal complexes with Mn^{II}, Fe^{II}, Co^{II}, Ni^{II} and Cu^{II} sulphates were tested *in vitro* against six bacterial species, *(Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi* (Gram negative) and *Bacillus subtilis, Streptococcus pyogenes* and *Staphylococcus aureus* (Gram positive)), by disc diffusion method[14] using agar nutrient as medium and gentamycin as control. The antifungal activities of all the compounds were also measured by using potato dextrose agar medium and adopting disc diffusion technique.. *Aspergillus niger* and *Aspergillus flavus* were the fungi used. The zone of inhibition of the compounds is given in mm. The results obtained are tabulated in Tables 1 and 2.

Anticancer Screening

In-Vitro Bioactivity Test for Immunomodulatory and Cytotoxicity Assessment

Separation of human peripheral Blood Mononuclear Cells

Venous blood was drawn and defibrinated in a sterile flask containing stone beads, diluted

with equal volume of RPMI 1640 medium and carefully layered over histopaque in the ratio of 2:1. After centrifugation for 20 minutes at 25˚C, the whole mononuclear cell layer seen at the interface was carefully transferred to a tube containing RPMI 1640 medium. The cells were thoroughly mixed with the medium and washed by centrifugation. One drop of cell suspension and one drop of tryphan blue solution were mixed, fed into a haemocytometer. Live and dead cells were counted under phase contrast objective. The cell concentration was adjusted to the desired number of viable mononuclear cells/mL of RPMI 1640 medium. Care was taken to obtain a cell suspension with 95% to 98% mononuclear cells with less than 5% to 7% contamination of erythrocytes, granulocytes, platelets and dead cells [15].

MTT Assay Method

A known concentration of each of the complexes was reconstituted with a known volume of PBS at a pH-7.2 for preparing this stock concentration of the complexes. This was centrifuged and the supernatant liquid was membrane filtered and used. From the stock, various concentrations of the complexes were prepared and subjected to the *in-vitro* analysis on human PBMC. Constant cell number was maintained [16]. The assay was performed in a 96 well tissue culture plate using various negative controls like plain media, complete media, vehicle, cell and solutions of the complexes. Positive controls like a known immunomodulator, PHA and a known cytotoxic compound, LPS were also maintained. After the addition of cell media and complex solution, the cultures were incubated in an incubator with 95% air, 5% CO_2 and humidified atmosphere at 37˚C.

The assay was monitored after 72 hours based on spectrophotometer method. After reading the plates, the tetrazolium compound was added to the wells and incubated. After incubation period, the MTT was reduced by mitochondrial dehydrogenase as a result of which the colour changed. Detergent was added to the wells to solubilize the formazan crystals. The absorbance was read by making use of an ELISA reader at 570 nm. The data were analyzed by plotting cell number versus absorbance in a graph. The rate of tetrazolium reduction is directly proportional to the rate of cell proliferation.

Cancer Screening

Cell Lines

Raji and Jukart cell lines[17] were used in this study. The number of cells is massively expanded in a minimal number of passages, and the cells are cryopreserved to provide a consistent, long-term frozen stock for future use. Cells are grown and passaged in antibiotic-free growth medium to ensure the absence of microbial contaminants.

Preparation and Inoculation of cells

The cells were separated into single cell suspensions, and then counted using tryphan-blue exclusion on a Hemocytometer. After counting, dilutions were done to give the appropriate cell densities for inoculation onto the microtiter plates. The cells were inoculated in a volume of 100 µL per well at densities between 5000 and 4000 cells per well. A 100 µL aliquot of complete medium was added to cell free wells. Prior to inclusion of cell lines in the screening panel, their growth and compatibility were determined.

Sample preparation

All the samples were initially solubilized in phosphate buffer saline at 1:1 ratio and were filtered using membrane filter to avoid microbial contamination.

MTT Assay

The assay was performed in a well tissue culture plate, using various negative controls and positive controls. After the addition of cell, media and complexes, the cultures were incubated in an incubator with 95% air, 5%

CO_2 and humidified atmosphere at 37˚C for 72 hours. The assay was monitored based on spectrophotometer method. After reading the plates, the 10 µL the tetrazolium compound MTT, was added to the wells and incubated. Finally, the MTT was reduced by mitochondrial dehydrogenase as a result of which the colour changed. Detergent SDS was added to the wells to solubilize the formazan crystals. The absorbance was read by making use of an ELISA reader at 570 nm. The rate of tetrazolium reduction is directly proportional to the rate of cell proliferation[18].

RESULTS AND DISCUSSION

IR spectrum of the free ligand showing a sharp band at 3295 cm^{-1} is assigned to (N-H) stretching vibration[19]. The medium band at 3042 cm^{-1} is attributed to (C-H) stretching vibration of aromatic ring and vinyl group. The amide I band due to $\nu_{(C=O)}$ appears at 1655 cm^{-1} and amide II band due to the $\delta_{(N-H)}$ in plane and $\nu_{(C-N)}$ vibrations appears at 1534 and 1490 cm^{-1}. The strong band at 1593 cm^{-1} may be assigned to $\nu_{(C=C)}$. The presence of absorption bands in the region 898-689 cm^{-1} are due to out of plane bending vibrations of (C-H) bonds of aromatic ring. In plane bending bands appears in the region 1383-1088 cm^{-1}. The absorption in the region of 2912 cm^{-1} is due to the $\nu_{(CH2)}$ group. The medium bands at 1218 and 1088 cm^{-1} may be assigned to the new C-N-C bond formed due to the formation of Mannich base by the insertion of diphenylaminomethyl group on acrylamide. The strong absorption band observed at 755 cm^{-1} is due to monosubstituted aromatic ring.

UV-visible spectrum of the ligand in DMF registers an intense split bands centered at 302 nm and 250 nm which are presumably due to $n\rightarrow\pi^*$ transition of the carbonyl group and $\pi\rightarrow\pi^*$ transition of the carbonyl group and the benzene ring[20].

The ^1H NMR and ^{13}C NMR spectra were recorded in DMSO-d_6. According to the proton NMR signals, the chemical shift of the proton of (N-H) group occurs at δ8.66 ppm as a broad band. The (CH_2) proton of vinyl group appears as a doublet at δ6.17 ppm (H cis to CO) and δ5.63 ppm (H trans to CO)[21]. The signal at δ6.31 ppm may be assigned to the (CH) proton of vinyl group. The methylene (CH_2) proton appears as a doublet at δ5.17 and δ5.16 ppm. The multiplet in the range δ7.31-6.92 ppm centered at δ7.07 ppm is attributed to the protons of the benzene ring[21].

Based on the ^{13}C NMR spectrum obtained, the carbonyl carbon shows a signal at δ164.78 ppm. The signals observed between δ146.58-120.95 ppm are due to aromatic carbons of diphenylamine. The resonance signals at δ146.58, 129.26, 121.81 and 120.95 ppm are assigned to the carbons of the phenyl group at 1, 3&5, 2&6 and 4 positions respectively. The substituted carbon of the aromatic ring can be distinguished[22] by its decreased peak height (δ146.58 ppm). The methylene carbon which is bonded to N exhibits signal at δ56.12 ppm. The signal due to terminal carbon of vinyl group appears at δ125.91 ppm. The peak at δ131.45 ppm may be assigned to the vinyl carbon attached to CO group.

The mass spectrum[23] was obtained on electron ionization mode, it shows a very weak molecular ion peak at m/z = 252, which confirms the assigned molecular mass to this Mannich base. Thereupon, on fragmentation, it records intense signals at m/z = 197 & m/z = 182 which are due to the removal of CH_2=$CHCO^-$ and NH^- groups respectively. The next m/z signal at 168 is due to diphenylamine ion.

To find out the stoichiometry of the complexes, the percentage of the metal ions, anions and CHN (Table 3) were determined[24]. The electrical conductance measurements were done in order to ascertain

whether the anion is within or outside the coordination sphere of the complex[25]. The molar conductance values predict that all the complexes are non-electrolytes. The CHN analyses are also in good agreement with the calculated values.

In the IR spectra of all the complexes (Table 4), the stretching frequencies of C=O and C-N-C bonds are found lowered showing that both carbonyl oxygen and CNC nitrogen atoms are coordinated to the metal ions. So, that the ligand acts as ON donor[26]. The IR spectrum of the sulphato complexes shows the presence of coordinated sulphato group. The bands at the ranges of 1150, 1000 and 900 cm^{-1} are due to 'SO' stretching mode, ν_3 of sulphato group and the triply degenerate 'OSO' bending mode, ν_4 and splits up into its components at the ranges of 650, 600 and 580 cm^{-1} in the complex. The frequencies 750(ν_1) and 500(ν_2) cm^{-1} are also observed. These frequencies are due to coordinated sulphato group which are consistent with the normally associated bidentate chelation[27]. The bands around 3300-3500, 1600-1650, 800-880, 600-690 and 460-530 cm^{-1} found in the spectra of Mn^{II}, Fe^{II}, Co^{II} and Ni^{II} sulphato, Cu^{II} nitrato complexes indicate the presence of coordinated water molecule[28].

The SCN stretching frequency are generally greater than 2100 cm^{-1} in the 'S' bonded complexes and it is below or equal to 2100 cm^{-1} in the 'N' bonded complexes[29]. The ν_{CN} absorption is strong and exhibits a broad band for isothiocyanato (M-NCS) and a sharp band for thiocyanato (M-SCN) complexes. The ν_{CS} is at 720-690 cm^{-1} for M-SCN and at 860 - 780 cm^{-1} for M-NCS complexes. The NCS bending mode in an isothiocyanato complex consists of a single band of medium intensity in the 490-460 cm^{-1} region while in the thiocyanato complex consists of a medium band in the 440-410 cm^{-1} region[30].

The electronic spectrum[31] of Mn^{II} complex recorded in solution shows the three absorption bands at 12655, 21279 and 24390 cm^{-1} are attributable to the three spin allowed transitions viz., $^6A_{1g}\rightarrow{}^4T_{2g}$, $^6A_{1g}\rightarrow{}^4E_g$ and $^6A_{1g}\rightarrow{}^4E_g$. The measured magnetic moment of Mn^{II} complex is 1.76 B.M., which indicates that the complex is paramagnetic and is a low spin one with hexa coordination.

The Fe^{II} complex registers two sharp bands at 11548 and 32006 cm^{-1} may be assigned to $^5T_{2g}\rightarrow{}^5E_g$ and charge transfer transitions. The effective magnetic moment is 5.52 B.M. which is in close agreement to high spin octahedral stereochemistry[32].

The Co^{II} nitrato and sulphato complexes exhibit electronic transition bands at 9705 & 7210(ν_1), 16163 & 15344(ν_2) and 19697 & 18959(ν_3) cm^{-1} respectively, corresponding to $^4T_{1g}(F)\rightarrow{}^4T_{2g}(F)(\nu_1)$, $^4T_{1g}(F)\rightarrow{}^4A_{2g}(F)(\nu_2)$, $^4T_{1g}(F)\rightarrow{}^4T_{1g}(P)(\nu_3)$ respectively. The bands at 30212 and 32878 cm^{-1} are due to charge transfer transition of the complexes. The order of Dq values among the Co^{II} complexes was found to be $Co(NO)_3.2L > CoSO_4.L.2H2O$. The ν_2/ν_1 ratio for Co^{II} nitrato complex is 1.67 and for Co^{II} sulphato complex is 2.63. The μeff. values for the nitrato and sulphato complexes of Co^{II} are 5.37 and 5.24 B.M. respectively indicating octahedral Co^{II} complex[32] with the ground state as 4T1g.

Diffused Reflectance Spectra[33] recorded for Ni^{II} thiocyanato complex exhibits two intense absorption bands at 8183 and 15452 cm^{-1}. These bands are characteristic of Ni^{II} in a tetrahedral environment. The former band in the near IR region is assigned to the $^3T_1\rightarrow{}^3A_2$ transition and the other band is due to $^3T_1\rightarrow{}^3T_2$ transition lies in the IR region. The room temperature magnetic moment value for the complex is found to be 3.86 B.M. which is slightly higher than the spin only value. The Ni^{II} sulphato complex exhibits three bands, at 9198 cm^{-1} due to $^3A_{2g}(F)\rightarrow{}^3T_{2g}(F)$, at

15151cm^{-1} due to $^3A_{2g}(F)\rightarrow{}^3T_{1g}(F)$ and at 25947 cm^{-1} due to $^3A_{2g}(F)\rightarrow{}^3T_{1g}(P)$ transitions respectively. The band appearing at 35466 cm^{-1} in the sulphato complex of Ni^{II} are attributed to charge transfer transitions. The ν_2/ν_1 ratio for the Ni^{II} thiocyanato and sulphato complexes is 1.19 and 1.65. The order of Dq values among the Ni^{II} complexes was found to be $Ni(NCS)_2.L < NiSO_4.L.H_2O$. The percentage covalency is more for the sulphato complex. There is a reduction to about 11and 14 for the thiocyanato and sulphato complexes of Ni^{II}, when the free ion value for the inter-electronic repulsion parameter is incorporated. The μ_{eff}. values for the thiocyanato and sulphato complexes of Ni^{II} are 3.86 and 3.15 B.M. respectively indicating distorted tetrahedral environment around Ni^{II} ion in thiocyanato complex and distorted octahedral geometry[34] for the sulphato complex.

The chloro complex of Cu^{II} exhibit electronic absorption bands at 9267 cm^{-1} due to $^2B_{1g}\rightarrow{}^2A_{1g}$, at 10354 cm^{-1} due to $^2B_{1g}\rightarrow{}^2B_{2g}$, at 12585 cm^{-1} due to $^2E_g\rightarrow{}^2T_{2g}(F)$ transitions and the bands observed at 24341 and 28313 cm^{-1} may be due to charge transfer transitions. The chlorine atom bonded to Cu^{II} to form a terminal Cu-Cl bond showing Far-IR bands around 320 & 280-260 cm^{-1}. The bands at 524 and 358 cm^{-1} due to ν(M-O) & ν(M-N) vibrations respectively. The band positions and multi-component nature of the spectra suggest a tetrahedral geometry. Nitrato and sulphato complexes of Cu^{II} exhibit electronic absorption[35] bands at 9924&9142 cm^{-1} due to $^2B_{1g}\rightarrow{}^2A_{1g}$ and $^2B_{1g}\rightarrow{}^2A_{2g}$ respectively, at 11853&12687 cm^{-1} due to $^2B_{1g}\rightarrow{}^2B_{2g}$ and at 15061&15015 cm^{-1} due to $^2E_g\rightarrow{}^2T_{2g}(F)$ and the bands at 32070 and 33062 cm^{-1} are assigned due to charge transfer transitions respectively. The band positions and multi-component nature of the spectra suggest a tetragonally distorted octahedral geometry to Cu^{II} nitrato and sulphato complexes.

The EPR spectra of polycrystalline chloro, nitrato and sulphato complexes of Cu^{II} are recorded at LNT (77 K)(Table 5). The g values of the chloro, nitrato and sulphato complexes of Cu^{II} are in the trend, $g_{\parallel} > g_{\perp} > g_{DPPh}$ suggesting that the unpaired electron lies predominantly in the d_{x2-y2} orbital and they showed EPR spectra of axial symmetry type indicating planar based distorted octahedral geometry around copper centre[36]. The $g_{\parallel}$ values of chloro, nitrato and sulphato complexes are less than 2.30 indicating the covalent nature. The axial symmetry parameter G which is a measure of interaction between the metal centers in the crystalline solids is less than 4 for complexes of Cu^{II} and it show a considerable coupling and appreciable misalignment of the local tetragonal axes leading to an exchange interaction of free electron between two copper centers in the solid state[36]. The G value for the chloro, nitrato and sulphato complexes of Cu^{II} is 37.36, 8.91 and 15.29 and this suggests the lack of change interaction between two Cu^{II} centers in the unit cell of the complex. The μ_{eff}. value of chloro complex is 2.40 B.M. suggests the distorted tetrahedral geometry. The μ_{eff}. value of nitrato (0.41 B.M.) and sulphato (1.01 B.M.) complexes indicate that they have distorted octahedral geometry.

On the basis of the above analytical and spectral data the structures (Figure 2 - 8) have been suggested for the complexes.

A comparison of diameters of the inhibition zones of the compounds investigated and listed in Table (1) and (2), shows that Co^{II} sulphato complex exhibits highest antibacterial and antifungal activity against all the bacterial and fungal species studied. They have larger diameters of inhibition zones than the control: gentamycin at the same concentration and identical conditions. This observation clearly indicates that chelation

increases the activity. The higher activity of the Co^{II} complex may be due to the fact that, Co^{II} is an essential micronutrient during transcription and transformation of nuclei acids. Co^{II} complex was shown to inhibit cellular protein and RNA synthesis. In Co^{II} complex the labile water can exchange its coordination sites with the enzymatic –SH group and hence inhibits the bacterial growth [37].

The fungi toxicity of the free ligand is less severe than that of the metal chelates. A possible mechanism of toxicity may be speculated in the light of chelation theory. Chelation reduces considerably the polarity of the metal ion mainly because of partial sharing of its positive charge with donor groups and possible π-delocalization of electron over the chelate ring. This increases the liphophilic character of the neutral chelate which favours its permeation through lipoid layers of fungus membranes. Furthermore, the mechanism of action of the compounds may involve the formation of hydrogen bond through the uncoordinated hetero atoms O, S and N with the active centers of the cell constituents resulting in the interference with the normal cell process[38]. Presence of lipophilic and polar substituents like C=O, NH, etc., are expected to enhance the fungi toxicity.

The results of cytotoxic studies of DPAMAcry and its metal complexes of Mn^{II}, Fe^{II} ,Co^{II} , Ni^{II} and Cu^{II} sulphates against peripheral blood mononuclear cells reveal that the cytotoxicityincreases as the concentration of test compound increases[42]. A very few metal complexes are found to be more active than the ligand. The ligand acts as very good immunopotentiator. When complexed with metal salts, the immunopotentiating property increases[39] (Table 6).

When the compounds were analyzed for anticancer activity, the results (Table 7) revealed that $CoSO_4$ and $NiSO_4$ down regulated[40] Raji and Jukart cell line at 50 ng. MnSO4 and DPAMAcry were found to be non toxic against both the cell lines tested. CuSO4 down regulated Raji cell line at 50ng, as against Jukart at 25ng down regulated. In the present study, it appears that there is a relationship between the lability of M-O and M-N linkages and the activity as discerned from the thermodynamic and kinetic stability. Based on the above preliminary studies, the following possible explanations are drawn

1. Compound may affect the specificity of DNA by altering the hydrogen bonding interactions[41] by accidental incorporation of antimetabolite in DNA in the place of the essential metabolite(thymine) and slow down the rapid multiplication.
2. The metal ion may act as an intermediate to keep the protein bound to RNA and retain the configuration or may inhibit the protein and nucleic acid synthesis[41].

So, metal chelation augments the activity of the drug.

3. The lability of the M-O and M-N bonds and thermodynamic stability appear to influence the activity of metal complexes. The activity is proportional to the lability of the metal-donor bond.

In general, the intake of a drug depends on the balance between hydrophilic and lipophilic properties and the solubility which are substituent dependent. Metal coordination increases the lipophilicity of a drug and this may be the reason for the enhanced activity upon complexation. Hydrogen bonding and the antimetabolite action of the compound may be an important factor in anticancer mechanism [42].

CONCLUSION

The ligand, *N*-[(Diphenylamino) methyl] acrylamide and its complexes of Mn^{II}, Fe^{II}, Co^{II}, Ni^{II} and Cu^{II} were synthesized and characterized. The spectral data revealed that

the ligand acted as bidentate to the metal ion through the azomethine nitrogen and carbonyl oxygen atoms. Tetrahedral geometry was assigned to the thiocyanato complex of Ni^{II} and chloro complex of Cu^{II}. The remaining seven complexes were assigned distorted octahedral geometry. The ligand and the complexes were screened for their antibacterial, antifungal and anticancer activities. All the antimicrobial studies showed that theCo^{II} complex was more active than the rest.

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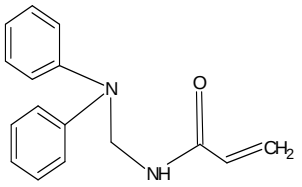

Figure 1

N-[(Diphenylamino)methyl]acrylamide(L)

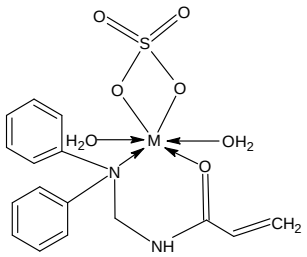

Figure 2

$M.SO_4.L.2H_2O$

(M= Mn, Fe, Co, Ni)

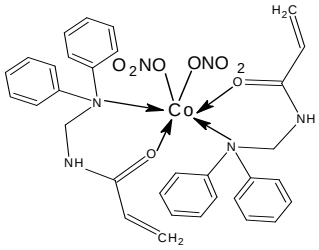

Figure 3

$Co(NO_3)_2.2L$

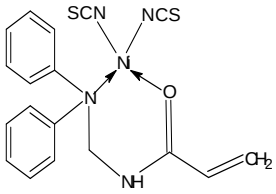

Figure 4

$Ni(NCS)_2.L$

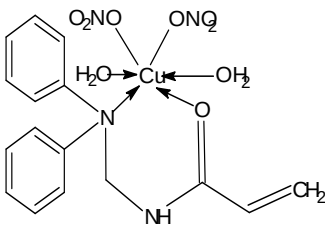

Figure 5

$Cu(NO_3)_2.L.\ 2H_2O$

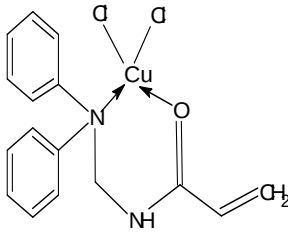

Figure 6

$CuCl_2.L$

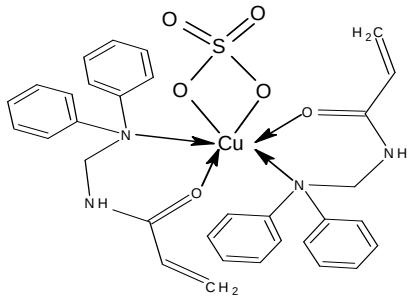

Figure 7

CuSO4.2L

Table 1 Antibacterial Activity of Ligand and its Complexes

Compound	*Escherichia coli*			*Pseudomonas aeruginosa*			*Salmonella typhi*			*Bacillus subtilis*			*Streptococcus pyogenes*			*Staphylococcus aureus*		
Conc.(µg/disc)	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30
Control	12	15	20	10	13	18	14	17	22	11	14	18	10	12	16	12	17	20
L	-	-	08	04	08	09	04	06	06	-	06	09	04	04	06	-	07	10
$MnSO_4.L.2H_2O$	16	20	22	14	18	20	10	18	22	16	20	20	18	18	24	14	20	26
$FeSO_4.L.2H_2O$	16	18	20	14	16	17	11	14	19	12	16	21	11	13	17	16	18	24
$CoSO_4.L.2H_2O$	18	21	24	17	22	27	20	28	30	23	26	32	18	24	29	25	28	35
$NiSO_4.L.\ 2H_2O$	09	12	17	06	09	15	10	12	16	08	14	18	14	19	28	10	10	18
$CuSO_4.2L$	18	20	21	17	19	24	14	15	21	12	18	24	16	21	26	12	19	25

Table 2 Antifungal Activity of Ligand and its Complexes

Compound	***A. niger***			***A. flavus***		
Conc. (µg/disc)	10	20	30	10	20	30
L	04	04	05	04	05	05
$MnSO_4.L.2H_2O$	-	-	05	-	11	13
$FeSO_4.L.2H_2O$	8	9	12	5	10	12
$CoSO_4.L.2H_2O$	15	19	26	16	19	28
$NiSO_4.L.2H_2O$	11	18	20	10	14	19
$CuSO_4.2L$	14	18	21	13	15	23

Table 3 Analytical and Conductance Data for Mn^{II}, Fe^{II}, Co^{II}, Ni^{II} and Cu^{II} Complexes of Ligand

Complex	**% C Obs. (Cal.)**	**% H Obs. (Cal.)**	**% N Obs. (Cal.)**	**%Metal Obs. (Cal.)**	**%Anion Obs. (Cal.)**	**Λ_M $ohm^{-1}cm^2$ mol^{-1}**	**Colour (μ_{eff}. B.M)**
$C_{16}H_{16}N_2O.$ $MnSO_4.(H_2O)_2$	37.64 (37.57)	3.00 (3.13)	5.71 (5.48)	11.0 (10.75)	18.14 (18.80)	25.69	Yellow (1.76)
$C_{16}H_{16}N_2O.$ $FeSO_4.(H_2O)_2$	34.23 (33.92)	3.02 (2.83)	5.28 (4.95)	10.54 (9.84)	17.12 (16.97)	31.04	Dark blue (5.52)
$(C_{16}H_{16}N_2O)_2.$ $Co(NO_3)_2$	48.83 (48.30)	3.56 (4.03)	6.84 (7.04)	8.18 (7.41)	15.42 (15.60)	33.19	Brown (5.37)

$C_{16}H_{16}N_2O.$ $CoSO_4.(H_2O)_2$	33.99 (33.74)	2.67 (2.81)	5.06 (4.92)	10.22 (10.36)	17.00 (16.88)	06.47	Pink (5.24)
$C_{16}H_{16}N_2O.$ $Ni(NCS)_2$	45.12 (44.99)	3.60 (3.75)	6.43 (6.56)	13.58 (13.76)	54.49 (54.37)	12.52	Dark green (3.86)
$C_{16}H_{16}N_2O.$ $NiSO_4.(H_2O)_2$	34.54 (34.85)	3.11 (2.90)	4.91 (5.08)	9.45 (9.69)	17.61 (17.43)	27.81	Green (3.15)
$C_{16}H_{16}N_2O.$ $CuCl_2$	45.88 (45.50)	4.05 (3.79)	6.47 (6.64)	14.92 (15.06)	16.98 (16.80)	42.32	Sky blue (2.40)
$C_{16}H_{16}N_2O.$ $Cu(NO_3)_2.(H_2O)_2$	36.41 (36.23)	3.55 (3.02)	5.11 (5.28)	12.12 (11.99)	23.32 (23.40)	17.90	Brown (0.41)
$(C_{16}H_{16}N_2O)_2.$ $CuSO_4$	50.75 (51.00)	3.98 (4.25)	7.21 (7.44)	9.03 (8.44)	13.19 (12.76)	08.55	Blue (1.01)

Table 4 Important IR Absorption Bands (cm^{-1}) of Ligand and of Mn^{II}, Fe^{II}, Co^{II}, Ni^{II} and Cu^{II} Complexes

Compound	ν_{NH}	$\nu_{C=O}$	ν_{CNC}	ν_3	ν_4	ν_1	ν_2	ν_5	ν_6
L	3295	1655	1218, 1088	-	-	-	-	-	-
$MnSO_4.L.2H_2O$	3296	1622	1170	1123, 1069, 1020	696, 652, 605	808	518	-	-
$FeSO_4.L.2H_2O$	3387	1598	1170	1104, 1018, 919	693, 666, 626	827	528	-	-
$Co(NO_3)_2.2L$	3392	1606	1174	-	-	1309	1017	1384	806
$CoSO_4.L.2H_2O$	3349	1630	1144	1144, 1099, 984	695, 632, 591	847	462	-	-
$Ni(NCS)_2.L$	3390	1598	1176	-	-	1311	1028	1384	805
$NiSO_4.L.2H_2O$	3351	1631	1143	1108, 990, 912	760, 631, 616	897	477	-	-
$CuCl_2.L$	3340	1574	1164	-	-	-	-	-	-
$Cu(NO_3)_2.L.\ 2H_2O$	3392	1596	1172	-	-	1319	1047	1384	814
$CuSO_4.2L$	3350	1620	1199	1156, 1095, 997	659, 604, 586	796	518	-	-

Table 5 EPR Spectral Parameters of Cu^{II} Complexes

Complex	$g_{\parallel}$	$g_{\perp}$	g_{av}	G
$CuCl_2.L$	2.29	2.01	2.17	37.36
$Cu(NO_3)_2.L.2H_2O$	2.16	2.02	2.09	08.91
$CuSO_4.2L$	2.12	2.01	2.06	15.29

Table 6 In-Vitro Bioactivity Test for Immunomodulatory and Cytotoxicity Assessment of ligand and its complexes(MTT Method)

S.No	Sample	Dye Exclusion	MTT
1	L	Non-toxic	Immunopotentiator at 100 ng
2	MnSO4.L.2H2O	Non-toxic	Immunopotentiator

3	FeSO4.L.2H2O	Non-toxic	Immunopotentiator
4	CoSO4.L.2H2O	Non-toxic	Non-toxic
5	NiSO4.L.2H2O	Non-toxic	Cytotoxic
6	CuSO4.2L	Non-toxic	Non-toxic up to 200ng Cytotoxic at 400 ng

(L- DPAMAcry)

Table 7 Consolidated results of the compounds against cancer cell lines

S.No	Sample	Cell Lines	
		Raji	Jukart
1	L	Non toxic	Non toxic
2	MnSO4.L.2H2O	Non toxic	Non toxic
3	FeSO4.L.2H2O	Up regulation at 50 ng	Up regulation at 50 ng
4	CoSO4.L.2H2O	Down regulation at 50 ng	Down regulation at 50 ng
5	NiSO4.L.2H2O	Down regulation at 50 ng	Down regulation at 50 ng
6	CuSO4.2L	Down regulation at 25 ng	Down regulation at 50 ng