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Investigation of divalent tetraazamacrocyclic complexes as potent antimicrobial and antioxidant agents

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ABSTRACT

A novel series of symmetric compartmental 16-membered tetraaza macrocyclic complexes were synthesized using template method with the help of ligands 1,8-diaminonaphthalene, 2,4-dioxopentane and metal ions in 2:2:1 ratio. The synthesized complexes afforded hexacoordinated geometry of the general formula [M(TML)X_2] where TML= Tetraazamacrocyclic ligand; M = Co(II), Ni(II) and Cu(II); X = Cl^-, CH_3COO^- or NO_3^-or M= Zn (II)and Cd(II); X = CH_3COO^-. The resulting complexes have been characterized with the help of elemental analyses, conductance measurements, magnetic measurements, TGA, electronic, infrared, far infrared, NMR, ESR and Mass spectral studies. The optimized energy calculations were also carried out. Powder X-ray diffraction points towards the presence of triclinic crystal system. The metal complexes have also been identified for antioxidant activities as well as screened for in vitro antimicrobial activities.

Keywords: Tetraazamacrocyclic ligand, biological screening, spectral characterization, metal complexes, powder XRD, TGA.

INTRODUCTION

Schiff base macrocycles have fetched a massive significance in macrocyclic and supramolecular chemistry. Research on diverse aspects of new macrocyclic compounds has evoked considerable worldwide interest in recent years [1, 2]. The relationship of electronic properties and reactivity of these synthetic macrocyclic complexes to those of naturally occurring macrocycles, such as porphyrin [3, 4] and corrins continue to promote great interest in their design and preparation. Because of the numerous areas of chemistry where aza-macrocyclic complexes have found a niche, the preparation of new macrocyclic complexes with ever more elaborate structures is also a vital area of research [5, 6]. Transition metal macrocyclic complexes have received much attention as an active part of metalloenzymes [7] and as biomimic model compounds [8]. It is due to their resemblance with natural proteins like hemerythrin and enzymes. The chemistry of macrocyclic compounds in particular is also important due to their catalytic [9] and biological applications [10]. In the light of above facts, the present study deals with the synthesis, spectroscopic and antioxidant as well as antimicrobial evaluation of Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) complexes synthesized from 1,8-diaminonaphthalene and 2,4-dioxopentane.

MATERIALS AND METHODS

Materials

The reagents and solvents employed were used as received without further purification. The precursors 1,8-diaminonaphthalene and 2,4-dioxopentane were procured from Acros, New Jersey, USA. The metal salts were purchased from S. D. fine Mumbai, India; Merck, Ranbaxy, India. These chemicals were used as received.

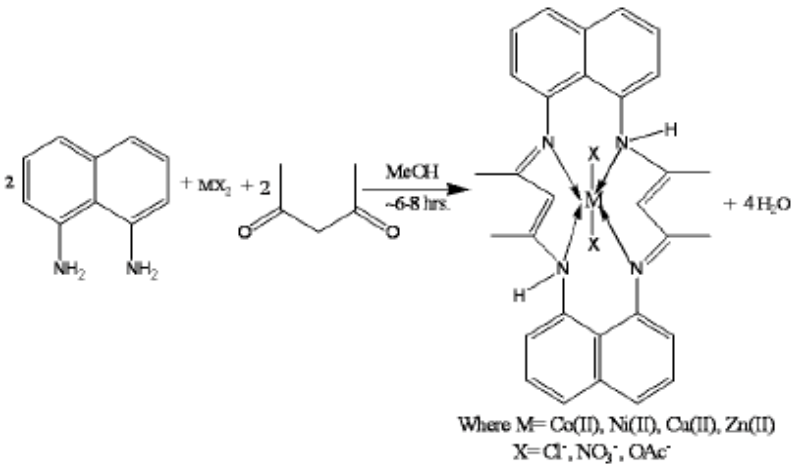
Physical measurements

The microanalysis of C, H and N was determined with a thermo quest elemental analyser at STIC, Cochin. Melting points were determined using capillaries in electrical melting point apparatus. Molar Conductance of the complexes was measured on a digital conductivity meter (HPG System, G-3001) in DMSO. The metal content in the complexes were determined by literature methods [11]. The magnetic susceptibility measurements were made at SAIF, IIT Rorkee, on a vibrating sample magnetometer (model PAR 155). The IR and Far IR spectra were recorded on FTIR spectrophotometer (Perkin Elmer RX-1), in the range 4000-200 cm^{-1} using KBr pellets at SAIF, Punjab University, Chandigarh. All the powder X-ray diffraction (PXRD) analysis was carried out on Bruker D8 X-ray diffractometer. Electronic spectra (DMSO) were recorded on a cary 14 spectrophotometer. ESR was obtained at IIT, Bombay. NMR and Mass spectra were recorded at SAIF, Punjab University, Chandigarh.

Synthesis of complex

All the complexes were synthesized by template method i.e. by condensation of 1, 8-diaminonaphthalene and 2,4-dioxopentane in the presence of the respective divalent metal salt. To a hot stirring methanolic solution (~50 cm^3) of 1, 8-diaminonaphthalene (10 mmol) was added divalent cobalt, nickel, copper, zinc or cadmium salt (5.0 mmol) dissolved in the minimum quantity of methanol (~20 cm^3). The resulting solution was refluxed for 0.5h. Subsequently, 2, 4-dioxopentane (10 mmol), in methanol, was added to the refluxing mixture and refluxing was continued for 6-8 hrs. The mixture was cooled to room temperature whereby dark colored precipitates formed which were filtered, washed with methanol, acetone and diethyl ether and dried in vacuum. The obtained yields were ~ 45-60%.

The synthesis of complexes may be shown by the following scheme:



Scheme: 1. Synthesis of Complexes derived from 1, 8-diaminonaphthalene and 2, 4-dioxopentane with divalent metal salts

Complex 1: [Co ($C_{30}H_{28}N_4$) Cl_2]: Yield-60%, Shiny Black, Calc. M=10.26, C=62.71, H=4.91, N=9.75; Found M=10.24, C=62.66, H=4.39, N=9.59; ^M= 04, $\mu_{eff.}$ = 4.80B.M.

Complex 2: [Co ($C_{30}H_{28}N_4$) $(NO_3)_2$]: Yield-52%, Dark Brown, Calc. M=9.39, C=57.42, H=4.50, N=13.39; Found M=9.28, C=57.32, H=4.37, N=13.21; ^M= 10, $\mu_{eff.}$ = 4.75B.M.

Complex 3: [Co ($C_{30}H_{28}N_4$) $(OAc)_2$]: Yield-47%, Black, Calc. M=9.48, C=65.70, H=5.51, N=9.01; Found M=9.46, C=65.60, H=5.47, N=9.02; ^M= 12, $\mu_{eff.}$ = 4.60B.M.

Complex 4: [Ni ($C_{30}H_{28}N_4$) Cl_2]: Yield-54%, Black, Calc. M=10.22, C=62.75, H=4.91, N=9.76; Found M=10.15, C=62.10, H=4.48, N=9.54; ^M= 11, $\mu_{eff.}$ = 3.15B.M.

Complex 5: [Ni ($C_{30}H_{28}N_4$) $(NO_3)_2$]: Yield-62%, Brown, Calc. M=9.36, C=57.44, H=4.50, N=13.38; Found M=9.23, C=57.08, H=4.38, N=13.30; ^M= 14, $\mu_{eff.}$ = 3.09B.M.

Complex 6: [Ni ($C_{30}H_{28}N_4$) $(OAc)_2$]: Yield-65%, Black, Calc. M=9.45, C=65.72, H=5.52, N=9.02; Found M=9.42, C=65.45, H=5.42, N=9.00; ^M=19, $\mu_{eff.}$ = 2.99B.M.

Complex 7: [Cu ($C_{30}H_{28}N_4$) Cl_2]: Yield-57%, Dark brown, Calc. M=10.97, C=62.22, H=4.87, N=9.67; Found M=10.95, C=62.12, H=4.66, N=9.62; ^M= 08, $\mu_{eff.}$ = 1.75B.M.

Complex 8: [Cu ($C_{30}H_{28}N_4$) $(NO_3)_2$]: Yield-48%, brownish black, Calc. M=10.05, C=57.00, H=4.46, N=13.29; Found M=10.02, C=56.89, H=4.38, N=13.28; ^M= 15, $\mu_{eff.}$ = 1.78B.M.

Complex 9: [Cu ($C_{30}H_{28}N_4$) $(OAc)_2$]: Yield-55%, Dark brown, Calc. M=10.15, C=65.21, H=5.47, N=8.95; Found M=10.11, C=65.13, H=5.39, N=8.85; ^M= 07, $\mu_{eff.}$ = 1.70B.M.

Complex 10: [Zn ($C_{30}H_{28}N_4$) $(OAc)_2$]: Yield-47%, Black, Calc. M=10.41, C=65.02, H=5.46, N=8.92; Found M=10.40, C=65.00, H=5.43, N=8.89; ^M= 18, $\mu_{eff.}$ = 0B.M (Diamagnetic)

Complex 11: [Cd ($C_{30}H_{28}N_4$) $(OAc)_2$]: Yield-47%, Black, Calc. M=16.65, C=60.50, H=5.08, N=8.30; Found M=16.60, C=60.45, H=5.03, N=8.19; ^M= 18, $\mu_{eff.}$ = 0B.M (Diamagnetic)

BIOLOGICAL ASSAY

Antimicrobial assay

Test microorganisms

Total five microbial strains were selected on the basis of their clinical importance in causing diseases in humans. one Gram-positive bacteria, *Bacillus subtilis* (MTCC 121); two Gram-negative bacteria, *Escherichia coli* (MTCC 1652) and *Pseudomonas aeruginosa* (MTCC 741) and two yeast, *Candida albicans* (MTCC 3017), and *Saccharomyces cerevisiae* (MTCC 170) were screened for evaluation of antibacterial and antifungal activity of the chemical complexes. All the microbial cultures were procured from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh. The bacteria were sub cultured on nutrient agar whereas yeast on malt yeast agar.

Primary Screening

The antimicrobial activities of all the 11 complexes were evaluated by the agar well diffusion method [12]. All the microbial cultures were adjusted to 0.5 McFarland standard, which is visually comparable to a microbial suspension of approximately 1.5 $\times 10^8$cfu/ml. 20ml of agar medium was poured into each petri plate and plates were swabbed with 100 µl inocula of the test microorganisms and kept for 15 min for adsorption. Using sterile cork borer of 8mm diameter, wells were bored into the seeded agar plates and these were loaded with a 100 µl volume with concentration of 2.0 mg/ml of each compound reconstituted in the DMSO. All the plates were incubated at 37^0C for 24 hrs. Antimicrobial activity of each compound was evaluated by measuring the zone of growth inhibition against the test organisms with zone reader (HiAntibiotic zone scale). DMSO was used as a negative control whereas Ciprofloxacin was used as positive control for bacteria and amphotericin-B for yeast. This procedure was performed in three replicate plates for each organism.

Determination of Minimum Inhibitory Concentration (MIC)

MIC is the lowest concentration of an antimicrobial compound that will inhibit the visible growth of a microorganism after overnight incubation**.** MIC of the synthesized complexes against bacterial and yeast strains was tested through a modified agar well diffusion method [13]. In this method, a twofold serial dilution of each chemically synthesized compound was prepared by first reconstituting the compound in DMSO followed by dilution in sterile distilled water to achieve a decreasing concentration range of 512 to 1.0 µg/ml. A 100 µl volume of each dilution was introduced into wells (in triplicate) in the agar plates already seeded with 100 µl of standardized inoculum (10^6 cfu/ml) of the test microbial strain. All test plates were incubated aerobically at 37^oC for 24 hrs and observed for the inhibition zones. MIC, taken as the lowest concentration of the chemical compound that completely

inhibited the growth of the microbe, showed by a clear zone of inhibition, was recorded for each test organism. Ciprofloxacin and amphotericin B was used as positive control while DMSO as negative control.

Antioxidant assay

The free radical scavenging potential of macrocyclic metal complexes were assayed using DPPH (2, 2-dipheny-1-picryhydrazyl) radical by literature reported method [14]. DPPH is a stable free radical containing an odd electron and it is utilized for the detection of radical scavenging potential. In the antioxidant activity assay the ability to scavenge the stable free radical DPPH is determined by decrease in absorbance at 517nm. The complexes were dissolved in DMSO. Briefly 1 ml of each complex solution, prepared in DMSO, was added to the 3 ml of methanolic solution of DPPH (0.1 mM). After 30 min., the absorbance of the complexes was taken at 517 nm. Ascorbic acid was used as positive control and methanol was used as negative control whereas DPPH solution was used as Blank. The % of scavenging activity of DPPH free radical was measured by using the following formula [15]:

$$\% \text{ Radical scavenging Activity} = \left[(A_o - A_c)/A_o\right] \times 100$$

Where A_o is the absorbance of the control and A_c is the absorbance of the sample at concentration c.

RESULTS AND DISCUSSION

Chemistry

The analytical data of the metal complexes shows that the formula of macrocyclic complexes may be represented as: [M ($C_{30}H_{28}N_4$) X_2]; where M= Co (II), Ni (II), Cu (II), Zn (II) and Cd (II) and X= Cl^{-1}, NO_3^{-1} and CH_3COO^{-1}. The test for anions was positive only after decomposing the complexes, indicating their presence inside the coordination sphere. The complexes were soluble in DMSO & DMF. The complexes were stable up to 320°C and after that the colour of the compound changed to black. The monomeric nature of these complexes was confirmed by the molecular mass found from the mass spectral data. Conductivity measured in DMSO indicated them to be non-electrolyte (10-20 $ohm^{-1}cm^2mol^{-1}$) [16]. Several attempts to obtain a single crystal, suitable for x-ray crystallography were unsuccessful that why we have gone for powder XRD. However the analytical, spectroscopic and magnetic data indicated octahedral geometry of the complexes.

IR Spectra

The preliminary identification regarding formation of the complexes was obtained from the IR spectral findings. The presence of single medium intensity band in the range 3206-3309 cm^{-1} may be assigned to (N-H) stretch [17] of 2,4-dioxopentane indicating the presence of imine-enol tautomeric form. The IR spectra of all the complexes do not show bands corresponding to free amino or carbonyl group rather a strong intensity band appeared in the region 1590-1630 cm-1 confirms the condensation of the carbonyl group of acetyl acetone and the amino group of diaminonaphthalene and the formation of macrocyclic Schiff's base [18] as these bands may be assigned to *ν*(C=N) stretching vibrations [19, 20]. The lower value of *ν*(C=N) may be explained on the basis of drift of the lone pair electron density of the azomethine nitrogen towards the metal atom [21, 22] indicating that coordination occurred through the nitrogen of the C=N groups. The medium intensity bands present in the region 2830- 2950 cm-1 may be assigned to *ν*(C-H) stretching vibrations of the methyl groups of the 2,4-dioxopentane moiety.

The far infrared spectra show bands in the region 440-470 cm^{-1} corresponding to *ν* (M-N) vibrations [23-25]. The presence of bands in all complexes in the region 440-470 cm^{-1}, originating from (M-N) azomethine vibrational modes, identifies coordination of the azomethine nitrogen [26]. The bands present in the range 300-320 cm^{-1} may be assigned to *ν*(M-Cl) vibrations [23-25]. The bands present in the region 220-250 cm^{-1} in all nitrato complexes are related to *ν* (M-O) stretching vibrations [23, 24].

^{1}H NMR Spectra

The ^{1}H NMR Spectra of the Zinc (II) complex shows multiplet at 6.55-7.10 ppm, corresponding to the aromatic ring protons of the naphthalene moiety [27]. The singlet at 0.9 ppm may be assigned to the protons of the one of the two methyl group of 2,4-dioxopentane moiety and the other methyl group appears at 1.96 ppm. The methine hydrogen (-CH-) of 2,4-dioxopentane gives singlet at 2.14 ppm. The 1H NMR Spectra also shows a broad singlet at 8.15 ppm corresponding to the protons of –NH group [28]. No peak corresponding to –NH2 (of naphthalene moiety) was

observed indicating the absence of –NH2 group in the complex and hence formation of macrocyclic frame by condensation of carbonyl group of 2,4-dioxopentane and –NH2 group of diaminonaphthalene.

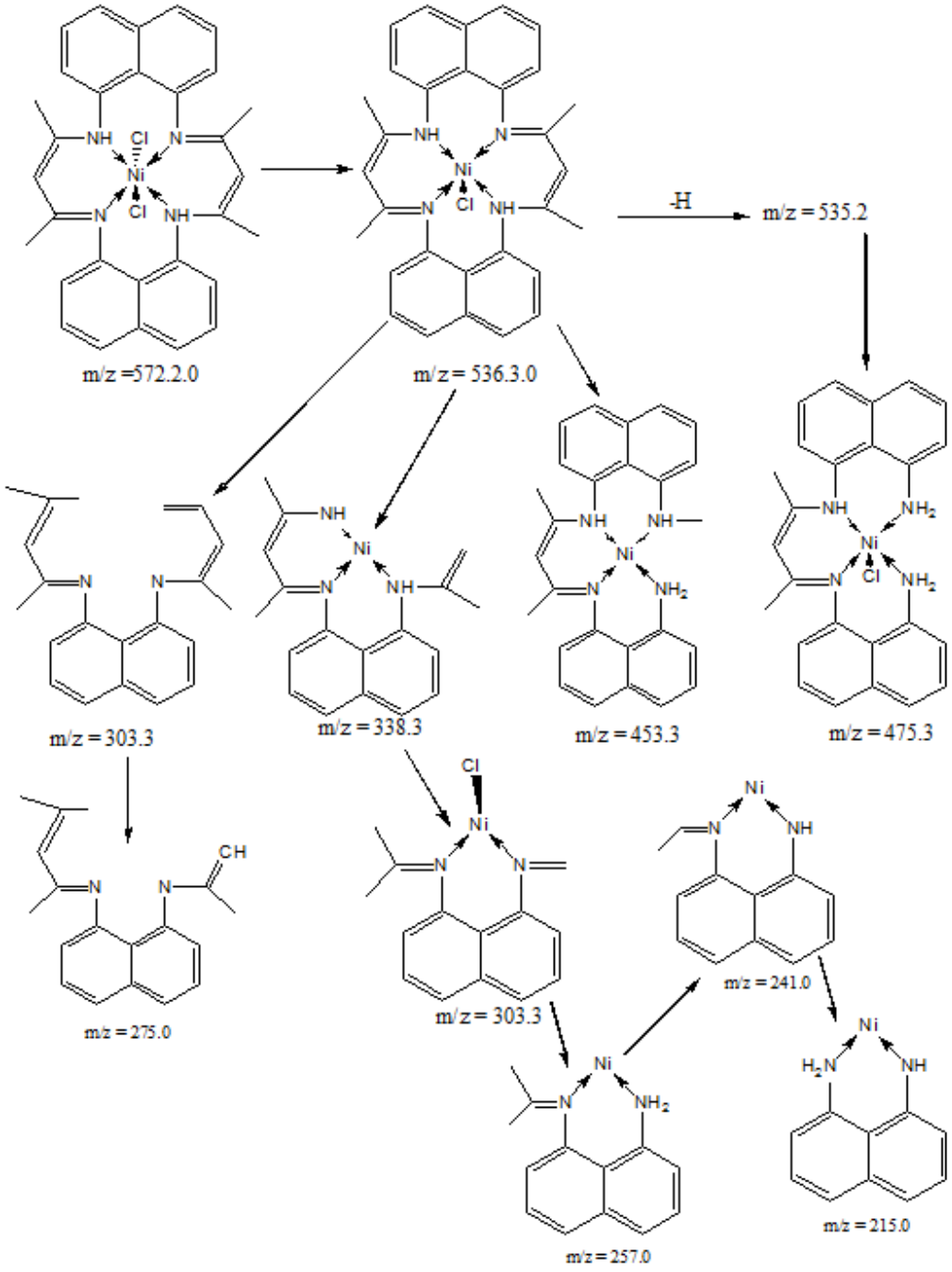


Figure: 1. Mass fragmentation of [Ni ($C_{30}H_{28}N_4$) Cl_2]

Mass Spectra

The EIMS mass spectra of Co (II), Ni (II), Cu (II), Zn (II) and Cd(II) macrocyclic complexes exhibit parent peaks due to molecular ions $[M]^+$ and $[M+2]^+$. The proposed molecular formulas of these complexes are confirmed by comparing their molecular formula weights with m/z values. The molecular ion peak $[M]^+$ peaks obtained for various complexes are as: at 573.1 for $[Co(C_{30}H_{28}N_4)Cl_2]$, [Mol.wt.-574.4]; 572.2 for $[Ni(C_{30}H_{28}N_4)Cl_2]$, [Mol.wt.-574.1]; at 577.0 for $[Cu(C_{30}H_{28}N_4)Cl_2]$, [Mol.wt.-579.0]; and at 626.1 for $[Zn(C_{30}H_{28}N_4)(OAc)_2]$, [Mol.wt.-628.0].

Similarly the molecular ion peaks are obtained for other complexes also. The mass fragmentation pattern of $[Ni(C_{30}H_{28}N_4)Cl_2]$ complex is shown in figure 1. The data were in good agreement with the proposed molecular formula of these complexes that is [M $(C_{30}H_{28}N_4)$ X_2]. This indicates the formation of the macrocyclic frame. In addition to the molecular ion peaks, the spectra also exhibit other peaks assignable to various fragments arising from the thermal cleavage of the complexes.

Magnetic Measurements & Electronic Spectra

Cobalt Complexes

The magnetic moment of cobalt complexes at room temperature were 4.60-4.75 B.M., corresponding to three unpaired electrons [29]. The electronic spectra of cobalt complexes showed bands at ~ 9065-11,500 (ν_1), 13,450-15,650(ν_2) and 19,395-21,550 cm-1(ν_3), similar to those reported for distorted octahedral complexes [29]. Thus, the bands may be assigned to $^4T_{1g} \rightarrow {}^4T_{2g}$ (F), (ν_1); $^4T_{1g} \rightarrow {}^4A_{2g}$ (F), (ν_2) and $^4T_{1g}$ (F) $\rightarrow {}^4T_{1g}$ (P), (ν_3); respectively. The assignment of the first spin allowed band seems plausible since the first band appears at approximately half the energy of the visible band [30].

Nickel Complexes

The magnetic moment of nickel complexes at room temperature were observed in the range of 2.70-3.30 B.M. These values were in tune with high spin configuration and show the presence of an octahedral environment around the Ni (II) ion in all the complexes [31]. The spectra of Ni (II) complexes recorded in DMSO solution exhibit a well discerned band with a shoulder on the low energy side. The other two bands generally observed in the region 16,525-17,570 cm^{-1} (ν_2) and 23,075-26,083 cm^{-1} (ν_3) are assigned to $^3A_{2g} \rightarrow {}^3T_{1g}$ (F), (ν_2) and $^3A_{2g} \rightarrow {}^3T_{1g}$ (P), (ν_3) respectively. The first two bands result from the splitting of one band, ν_1 and were in the range of ~ 9,210-10,610 and 11,270-12,540 cm^{-1} which may be assigned to $^3B_{1g} \rightarrow {}^3E_g$ and $^3B_{1g} \rightarrow {}^3B_{2g}$, assuming the effective symmetry to be D_{4h} (component of $^3T_{2g}$ in octahedral symmetry) [30]. The intense higher energy bands at 34570 cm^{-1} may be due to $\pi \rightarrow \pi^*$ transition of the (C=N) group. The spectra were consistent with distorted octahedral nature of these complexes. **(Figure 2)**

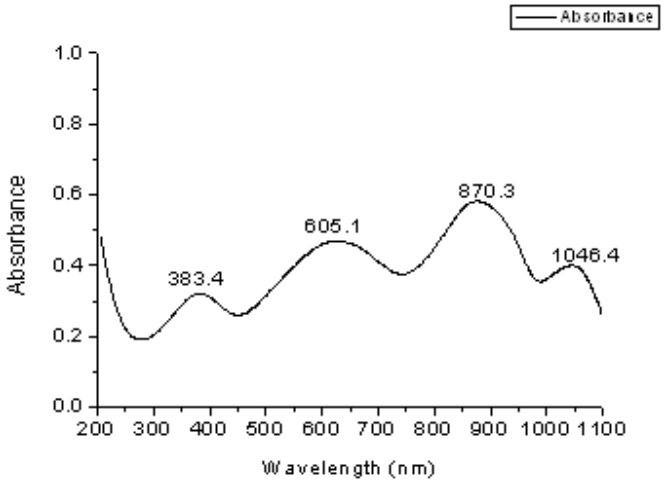


Figure: 2. Electronic Spectra of [Ni $(C_{30}H_{28}N_4)$ $(NO_3)_2$]

Copper Complexes

The magnetic moment of copper complexes at room temperature were observed in the range of 1.74-1.80 B.M., corresponding to one unpaired electron. The electronic spectra of the copper complexes exhibit bands at ~ 17,610-19,530 cm^{-1} with a shoulder at ~ 14,250-15,075 cm^{-1} and showed that these complexes have distorted octahedral geometry [30]. Assuming tetragonal distortion in the molecule, the d-orbital energy level sequence may be represented as: x^2-y^2>z^2>xy>xz>yz and the shoulder may be assigned to $z^2 \rightarrow x^2$-y^2 ($^2B_{1g} \rightarrow {}^2B_{2g}$) and the broad band contains both xy$\rightarrow$ x^2-y^2 ($^2B_{1g} \rightarrow {}^2E_g$) and xz, yz$\rightarrow$ x^2-y^2 ($^2B_{1g} \rightarrow {}^2A_{2g}$) transitions[29] Band separation in the spectra of these complexes was of the order of 2,530 cm^{-1}, which was consistent with the proposed geometry [32]. Therefore, it may be concluded that all the copper complexes are distorted octahedral.

ESR Spectra

ESR spectra of the Cu (II) complexes were measuredat X-band frequency of 9.1GHz under the magnetic field strength of 3,000G. ESR measurements have been done for Cu complexes using powder sample at Liquid Nitrogen Temperature in the solid state.The spectra provide information about the coordination environment around Cu(II) ion and showed a broad isotropic signal. The spectra provide only value of g_{iso} and do not give any idea about the individual g perpendicular and g parallel values. The g_{iso}value of the complexes lies in the range 2.121-2.157, Which shows that the Cu(II) complexes are in octahedral environment.

TGA (Thermogravimetric Studies)

The thermal stabilities of the complexes were investigated using TGA technologies. The thermogravimetric analysis (TGA) was obtained at a heating rate of 10°C/min in a nitrogen atmosphere over a temperature range of 50-800 °C. The complex [Ni ($C_{30}H_{28}N_4$) $(OAc)_2$] decompose above 800° C as illustrated by figure 3. The decomposition takes place in two steps. Absence of any decomposition step in the temperature range 100-250°C suggests absence of coordinated water molecule [33]. The first step in temperature range 250-400° C may correspond to the loss of an acetate ligand (Calc. - 9.52%; Obs. - 9.6%). The second step in the range 475-800°C may correspond to the slow decomposition of organic part involving the moiety $C_{17}H_{16}N_2NiO_4$ (Calc. – 59.67%; Obs. – 59.7%).

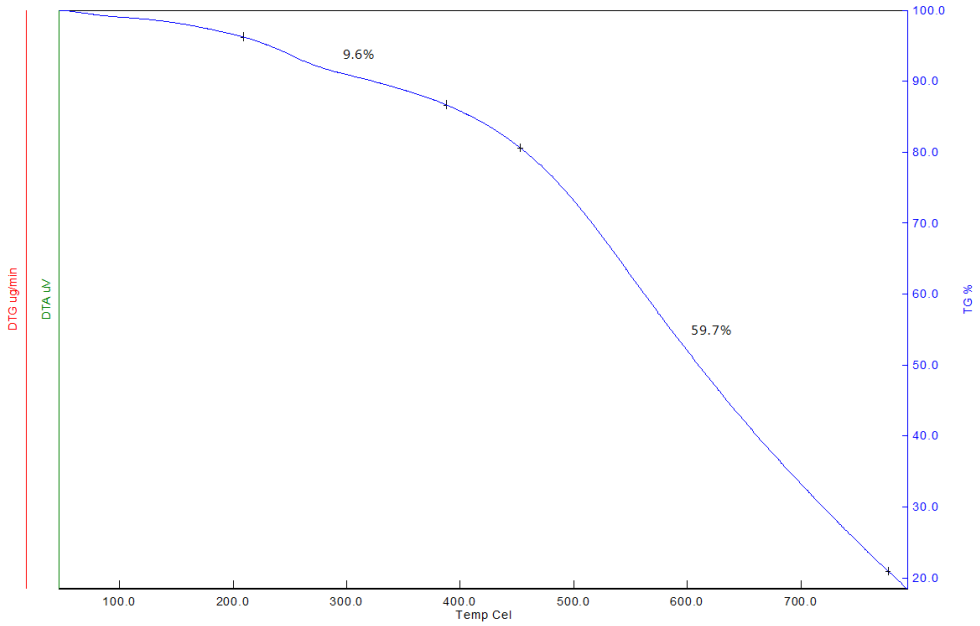


Figure: 3. Thermogram (TG) Curve of [Ni ($C_{30}H_{28}N_4$) $(OAc)_2$] complex

Molecular Modelling

The ligand-M(II) complexes were optimized using B3LYP (Becke-3–Lee-Yang-Parr) functional with 6-31G(d, p) basis sets in Gaussian09W program [34]. The geometry optimized structure of [Co ($C_{30}H_{28}N_4$) Cl_2] complex is shown in figure 4. The HOMO and LUMO energy gap of the complexes was calculated at B3LYP/6-31G(d,p) level, which reflects the chemical activity of the molecule. The calculated self-consistent field (SCF) energy of [Co ($C_{30}H_{28}N_4$) Cl_2] is -1552.38 a.u. at B3LYP/6-31G(d, p). The HOMO and LUMO energy gap explains the fact that eventual charge transfer interaction is taking place within the molecule. The HOMO is located over all the heteroatoms including central metal ion and the HOMO-LUMO transition implies an electron density transfer to the central metal ion. The atomic compositions of the frontier molecular orbital. The HOMO and LUMO orbitals are distributed not only on the conjugated molecular backbones, but also on substituents. The selected bond lengths and bond angles are as listed in table-1.

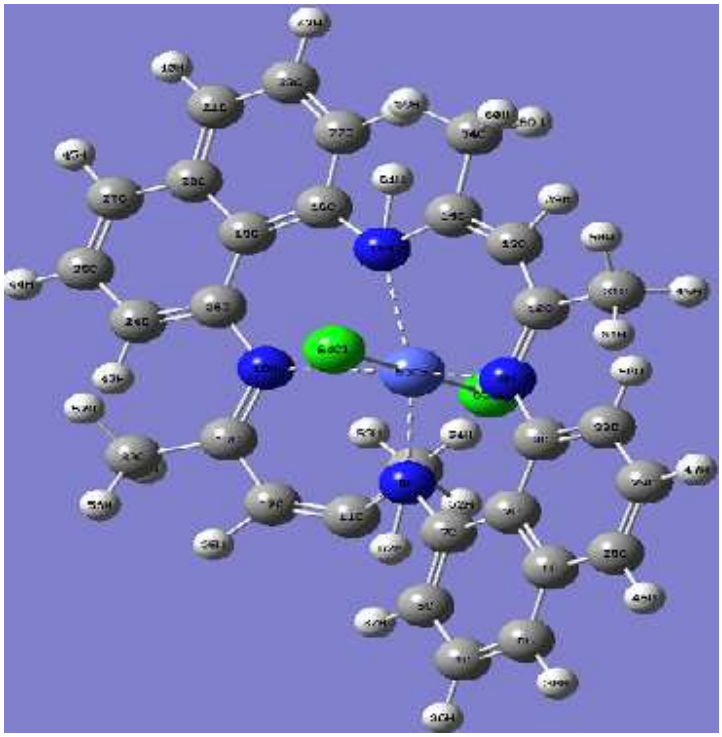


Figure: 4. Geometry optimized structure of [Co ($C_{30}H_{28}N_4$) Cl_2] complex

Table: 1. Structural parameters of [Co ($C_{30}H_{28}N_4$) Cl_2] complex

Parameters	Bond Length(Å)	Parameters	Bond Angles (Degree)
15N-63Co	2.52	65Cl-63Co-15N	89.18
18N-63Co	3.06	8N -63Co-15N	115.48
8N-63Co	1.96	8N -63Co-64Cl	77.82
9N-63Co	2.11	65Cl-363Co-18N	76.25
65Cl-63Co	2.15	64Cl-63Co-9N	101.97
64Cl-63Co	2.15	9N-63Co-18N	88.46
17C=18N	1.46	15N-63Co-64Cl	90.82
14C-15NH	1.47	18N-63Co-15N	67.20
11C-9NH	1.44	10C-8N-63Co	106.13
10C=8N	1.45	8N-63Co-9N	88.70

Powder XRD

The X-ray diffractogram of metal complex [Cd ($C_{30}H_{28}N_4$) $(OAc)_2$], as shown in figure 5, was scanned in the range 4-85° degree at wavelength 1.54060 Å and the generator setting 30mA, 40 kV. The Interplanar spacing (d-value), h, k, l and lattice parameters were calculated running Dicvol04 in computer programmer FullProf suite [35]. The indexing was confirmed from high figure of merit i.e., 18.4. The diffractogram of Cd(II) complex shows 16 reflections. The X-ray diffraction pattern of the complex has been indexed with the peaks having greater than 10% intensity. The lattice parameters for the complex was a= 30.2464, b=3.5106, c=4.1645, α=79.728, β=114.950, γ= 107.264 and volume=382.14. The condition a≠b≠c and α≠γ≠β for these complexes satisfy triclinic crystal system.

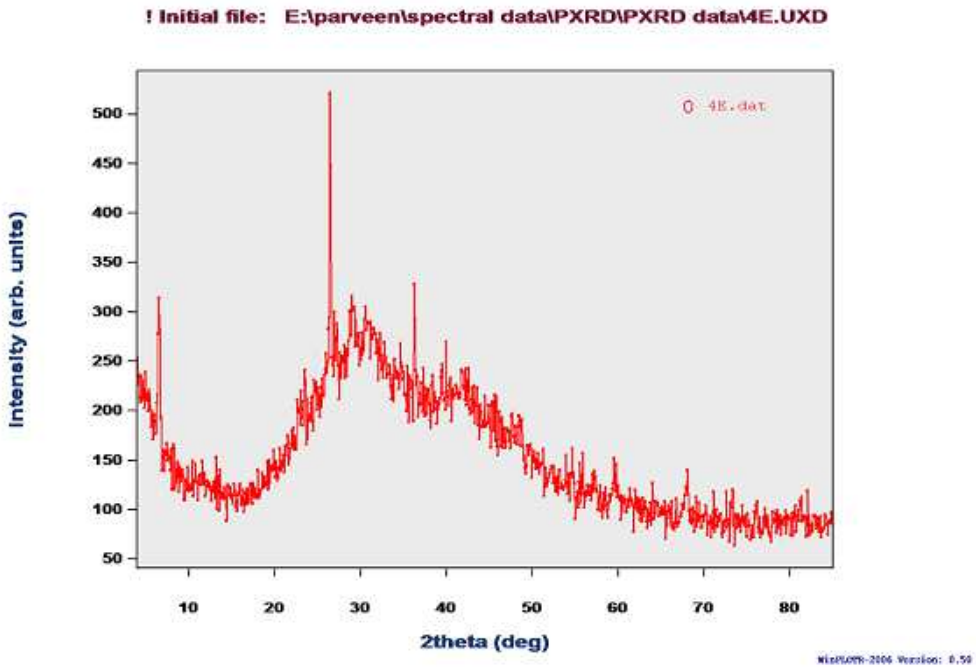


Figure: 5. XRD graph of [Cd ($C_{30}H_{28}N_4$) ($OAc)_2$]

Table: 2. X-ray diffraction data of the [Cd ($C_{30}H_{28}N_4$) ($OAc)_2$] complex

2TH. Obs	2TH. Cal	Diff. 2TH.	d-spacing	h	K	l
6.676	6.667	0.009	13.22947	2	0	0
13.329	13.339	-0.010	6.63721	4	0	0
23.598	23.606	-0.008	3.76706	0	0	1
26.636	26.636	-0.001	3.34398	0	1	0
29.095	29.089	0.006	3.06673	2	1	0
30.830	30.816	0.015	2.89792	3	1	0
34.736	34.732	0.003	2.58054	0	1	1
36.471	36.467	0.004	2.46161	1	1	1
39.653	39.642	0.011	2.27110	5	1	-1
40.087	40.094	-0.007	2.24752	7	1	0
42.691	42.690	0.000	2.11628	7	1	-1
45.873	45.888	-0.016	1.97661	2	0	-2
54.840	54.845	-0.006	1.67271	0	2	0
59.613	59.620	-0.007	1.54967	2	-1	2
64.241	64.250	-0.008	1.44874	5	-1	2
68.291	68.249	0.042	1.37236	6	1	2

BIOLOGICAL STUDIES

Antimicrobial results

The synthesized macrocyclic complexes were screened for their antibacterial and antifungal activity. Of the tested complexes, all the complexes showed some notable antibacterial activity against the Gram-positive (*Bacillus subtilis*), Gram-negative (*E. coli)* bacteria and yeast (*S. cerevisiae* and *Candida albicans*). However, these complexes were not effective and did not exhibit any activity against Gram negative bacteria (i.e. *P. aeruginosa)*. (Table-3).

Table: 3. *In vitro* antimicrobial activity of synthetic chemical compounds through agar well diffusion method

Compound No.	Diameter of growth of inhibition zone (mm)[a]				
	Bacillus subtilis	***Escherichia coli***	***Pseudomonas aeruginosa***	***Saccharomyces cerevisiae***	***Candida albicans***
1	-	13.3	-	-	14.3
2	11.3	14.6	-	24.6	12.6
3	13.6	-	-	15.3	-
4	13.3	12.6	-	-	**24.6**
5	12.6	13.0	-	17.3	13.3
6	13.3	16.3	-	20.6	12.6
7	14.6	15.6	-	-	15.3
8	11.6	15.3	-	-	13.6
9	16.3	20.6	-	-	12.3
10	20.3	20.3	-	-	15.0
11	**24.3**	**25.6**	-	20.6	21.3
Ciprofloxacin	24.0	25.0	22.0	-	-
Amphotericin-B	-	-	-	19.3	16.6

- No activity; [a] Values, including diameter of the well (8mm), are means of three replicates

MIC of all complexes were determined against Gram positive (i.e. *Bacillus subtilis*) Gram-negative (*E. coli*) bacteria and yeast (i.e. *S. cerevisiae* and *Candida albicans*) and then compared with MIC shown by standard antibiotic i.e. Ciprofloxacin and standard antifungal drug i.e. Amphotericin-B, respectively as shown in Table 4, figure-6

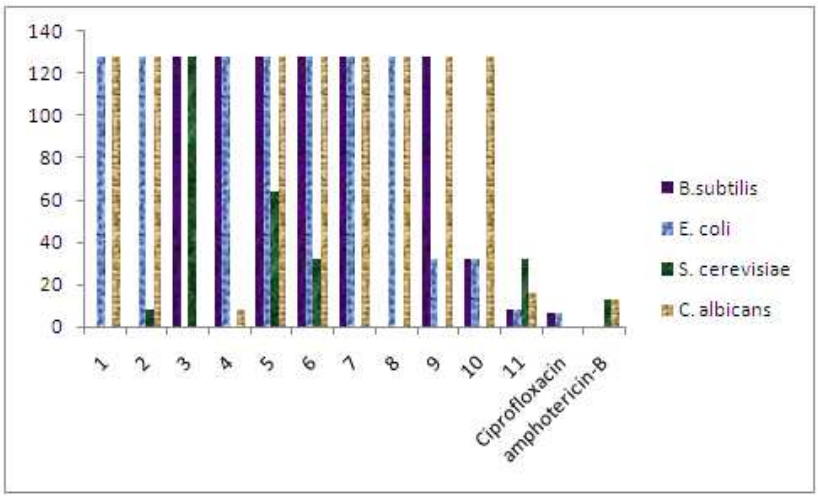


Figure: 6. Bar Graph representation of Minimum Inhibitory Concentration (MIC) of Complexes

Table 4: Minimum inhibitory concentration (MIC) (in µg/ml) of compounds by using modified agar well diffusion method

Compound No.	***Bacillus subtilis***	***Escherichia coli***	***Saccharomyces cerevisiae***	***Candida albicans***
(1)	nt	128	nt	128
(2)	32	128	**8**	128
(3)	128	nt	128	nt
(4)	128	128	nt	**8**
(5)	128	128	64	128
(6)	128	128	32	128
(7)	128	128	nt	128
(8)	nt	128	nt	128
(9)	128	32	nt	128
(10)	32	32	nt	128
(11)	**8**	**8**	32	16
Ciprofloxacin	6.25	6.25	nt	nt
Amphotericin-B	nt	nt	12.5	12.5

-= No activity; nt=not tested.

It may be noted that out of all the macrocyclic complexes, complex 11 was found to be most potent against pathogens under test and can be used as antimicrobial agent in pharmaceutical industries after testing it's toxicity to human beings.

Antioxidant activity

The antioxidant activity of newly synthesized complexes were tested by free radical scavenging activity using DPPH. The complex 1 i.e. [Co ($C_{30}H_{28}N_4$) Cl_2] have lowest IC_{50} value or highest % inhibition i.e. the concentration of the complexes required to reduce the initial absorption by 50% as shown by table-V. The tested complexes contain the azomethine hydrogen which is acidic in nature and can be donated to the DPPH free radical thus converting it to stable free radical [36]. The antioxidant activity of nitrate complexes is higher than the acetate complexes. This may be explained on the basis of higher electron withdrawing effect of nitrate ligand as compared to others and thus enhancing the easy release of proton. (Figure 7)

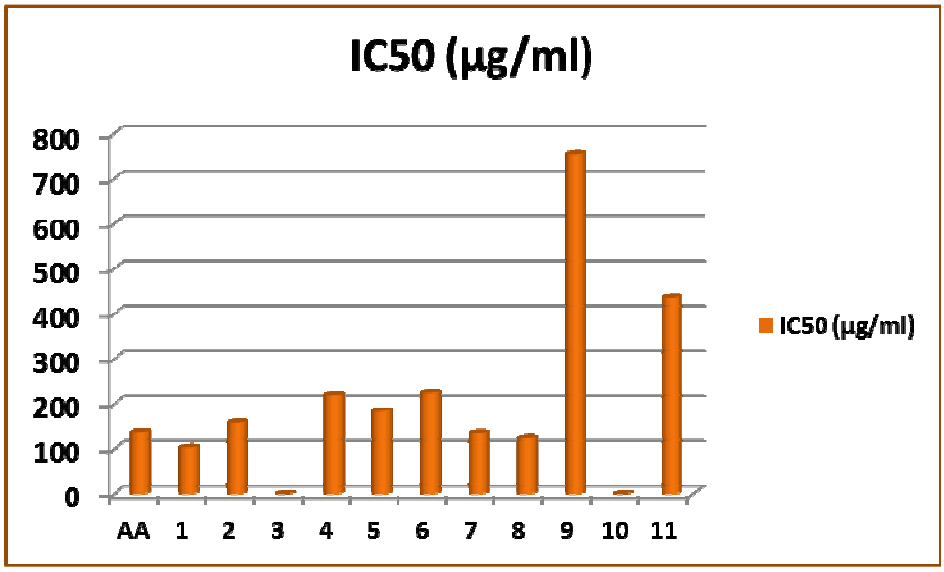


Figure: 7. Graphical representation of IC_{50} values of test complexes (µg/ml)

CONCLUSION

The newly synthesized complexes are hexacoordinated. The analytical, spectral and magnetic moment studies confirm the formation of macrocyclic frame and it's binding with the metal ions. Based on all these studies a distorted octahedral geometry may be proposed for all of these complexes. The powder X-ray diffraction studies suggest that the system belongs to triclinic system [37]. All the complexes were found to be potentially active towards microbial strains up to different levels and complex 11 was found to be best antimicrobial agent. The antioxidant activity of complex 1 was found to be upto significant level and it is the best antioxidant agent in the series. It has been suggested that chelation/coordination reduces the polarity of the metal ion mainly because of partial sharing of its positive charge with donor group within the whole chelate ring system [38]. This process of chelation thus increases the lipophilic nature of the central metal atom, which in turn, favours its permeation through the lipoid layer of the membrane thus causing the metal complex to cross the bacterial membrane more effectively thus increasing the activity of the complexes. Besides from this many other factors such as solubility, dipole moment, conductivity influenced by metal ion may be possible reasons for remarkable antibacterial activities of these complexes [39].

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REFERENCES

[1] M. P. Sathisha, N. V. Kulkarni, S. Budagumpi, B. N. Kirasur and V. K. revankar, *Supramolecular Chemistry*, **2011**, 23, 342.
[2] A. K. Singh, A. Panwar, R. Singh and S. Baniwal, *Trans. Met. Chem.*, **2003**, 28, 160.
[3] T. Chandra, B. J. Kraft, J. C. Hffman, J. M. Zalesk, *Inorg. Chem.*, **2003**, 42, 5158.
[4] M. A. Panchbhai, L. J. Paliwal, N. S. Bhave, *E. J. Chem.*, **2008**, 5, 1048.
[5] D. P. Singh, M. Kamboj, K. Jain, *Int. J. Chem. Res.*, **2012**, 3, 21.
[6] D. P. Singh, K. Kumar, *J. Serb. Chem. Soc.*, **2010**, 75, 475.
[7] M. Canadas, E. L. Torres, A. M. Arias, M. A. Mendiola, M. T. Sevilla, *Polyhedron*, **2000**, 19, 2059
[8] C. N. Verani, E. Rentschler, T. Weyhermuller, E. Bill, P. Chaudhuri, *J. Chem. Soc. Dalton Trans.*, **2000**, 251.
[9] M. S. Niasari, A. Amiri, *J. Mol. Cat.* 235A, **2005**, 114.
[10] D. P. Singh, R. Kumar, P. Tyagi, *Trans. Met. Chem.* **2006**, 31, 970.
[11] A. I. Vogel, *A text book of quantitative chemical analysis*, 5th ed., 1989, Longman, London.
[12] A. U. Rahman, M. I. Choudhary, W. J. Thomsen, *Bioassay Techniques for Drug Development*, Hardwood Academic, Amsterdam, The Netherlands, 2001.
[13] K. R. Aneja, C. Sharma, R. Joshi, *Jund. J. Microbial.*, **2011**, 4, 175.
[14] I. Yoon, H. S. Park, B. C. Cui, J. Z. Li, J. H. Kim, B. Lkhagvadulam, and Y. K. Shim, *Bull. Korean Chem. Soc.*, **2011**, 32, 2981.
[15] G. C. Yen, P. D. Duh, *J. Agric. Food Chem.*, 1994, 42, 629.
[16] W. J. Geary, *Coord. Chem. Rev.*, **1971**, 7, 81.
[17] G. A. Bain, D. X. West, J. Krejci, J. V. Martinez, S. H. Ortega, R. A. Toscano, *Polyhedron*, **1997**, 16, 855.
[18] V. B. Rana, D. P. Singh, M. P. Teotia, *Polyhedron*, **1982**, 1, 377.
[19] K. Nakamoto, *Infrared and Raman spectra of Inorganic and coordination compounds*, Part B, Wiley Interscience, New York **1997**, 5th ed.
[20] B. Adhikary, S. K. Mandal, K. Nag, *J. Chem. Soc. Dalton Trans.*, **1988**, 935.
[21] S. Chandra, S. D. Sharma, *Trans. Met. Chem.*, **2002**, 27, 732.
[22] C. Lodeiro, R. Basitida, E. Bertolo, A. Macias, R. Rodriguez, *Trans. Met. Chem.*, **2003**, 28**,** 388.
[23] M. Shakir, O. S. M. Nasman, S. P. Varkey, *Polyhedron*, **1996**, 15, 309.
[24] M. Shakir, K. S. Islan, A. K. Mohamed, M. Shagufa, S. S. Hasan, *Trans. Met. Chem.* **1999**, 24, 577.
[25] S. Chandra, R. Kumar, *Trans. Met. Chem*. **2004**, 29, 269.
[26] V. B. Rana, D. P. Singh, P. Singh, M. P. Teotia, *Trans. Met. Chem*. **1982**, 7, 174.
[27] D. P. Singh, K. Kumar, C. Sharma, *Synth. Reac. Inorg. Met-org. Chem.* **2010**, 40, 378.
[28] A. Kalam, V. Tripathi, S. Srivastava, Y. Pandey, A. Kumar, A. Gupta, S. Srivastava, A. Purohit, *Turkish J. Chem.* **2010**, 34, 147.
[29] V. B. Rana, D. P. Singh, P. Singh, M. P. Teotia, *Trans. Met. Chem.* 1981, 6, 36.
[30] A. B. P. Lever, *Inorganic Electronic Spectroscopy*, Amsterdam Elsevier **1984**, 2nd ed..
[31] D. P. Singh, K. Kumar, S. S. Dhiman, J. Sharma, *J. Enz. Inhib. Med. Chem.*, **2009**, 24, 795.
[32] A. B. P. Lever, E. Mantovani, *Inorg. Chem.*, **1971**, 817.
[33] P. Kavitha, M. R. Chary, B. V. V. A. Singavarapu, K.L. Reddy, "Synthesis, characterization, biological activity and DNA cleavage studies of tridentate Schiff bases and their Co(II) complexes", *J. Saudi Chem. Soc.* Doi: org/10.1016/j.jscs.2013.03.005, 2013.
[34] M. J. Frisch et. Al. Gaussian 09, Gaussian Inc., *Wallingford CT*, 2009.
[35] A. Boultif, D.Louer, *J. Appl. Cryst.* **2004**, 37, 724.
[36] A. Chaudhary, R. Sharma, M. Nagar, M. Mohsin, H.S. Meena, *J. Chil. Chem. Soc.*, **2011**, 56, 911.
[37] P. Kavitha, M. Saritha, K. L. Reddy, *Spectrochim. Acta part A*, **2013**, 102, 159.
[38] Z. H. Chohan, H. A. Shad, M. H. Youssoufi, T. B. Hadda, *J. Enzym. Inhib. Med.Chem.* **2004**, 19, 85.
[39] Z. H. Chohan, A. U. Shaikh, M. M. Naseer, C. T. Supuran, *J. Enzym. Inhib. Med. Chem.*, **2006**, 21, 771.