Research Article

Synthesis, Characterization, Thermal and Biological Studies of Substituted Benzophenone Derived Schiff Base Metal(II) Complexes

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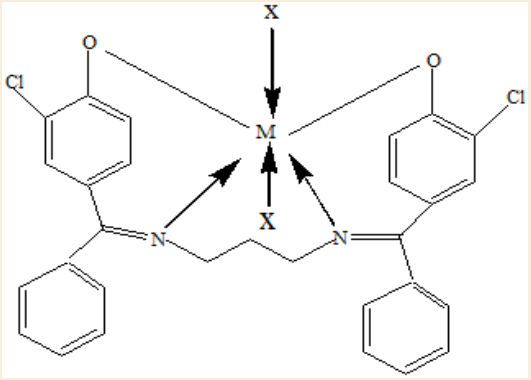
Abstract

The N,O type Schiff base ligand and its metal complexes have been synthesized from propylene diamine and chloro substituted hydroxy benzophenone in high yield, from which cobalt(II), nickel(II), zinc(II), cadmium(II), copper(II), complexes have been synthesized. All the complexes have been characterized by elemental analysis, FT-IR, Mass, UV-Vis, TGA/DTG, magnetic susceptibility data and molar conductance studies provide unambiguous evidence that complexes show an octahedral and square planar geometry. Metal complexes exhibited enhanced anti-inflammatory and antioxidant activity in comparison to their ligand.



Keywords: Benzophenone; Schiff base; Metal(II) complexes; Anti inflammatory and antioxidant

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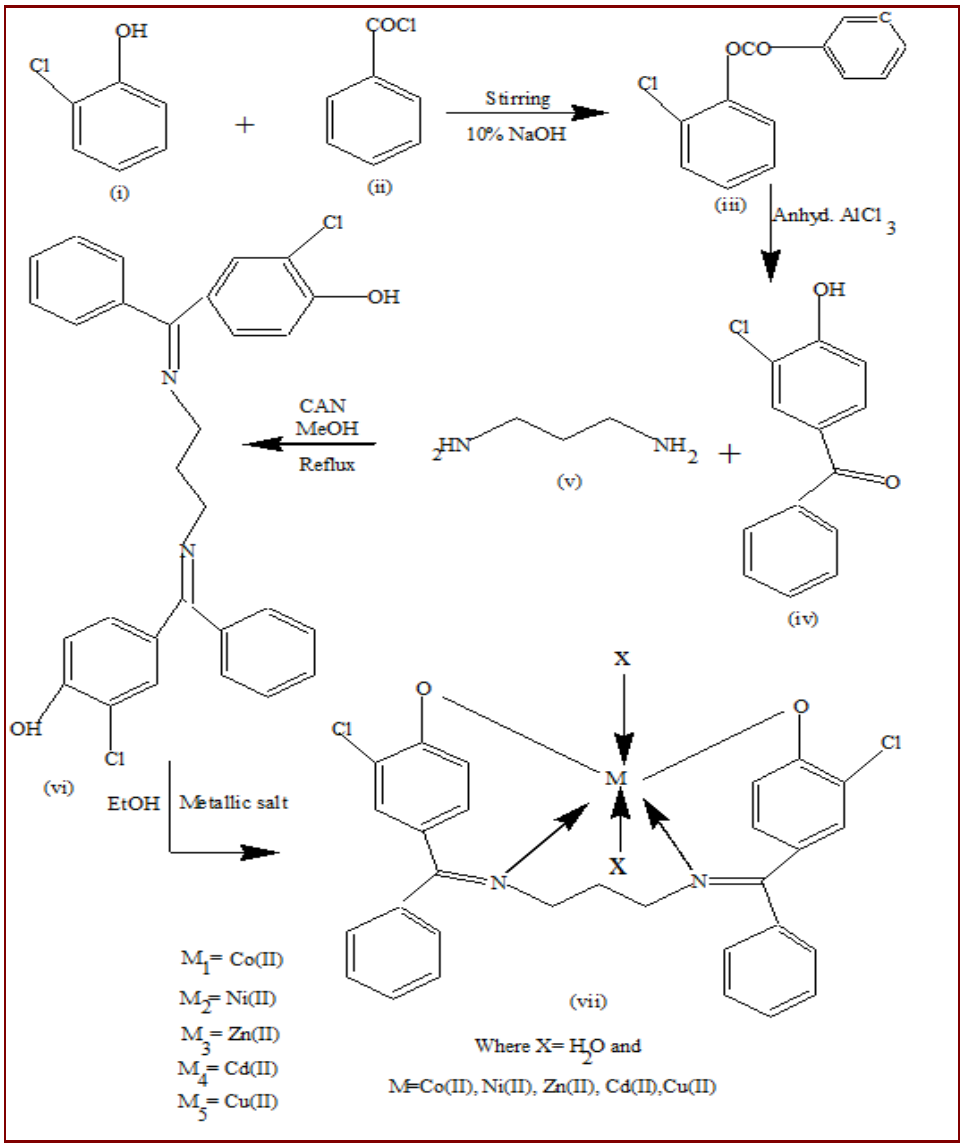
Introduction

The condensation of an amine with an aldehyde or ketone, forming what is called a Schiff base, is one of the oldest reactions in chemistry [1]. Tetradentate Schiff bases with N_2O_2 donor atoms are well known to coordinate with various metal ions and have attracted a great deal of interest in recent years due to their rich coordination chemistry [2–9]. Schiff base ligands coordinate to a metal through the imine nitrogen and another group, usually oxygen. The ligands have four donor sites. This makes the ligands ideal for the equatorial coordination of transition metals, leaving the two axial sites open for auxiliary ligands. Schiff base ligands have played an important role in the development of coordination chemistry, especially their metal complexes exhibit wide applications in biological and industrial systems [10-15]. Furthermore, the Schiff bases are very important tools for the inorganic chemists as these are widely used to design molecular ferromagnets, in catalysis, in biological modeling applications, as liquid crystals and as heterogeneous catalysts [16–18]. Schiff base ligands containing various donor atoms (like N, O, S,etc.) show broad biological activity and are of special interest because of the variety of ways in which they are bonded to the transition metal ions [19-20].Day by day Schiff bases are most frequentlyapplied for the betterment of human welfare. The importance of the Schiff base is due its versatile nature. The azomethine (-HC=N-) linkage present in Schiff base ligand and itsmetal(II) complexes show a wide range of biocidal activities such as antibacterial [21,22], antifungal [23,24], herbicidal, anti-inflammatory [25], anticancer, anti-diabetic [26] and antitumor [27] activities. Benzophenone derivatives are very important compounds due to their various biological and physicochemical properties such as electrochemical, spectroscopic,metal complexation, adsorptive and crystallographic properties [28-30] among others. The most important biological property of benzophenone derivatives is their ability to absorb a broad range of UV radiation (200 to 350 nm). Due to this property, the benzophenone derivatives like2-hydroxy-4-methoxybenzophenone and 2-hydroxy-4-methoxy-40-methoxybenzophenone were used as raw materials in the

manufacture of sunscreen creams [31]. These creams are helpful to avoid photosensitization, phototoxicity or allergic reactions of patients with various medicinal treatments [32].A comprehensive review [33] covers much of the Schiff base chemistry known up to 2007 and it has been followed by others [34]. Structure and mechanism of the formation of metal complexes and stereochemistry of four coordinate chelate compounds from Schiff bases and their analogues have been discussed in a review [35] with 250 references.

This fact tempted us to synthesize a benzophenone derivative. Hence, herein, we report the synthesis and characterization of a Schiff base ligand which is derived from the condensation of chloro substituted hydroxy benzophenone with 1-3,diamino propane and its metal(II) complexes [M_1 = Co(II), M_2= Ni(II), M_3=Zn(II),M_4=Cd(II) and M_5=Cu(II)]. The synthesized ligand and its Schiff base metal (II) complexes were characterized with the help of various spectral (FT-IR, Mass, UV-Vis, TGA/DTG, magnetic susceptibility data and molar conductance) studies. In addition, anti-infalammatory and antoxidant activities of ligand and its complexes are also described.

Experimental section



Synthesis of schiff base and its metal complexes

Materials and Methods

All the chemicals used in the preparation ofthe ligand and its metal complexes were of AR grade. The solventswere distilled before use. For the preparation and analyses, distilled water was used.

A Perkin-Elmer CHN analyzer (model 2400) was used for C, H and N analyses. The room temperature molarconductance was determined using a Century digital conductivity meter (model cc 601) with a dip type cell and a smooth platinum electrode. The electronic absorption spectra of the complexes were recorded as dilute solutions on a Shimadzu160A/240A UV-visible spectrophotometer. The ^{1}H NMR spectra were recorded using Bruker DRX 400 spectrometer at400 MHz with TMS as the internal standard. Mass spectra were obtained with a VG70-70H spectrophotometer.The infrared spectra of the solid samples were recorded in the range 4000-500 cm^{-1} on a Perkin-Elmer 597/1650 spectro photometer using KBr pellets. The magnetic moments were measured out using gouy balance. The TG-DTGexperiments were carried out in air using a Shimadzu DT-40 thermal analyzer. The heating rate employed was 10˚C perminute and platinum cups were used to hold about 5 mg of the samples.

General Procedure for the preparation of the ligand:

A mixture of chloro substituted hydroxy benzophenone and1,3 diamino propane in 2:1 molar ratios withmethanol was refluxed with constant stirring. This condensation reaction was carried by using catalyst CANfor three hours.The formed water was removed from the reaction mixture by using sodium sulphate as dehydrating agent. After completion of thereaction, the mixture was reduced to half of its original volume using a water bath and kept aside at room temperature. The white precipitate was formed on slow evaporation. This was filtered off and washed with ethanol. The product was recrystallized from hot ethanol and stored over anhydrous $CaCl_2$. They showed satisfactory melting point and elemental analyses.

Viii: Yield 89%; M.p. 157^{o}C; IR (Nujol): (C=N) 1661 cm^{-1}, (O-H) 1274 cm^{-1}, Phenolic(C-O) 1250 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.8, 2.6, 2.3 (s, 6H, $3CH_2$), δ6.9-8.8 (m, 16H, Ar-H), δ 12.1, 12.3 (bs, 2H, $-OH_{phenolic}$). $C_{29}H_{24}N_2O_2Cl_2$ (503): C, 69.21; H, 5.56; N, 4.77. Found: C, 69.18; H, 5.67; N, 4.81%.

General Procedure for the Synthesis of Schiff base metal(II) complexes ($M_1 – M_5$):

A solution of ligand (**vi,** 1 mmole) and metallic salt (0.5 mmole) in ethanol was refluxed for six hours. The resulting solution was reduced to half by a water bath and kept aside. On standing, the obtained solid products were filtered off and washed with water and ethanol. The products (**vii**) were recrystallized from a mixture of chloroform and ethanol (3:1). The product was stored over anhydrous $CaCl_2$.

(M_1): Yield75%; M.p. >250; IR (Nujol): 1635 cm^{-1} (C=N), Phenolic(C-O) 1265 cm^{-1}, 512 cm^{-1} (M-N), 458 cm^{-1} (M-O). $C_{29}H_{22}N_2O_2Cl_2Co(H_2O)_2$ (559.9): C, 58.42; H, 3.69; N, 4.71. Found: C, 48.49; H, 3.62; N, 4.77%, Molar conductance: 20.1, Magnetic moment: 4.70-4.78 B M.
(M_2): Yield78%; M.p. >250; IR (Nujol): 1640 cm^{-1} (C=N), Phenolic(C-O) 1268 cm^{-1}, 550 cm^{-1} , (M-N), 462 cm^{-1} (M-O). $C_{29}H_{22}N_2O_2Cl_2Ni(H_2O)_2$(559.6): C, 58.45; H, 3.69; N, 4.70. Found: C, 58.72; H, 3.02; N, 4.72%, Molar conductance: 16.3, Magnetic moment: 3.10-3.21B M.
(M_3): Yield72%; M.p. >250; IR (Nujol): 1637 cm^{-1} (C=N), Phenolic(C-O) 1270 cm^{-1}, 575 cm^{-1} (M-N), 484 cm^{-1} (M-O). $C_{29}H_{22}N_2O_2Cl_2Zn(H_2O)_2$(602.2): C, 57.78; H, 3.66; N, 4.64. Found: C, 57.60; H, 3.91;N, 4.98%, Molar conductance: 20.0.
(M_4): Yield74%; M.p. >250; IR (Nujol): 1644 cm^{-1} (C=N), Phenolic(C-O) 1272 cm^{-1}, 575 cm^{-1} (M-N), 496 cm^{-1} (M-O). $C_{29}H_{22}N_2O_2Cl_2Cd(H_2O)_2$ (649.2): C, 53.60; H, 3.38; N, 4.31. Found: C, 53.81; H, 3.61; N, 4.41%, Molar conductance: 14.20.

(M_5): Yield78%; M.p. >250; IR (Nujol): 1646 cm^{-1} (C=N), Phenolic(C-O) 1275 cm^{-1} ,632 cm^{-1} (M-N), 505 cm^{-1} (M-O). ^1H NMR ($CDCl_3$):δ 2.7, 2.5, 2.3 (s, 6H, $3CH_2$), δ 6.7-7.8 (m, 16H, Ar-H). $C_{29}H_{22}N_2O_2Cl_2Cu$ (564.5): C, 61.64; H, 3.89; N, 4.96. Found: C, 61.53; H, 3.81; N, 4.85%, Molar conductance: 19.2, Magnetic moment: 1.91-1.95 B M.

Biology:

Biological activity

DPPH radical scavenging activity was carried out according to Scherer R et al.., method. Briefly, 1ml of DPPH solution (dissolved in methanol) was mixed with different concentration (2,4,6,8 and 10µg/ml) of test samples(M_1, M_2, M_3, M_4, M_5 and vi) . After vigorous shaking, the mixture was allowed to stand for 20 min at room temperature. Absorbance of the resulting solution was measured at 517 nm with a UV-VIS spectrophotometer (HITACHI, U-2900) Butylated hydroxyl toluene (BHT) was used as positive control. The radical scavenging activity was measured as the decreased in the absorbance of DPPH and calculated using the following equation:

$$\text{Scavenging effect (\%)} = [1- A \text{ of sample}_{(517nm)} / A \text{ of control}_{(517nm)}] \times 100$$

Nitric oxide radical scavenging activity

Nitric oxide was generated from sodium nitroprusside and measured by Griess reaction. Sodium nitroprusside in phosphate buffer at physiological pH spontaneously generates nitric oxide, which in turn reacts with oxygen to produce nitrite ions that can be estimated by the Griess reagent [36] Nitric oxide scavengers compete with oxygen, leading to reduced production of nitric oxide. Sodium nitroprusside (5mM) in phosphate buffered saline was mixed with different aliquots of 2-10µg of M_1, M_2, M_3, M_4, M_5 and (vi) incubated at 25ºC for 3 hrs. The absorbance of the color formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthylethylenediamine was read at 546 nm and referred to the absorbance of BHT treated in the same way with the Griess reagent. The radical scavenging potential was calculated and expressed as EC_{50} value.

Ferrous ion chelating assay

Ferrous ion chelating ability was measured according to the method of [37]. Three sets of test tube were taken. One tube was taken as control to this $FeCl_3$ (200 mM) and $K_3Fe(CN)_6$ (400 mM) were added and the volume was made up to 1ml by adding distilled water. For the second tube, EDTA (40 mM), $FeCl_3$ (200 mM) and $K_3Fe(CN)_6$ (400 mM) were added and the volume was made up to 1ml by adding distilled water. For the third one, test compounds (M_1, M_2, M_3, M_4, M_5 and vi**)**with concentrations 2, 4, 6, 8 and 10 µg, $FeCl_3$ (200 mM) and $K_3Fe(CN)_6$ (400 mM) were added and the volume was made up to 1ml by adding distilled water. The tube was incubated for 10 min at 20°C and read the absorbance at 700 nm and ion chelating ability was calculated.

The antioxidant activity of all the compounds was compared with that of BHT. Radical scavenging activity was expressed as percentage activity using the formula.

$$[(\text{Control absorbance-sample absorbance})/\text{control absorbance})] \times 100.$$

Inhibition of Vipera russelli venom PLA_2

Assay of PLA_2 by indirect hemolytic activity

Protein concentrationin the venom was calculated, using bovine serum albumin fraction V as standard (0-75µg) [38]. A semi quantitative indirect hemolytic assay was employed [39]. Briefly, packed human erythrocytes, egg yolk and

phosphate buffer saline was mixed (1:1:8 V/V). 1ml of this suspension was incubated with 20µg enzyme for 10 min at 37^0C. The reaction was stopped by adding 10ml of ice cold phosphate buffer saline and centrifuged at 4^0C for 10 min at 800Xg. The amount of hemoglobin released in the supernatant was measured at 540 nm.

Protein glycation

The BSA/glucose model system was performed according to the procedure described by McPherson et al. (1988) with modifications. Briefly, the reaction mixture contained 0.9 mL of 200 mM phosphate buffer, pH 7.4 added with 0.02% sodium azide, 0.3 mL of 50 mg/mL BSA in phosphate buffer, 0.3 mL of 1.25 M glucose in phosphate buffer and 0.1 mL of each anti-glycation agent. The mixtures were incubated at 37 °C for 30 days in the dark. After incubation, 1.6 mL of 20% trichloroacetic acid was added to the reaction mixture before centrifugation at 10000g for 10 min. The supernatant was discarded and the precipitate was re-dissolved in 1.6 mL of phosphate buffer for fluorescence measurement (excitation 370nm/emission 440nm).

Results and Discussion

Chemistry

The ligand(vi) was prepared by condensation reaction of chloro substituted hydroxy benzophenone (i) with 1,3 diamino propane. TLC spots suggested the formation of the ligand. The complexes (M_1-M_5) were formed by the direct reaction of the ligand with cobalt(II), nickel(II), copper(II), zinc(II) and cadmium(II), copper(II) chloride.

The elemental analysis (**Table 1**) of the compounds is in good agreement with their proposed formulae and the conductance values indicate the non-electrolytic nature of the complexes [40]. The magnetic measurements of the complexes at room temperature reveal that all the complexes are paramagnetic and diamagnetic, consistent with octahedral and square planar geometry of cobalt (II), nickel (II), zinc (II), cadmium (II) and copper (II) yields light brown, yellow, white, white and algae green complexes. All the complexes are stable at room temperature and melt with decomposition above 250°C. Unfortunately, our efforts to obtain single crystals of complexes were not successful. Therefore the ligand, and its complexes were characterized on the basis of elemental analysis, ^{1}HNMR, IR, magnetic susceptibility measurement, electronic spectra data, TGA/DTA, colours, melting point, partial elemental analyses and molar conductivities.

FT IR spectra

To determine the way of chelation of Schiff base ligand to metal(II) ion, the IR spectrum of free Schiff base ligand is compared with IR spectra of its metal(II) complexes. The IR spectra of the ligand(vi) show a very strong band at 1278 cm $^{-1}$ was assigned to in-plane bending (O-H) vibration. The absence of (O-H)stretching andv(O-H) vibration in the spectra of the complexes indicated deprotonation of the –OH groups[41] The strong band observed in the region 1661cm $^{-1}$ in the ligand assigned to C=N stretching.The complexes (vii) show a strong band at 1635-1646 cm $^{-1}$ band at region, representing the C=N stretching moiety. This band is shifted to a lower frequency by ~ 15-26 cm $^{-1}$, as compared to the corresponding ligand indicating that the azomethine nitrogen of the C=N group has participated in coordination [42]. This canbeascribed to thewithdrawn of electron density from the nitrogen atom owing to coordination.The phenolic v(O-H) band is shifted from 1250 to 1265–1275 cm^{-1} as a consequence of the delocalization of double bond in chelate rings that has increased the bond order of the phenolic C-O. The phenolic oxygen, after the loss of O-H proton gets coordinated to the metal. This is supported by shift in the stretching frequency of v(C-O) to lower wave number by 15-25 cm^{-1} from its position in the free ligand [43]. In addition all the complexes showed additional weak to medium intensity bands in the region new 512-632 and 458-505 cm $^{-1}$ which were absent in the spectra of ligand, these can be attributed to v(M-N) and v(M-O) respectively [44, 45,46].

^{1}H NMR

The proton NMR spectrum of the Schiff base ligand shows **(Figure 1)** singlet peaks at 12.1and 12.3 ppm which corresponds to the two phenolic-OH groups which are absent in the spectrum of Cu(II) complex (M_5) **(Figure 2)**. This indicates that the deprotonated phenolic-O atom is involved in chelation. Both the ligand and Cu(II) complex show a group of multiplet (6.7–8.8 ppm). The methyl protons were appeared at 2.8, 2.6, 2.3 ppm and2.7, 2.5, 2.3 in the ^{1}H-NMR spectra of the ligand and its Cu(II) complex, respectively. The absence of a peak in the region 4.69–4.82 ppm reveals the absence of coordinated water molecules in the complex.

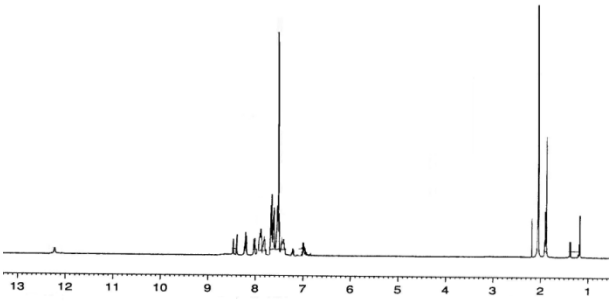


Figure 1 ^{1}H NMR Spectrum of ligand (vi)

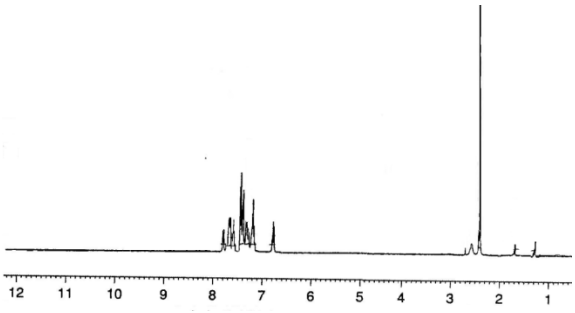


Figure 2 ^{1}H NMR Spectrum of Cu(II)complex (M_5)

Magnetic Measurements and Electronic Spectra

The magnetic moment of the complexes were measured at room temperature. The magnetic moment of the cobalt (II) lay in the range 4.70-4.78 BM which corresponds to 3 unpaired electrons. The solution spectra of the cobalt (II) complex exhibited absorption in the region 280-290, 330-360, 420-440 nm. The spectra resemble that reported for octahedral complex [47].Thus the various bands can be assigned to: $^4T_1g \rightarrow {}^4T_2g$, $^4T_1g \rightarrow {}^4A_2g$, $^4T_1g \rightarrow {}^4T_1g$.

The magnetic moment of the nickel (II) was lay in the range 3.10-3.21 BM which corresponds to 2 unpaired electrons. The solution spectra of the nickel (II) complex exhibited absorption in the region 340-360, 370-395, 430-450, 469-490 nm. The spectra resemble that reported for octahedral complex [48]. Thus the various bands can be assigned to: $^4T_1g \rightarrow {}^4T_2g$, $^4T_1g \rightarrow {}^4A_2g$, $^4T_1g \rightarrow {}^4T_1g$ and L→M charge transfer respectively.

The magnetic moment of the copper(II) lay in the range 1.91-1.95 BM which lie appreciably above the spin-only value of 1.73 BM for Cu(II) ion, indicating the presence of single unpaired electron and suggesting the square planar structure for the complex. The solution spectra of the copper(II) complex exhibited absorption in the region 320-356, 370-415, 490-540 nm. Thus for a square planar complex with $d_{x^2-y^2}$ ground state, three spin allowed transitions are possible, viz.,$^2B_{1g} \rightarrow {}^2A_{1g}(d_{x2-y2} \rightarrow d_{yz})$, $^2B_{1g} \rightarrow {}^2B_{2g}(d_{x^2-y^2} \rightarrow d_{xy})$ and $^2B_{1g} \rightarrow {}^2E_g(d_{x2-y2} \rightarrow d_{xy}, d_{yz})$. Since the four 'd' orbitals lie close together, the transitions cannot distinguished by their energy and hence, it is very difficult to resolve the bands into separate components [49].

The electronic spectra of Zn(II) and Cd(II) complexes reveal a charge transfer bands ∏→∏ and n→ ∏ transitions in the vicinity of the Schiff base ligand at 355 for both complexes;and 285 and 279 cm^{-1}, respectively suggesting anoctahedral structure which is common for d^{10} systems.

Table 1 Analytical, conductance and magnetic moment data of compound

Comp-ound	Mol. Formula (Colour)	M.pt. (°C)	Yield (%)	Found (Calc.) %			μ_{eff} (BM)	Λm_2 ($ohm^{-1}cm^2$ mol^{-1})
				C	H	N		
L	$C_{23}H_{19}N_2O_3$ White	157	89	69.18 (69.21)	5.67 (5.56)	4.81 (4.77)	–	–
M_1	$C_{48}H_{36}N_4O_6Cl_2Co(H_2O)_2$ Light brown	>250	75	58.49 (58.42)	3.62 (3.69)	4.77 (4.71)	4.70-4.78	20.1
M_2	$C_{48}H_{36}N_4O_6Cl_2Ni(H_2O)_2$ Yellow	290	78	58.72 (58.45)	3.02 (3.69)	4.72 (4.70)	3.10-3.21	16.3
M_3	$C_{48}H_{36}N_4O_6Cl_2Zn(H_2O)_2$ White	>250	72	57.60 (57.78)	3.91 (3.66)	4.98 (4.64)	–	20.0
M_4	$C_{48}H_{36}N_4O_6Cl_2Cd(H_2O)_2$ White	>250	74	53.81 (53.60)	3.61 (3.38)	4.41 (4.31)	–	14.2
M_5	$C_{48}H_{36}N_4O_6Cl_2Cu$ Algae green	>250	78	61.53 (61.64)	3.81 (3.89)	4.85 (4.96)	1.91-1.95	19.2

Thermal Studies

The study of the thermal behavior of all the complexes in air provides information about its thermal stability and nature of degradation of products produced at various temperatures. Thermal analyses of the complexes were carried out up to 800℃. All the complexes show a gradual mass loss indicating decomposition by fragmentation with increase in temperature and follow the similar pattern of their thermal decomposition. Thermogram of complex (M_1) **(Figure 3)** shows three stages of weight loss whereas Cu(II) complex (M_5) shows only two stages of weight loss. By comparison, the Co(II) complex shows an initial weight loss at 110–250℃ which corresponds to the coordinated water molecules [50] whereas Cu(II) complex shows no weight loss at this temperature range confirming the absence of these coordinated water molecules. The Co(II) complex shows a weight loss (80–82%) around 310–360℃ where as Cu(II) complex shows 240- 380℃ corresponding to continuoussublimation of organic ligand moieties and the formation of an air stable metal oxide as the end product in the range of above 400℃. The resultswell agreed with the composition of the metal complexes.The decomposition was completed at ≥800 °C.

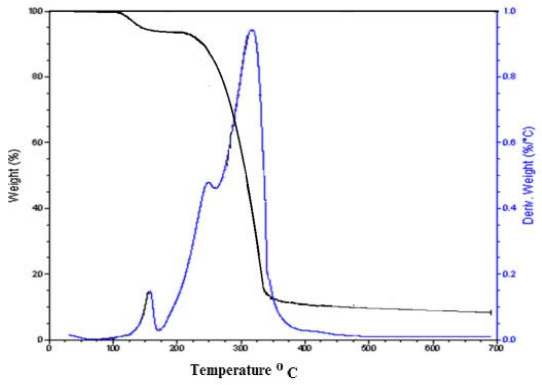


Figure 3 TGA Curve Of Co(II)Complex (M1)

Table 2 Stepwise Thermal Degradation Data obtained from TGA Curves and their Composition

Complex	Temp.range (℃)	Degradation Product	No.of Moles	% Weight loss		% Residue	
				Calcd	Expt	Calcd	Expt
M1	110-250 310-360	H_2O Ligand	2 1	6.5 80.1	6.5 80.2	13.3	13.0
M2	90-180 250-320	H_2O Ligand	2 1	6.4 80.3	6.5 80.5	13.2	12.9
M3	270-380	H_2O Ligand	2 1	6.4 79.0	7.0 81.5	14.5	11.0
M4	270-350	H_2O Ligand	2 1	5.8 73.3	6.0 74.0	20.8	20.0
M5	240-380	ligand	1	86.0	85.5	14.0	14.4

Biology

Table 3 DPPH radical scavenging activity

Test samples (DPPH)	BHT	M_1	M_2	M_3	M_4	M_5	Vi
IC_{50} value (µmol/ml)	0.019	0.019	0.017	0.019	0.019	0.135	0.014

DPPH radical scavenging activity evaluation is a rapid and convenient technique for screening the antioxidant activities. It can be seen from **(Table 3)** that, in DPPH assay, the compounds M_4 and M_5 showing scavenging activity which are slightly better than the reference compound BHT. Of all the compounds (vi) showed least DPPH radical scavenging activity compared to reference compound.

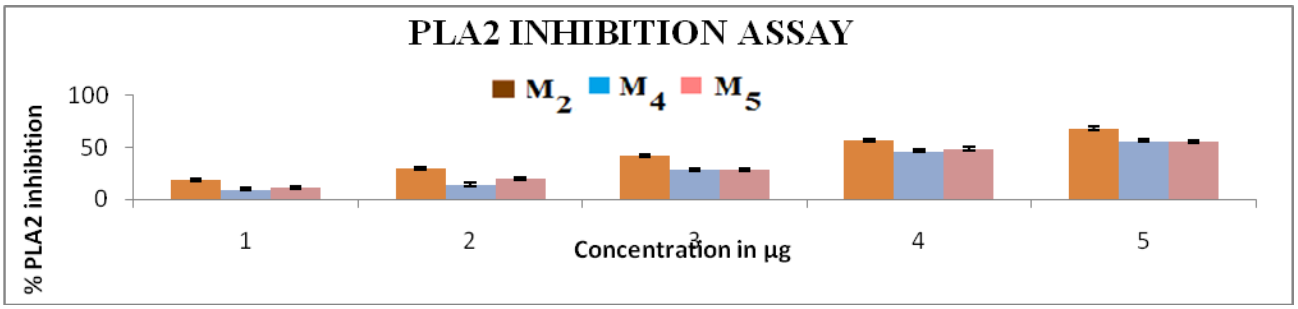
Table 4 Nitric oxide radical scavenging assay

Test samples	M_1	M_2	M_3	M_4	M_5	Vi
IC_{50} value (µmol/ml)	0.011	0.014	0.007	0.010	0.006	0.011

Under physiological condition, nitric oxide (NO) plays important role as a neurotransmitter, vasodilator and in the immunological system it fights against tumor cells and infectious agents.During inflammatory reactions, NO is produced by the inducible enzyme NO synthase (iNOS) in cells like macrophages, hepatocytes and renal cells after the stimulation with lipopolysaccharide (LPS), tumor necrosis factor (TNF-α), interleukin (IL-1) or interferon (INF-γ) and acts as a defense and regulatory signal molecule. However, NO is pathogenic when present in excess. NO per se as a reactive radical, directly damages normal tissues. [51]Further, nitric oxide can also react with superoxide anion

radical to form an even stronger oxidant peroxy nitrite.[52] The synthetic molecules M_1-M_4 showed significant activity in scavenging nitric oxide radical with an IC_{50} value ranging from0.0072 to 0.008µ moles/ml compared to BHT with an IC50 value of 0.0190 µ moles /ml.**(Table 4)**.

Table 5 PLA_2 inhibition assay



Test samples	M_2	M_4	M_5
IC_{50} value (µmol/ml)	0.012	0.014	0.015

The Russell viper venom PLA_2 is known to induce presynaptic neurotoxicity and myotoxicity in experimental mice (36). All these maladies are associated with PLA_2 activity. The compounds (M_1, M_2, M_3, M_4, M_5 and vi**)** were tested for in vitro anti-inflammatory properties by studying their inhibition by indirect hemolytic PLA_2 activity **(Table 5)**. The compoundsM_4 and M_5showed less inhibition with an IC50 value of 0.014 and 0.015µmoles/ml. The rest of the compounds did not show any inhibition.

Protein glycation

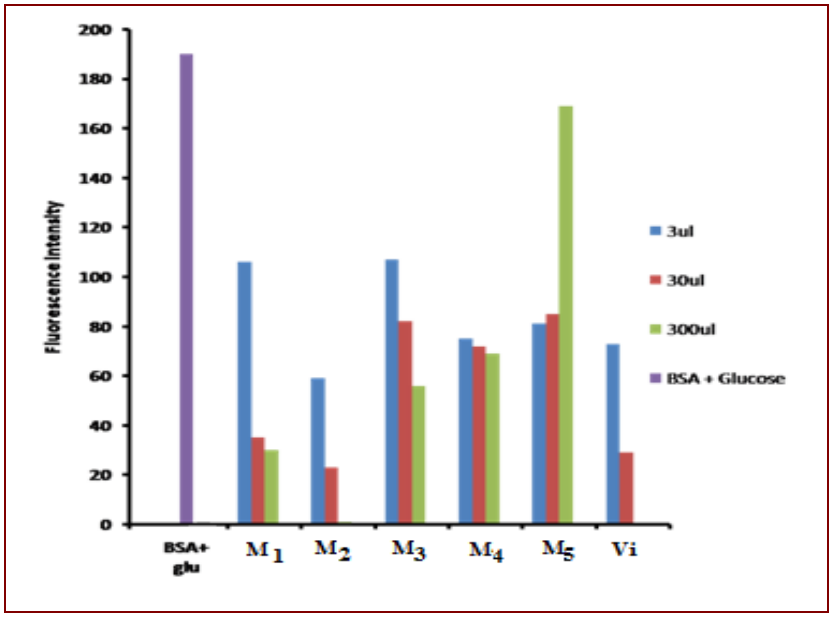


Figure 4 Effect of different compounds on non-enzymatic reaction between BSA/Glucose. Fluorescence intensity (excitation 370nm/emission 440nm)

Compounds showed significant antiglycating activity. Compounds showed a dose-dependent inhibition of protein glycation with concentrations ranging from 3 μg to 300 μg/ml, the response varied with the extract.

M_1, M_2, M_3, M_4, M_5 and vicompounds inhibited protein glycation. Inhibition by these compounds was significant at concentration 300 ug/ml. Among these compounds M_5 effectively controlled AGEs formation when compared to other compounds. In contrast, M_4 increased AGEs formation with increased concentration.

AGEs are nonenzymatically formed by reduction of glucose with proteins, lipids, and nucleic acids. The initial reaction between reducing sugars and proteins proceed through the formation of reversible intermediates of Schiff base and Amadori product, before a series of reactions that generate AGEs. It is now well accepted that the generation of AGEs in the body is an inevitable process. Formed during complex pathways, AGEs are chemical modifications of proteins, lipids, peptides, amino acids and nucleic acids by carbohydrates/reducing sugars including reactive carbonyl compounds as metabolic intermediates (e.g. 3-deoxyglucosone, glyoxal, methylglyoxal) (Bierhaus 1998). The reaction proceeds slowly through different stages, leading to a heterogeneous class of structures frequently characterized by brown colour, fluorescence and a tendency to polymerization.

Remarkable features of AGE-mediated cross-linked proteins include a decrease in solubility and a high resistance to proteolytic digestion. Thus, they are involved in altering biomechanical and biochemical properties of tissue components that can reduce tissue quality. Proteins with long half-lives including matrix and structural proteins in tissue compartments with slow biological turnover are particularly susceptible to develop AGE modifications (Miyata 1999).

As bioreactive molecules AGEs are formed during ageing as a physiological process, but are enhanced generated, and accumulate in a variety of chronic diseases such as diabetes mellitus, atherosclerosis, Alzheimer's disease and renal failure. AGE-specific receptors are present on a variety of different cells and serve in the regulation of AGE uptake and removal, but also modulate cell activation [52].

There are different ways to interfere with the pathophysiological effects of AGEs. Firstly, AGE formation can be prevented by certain inhibitors. Secondly, already formed AGEs may be broken down by certain drugs (Turgut 2010). Finally, RAGE activation can be blocked by sRAGE as a scavenger receptor or antibodies against RAGE. Hydrazine-derivatives such as aminoguanidin (AG) (Brownlee 1986) and OPB-9195 (Nakamura 1997) inhibited AGE-formation in vitro by the binding of reactive carbonyl compounds. M_1, M_2, M_3, M_4, M_5 and vi inhibit AGEs formation. The mode of their action still needs to be elucidated.

Conflict of Interest

The authors declare that they have no conflicts of interest with respect to the content of the manuscript.

Conclusion

The synthesized Schiff base ligand and its metal(II) complexes were characterized by microanalytical and various spectralstudies. The observed molar conductance, magnetic susceptibility and spectral data confirm octahedral and square planar environment. The presence of coordinatedwater molecules in all the complexes except Cu(II) has been further confirmed from thermal analysis. The anti-inflammatory and antioxidant activity of the complexes reveals higher activity than the ligand molecule.

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