Synthesis, X-ray powder diffraction, electrochemical and genotoxic properties of a new azo-Schiff Base and its metal complexes

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Abstract: A new, substituted 2-[(E)-{[4-(benzyloxy)phenyl]imino}methyl]-4-[(E)-(4-nitrophenyl)diazenyl]phenol azo-azomethine ligand (mbH) was synthesized from 2-hydroxy-5-[(4-nitrophenyl)diazenyl]benzaldehyde and 4-benzyloxyanilinehydrochloride in ethyl alcohol solution. These mononuclear Mn(II), Co(II), Ni(II), Cu(II), and Zn(II) complexes of the ligand were prepared and their structures were proposed by elemental analysis, infrared and ultraviolette-visible spectroscopy; the proton NMR spectrum of the mbH ligand was also recorded. The azo-azomethine ligand, mbH, behave as a bidentate ligand coordinating through nitrogen atom of the azomethine (–CH=N–) and oxygen atom of the phenolic group. Elemental analyses indicated that the metal:ligand ratio was 1:2 in the metal chelates. Powder X-ray diffraction parameters suggested monoclinic system for mbH ligand and its Ni(II), Cu(II), Co(II), Zn(II) complexes, and orthorhombic system for the Mn(II) complex. Electrochemical properties of the ligand and its metal complexes were investigated in the $1 \times 10^{-3} - 1 \times 10^{-4}$ M DMF and CH_3CN solvent in the range 200, 250 and 500 mV s^{-1} scan rates. The ligand showed both reversible and irreversible processes at these scan rates. In addition, genotoxic properties of the ligand and its complexes were also examined.

Key words: Azo dye, Schiff base, transition metal complexes, electrochemistry, X–ray powder diffraction, genotoxicity

1. Introduction

Schiff bases first reported by Hugo Schiff in 1864 are condensation products of primary amines with carbonyl compounds.[1] The common structural feature of these compounds is the azomethine group with a general formula R–HC=N–R. These compounds are an important class of ligands in coordination chemistry and found extensive application in various fields of science. d-block metal complexes of Schiff bases have expanded enormously and embraced wide and diversified subjects comprising vast areas of organometallic compounds and various aspects of biocoordination chemistry.[2-5] A number of Schiff base derivatives have shown interesting biological activities such as antibacterial, antifungal, anticonvulsant, antimalerial and anticancer.[6-9] Schiff base ligands and their metal complexes have been also investigated due to their interesting and important features such as their ability to reversibly bind oxygen and their use in catalyses for oxygenation and oxidation reactions of organic compounds and electrochemical reduction reactions.[10-13]

Azo dyes form an important class of organic colourants which consist of at least a conjugated azo (–N=N–) chromophore and the largest and most versatile class of dyes. Azo compounds have taken considerable attention due to their impressive and useful chemical physical properties. These compounds belong to one of the most intensively studied groups for non-linear optics, optical information storage, and optical switching.[14-17] Azo–azomethines have been extensively used as dyestuffs for wool, leather and synthetic fabrics because of their extraordinary coloring properties and in photonic devices, electro-optic modulators, components of optical communication systems of their second-order nonlinear optical properties.[18-19]

Previously, we have obtained and characterized various bidentate and/or polydentate ligands containing *N* and *O* donors.[6,20-22] In continuation of these studies, we discuss the synthesis of a new azo-azomethine ligand (mbH) and its mononuclear complexes with Mn(II), Co(II), Ni(II), Cu(II), and Zn(II). All the synthesized compounds were characterized by using various spectral (IR, ^{1}H NMR, and UV–Vis.) and physico-chemical techniques. The elemental analysis, type of chelation of ligand, and the geometry of the metal complexes were discussed in detail.

Insert Figure 1.

2. Experimental

2.1. Chemicals

All reagents and solvents were purchased from commercial sources and used without further purification unless otherwise noted. 2-hydroxy-5-[(E)-(4-nitrophenyl)diazenyl]benzaldehyde was prepared according to a previously published procedure.[23]

2.2. Physical measurements

Infrared spectra were obtained using KBr discs (4000-400 cm^{-1}) on a Perkin Elmer FT-IR spectrophotometer. The electronic absorption spectra of the compound in the 200-800 nm range were measured in DMSO on a T80 + UV-Vis. Spectrometer PG Instruments LTD spectrometer. Carbon, hydrogen and nitrogen elemental analyses was performed with a model LECO CHNS 932 elemental analyzer. ^{1}H NMR spectrum of the ligand was obtained in $CDCl_3$ as solvent on a Bruker FT–NMR AC–400 (400 MHz) spectrometer. All chemical shifts are reported in δ(ppm) relative to the tetramethylsilane as internal standard. Powder X–ray diffraction analysis was performed by PANanalytical X'Pert PRO instrument with Cu–K_α radiation (wavelength 0.154 nm) operating at 40 kV and 30 mA. Measurements were scanned for diffraction angles (2θ) ranging from 20° to 90° with a step size of 0.02° and a time per step of 1 s. Melting points were obtained with a Electrothermal LDT 9200 apparatus in open capillaries. Cyclic voltammograms studies were recorded according to literature method on a Iviumstat Electrochemical workstation equipped with a low current module (BAS PA–1) recorder.[24]

2.3. Synthesis of 2-[(E)-{[4-(benzyloxy)phenyl]imino}methyl]-4-[(E)-(4-nitrophenyl)diazenyl]phenol, (mbH).1/2H_2O

A solution of 4-benzyloxyanilinehydrochloride (433.50 mg, 2.176 mmol) in ethyl alcohol (10 mL) was mixed with a solution of 2-hydroxy-5-[(4-nitrophenyl)diazenyl]benzaldehyde (498.64 mg, 1.84 mmol) in ethyl alcohol (50 mL) and the reaction mixture was refluxed for 24 hours. The formed dark yellow product was dissolved in ethyl alcohol (25 mL) and left for crystallization at room temperature for a day. Then orange crystals were collected, washed with cold ethyl alcohol and dried in air. Yield, 650.00 mg (77%). Mp: 209–210 °C. Elemental analyses for $C_{26}H_{21}N_4O_{4,5}$ (461.46 g/mol): Found: C, 67.42; H, 4.34; N, 12.09 %. Calcd.: C, 67.67; H, 4.59; N, 12.14%. IR (cm^{-1}): 3448 υ(O–H hydrated water), 1637 υ(C=N), 1520 υ(–N=N–), 1342 υ(C=C), 1104 υ(C–O–C).

2.4. Synthesis of di(aqua)bis{2-[(E)-{[4-(benzyloxy)phenyl]imino}methyl]-4-[(E)-(4-nitrophenyl)diazenyl]phenolato}mangan(II), [Mn(mb)$_2$(H_2O)$_2$].4H_2O

A solution of $MnCl_2.4H_2O$ (1.40 mg, 0.011 mmol) in methyl alcohol (10 mL) was added to a solution of mbH (10.00 mg, 0.022 mmol) in dichloromethane (20 mL). The mixture was then heated in a water bath for another 30 min to complete the precipitation. The red complex was filtered, washed with cold ethyl alcohol and dried. Yield, 7.34 mg (64%). Mp: > 250 °C. Elemental analyses for $C_{52}H_{50}MnN_8O_{14}$ (1065.93g/mol): Found: C, 58.72; H, 3.90; N, 10.48 %. Calcd.: C, 58.59; H, 4.73; N, 10.51%. IR (cm^{-1}): 3419 υ(O–H hydrated water), 1630 υ(C=N), 1523 υ(–N=N–), 1342 υ(C=C), 1106 υ(C–O–C), 850 (coordinated water), ~650 υ(Mn–O), 545 υ(Mn–N).

2.5. Synthesis of di(aqua)bis{2-[(E)-{[4-(benzyloxy)phenyl]imino}methyl]-4-[(E)-(4-nitrophenyl)diazenyl]phenolato}nickel(II), [Ni(mb)$_2$(H_2O)$_2$].3H_2O

2-[(E)-{[4-(benzyloxy)phenyl]imino}methyl]-4-[(E)-(4-nitrophenyl)diazenyl]phenol ligand (10.00 mg, 0.022 mmol) was dissolved in dichloromethane (20 mL) at room temperature (Figure 1). A solution of $NiCl_2.6H_2O$ (2.70 mg, 0.011 mmol)) in methyl

alcohol (10 mL) was added dropwise into the solution of the ligand with continuous stirring. The mixture was refluxed for 3 h, the volume of the solution was then reduced to 10 mL and left to cool down to the room temperature. On addition of ethyl alcohol (10 mL), a precipitate formed and was collected and washed with small amount of ethyl alcohol. The orange product was recrystallized from hot ethyl alcohol and it was dried at room temperature. Yield, 8.00 mg (70%). Mp: 266–267 °C. Elemental analyses for $C_{52}H_{48}N_8NiO_{13}$ (1051.67 g/mol): Found: C, 59.29; H, 4.00; N, 10.29 %. Calcd.: C, 59.39; H, 4.60; N, 10.65 %. IR (cm^{-1}): 3409 υ(O–H hydrated water), 1627 υ(C=N), 1510 υ(–N=N–), 1376 υ(C=C), 1104 υ(C–O–C), 845 (coordinated water), 610 υ(Ni–O), ~540 υ(Ni–N).

2.6. Synthesis of di(aqua)bis{2-[(E)-{[4-(benzyloxy)phenyl]imino}methyl]-4-[(E)-(4-nitrophenyl)diazenyl]phenolato}copper(II), $[Cu(mb)_2(H_2O)_2].3H_2O$

$Cu(CH_3COO)_2{\cdot}H_2O$ (2.20 mg, 0.011 mmol) was dissolved in methyl alcohol (10 mL) and stirred under reflux for 45 min, followed by the addition of the mbH Schiff base (10.00 mg, 0.022 mmol) in dichloromethane (20 mL) and reaction mixture was refluxed for 3 hours. The obtained brown precipitate was filtered, washed with methyl alcohol and dried in air. Yield, 5.90 mg (51%). Mp: 250–251 °C. Elemental analyses for $C_{52}H_{48}N_8CuO_{13}$ (1056.53 g/mol): Found: C, 58.43; H, 3.86; N, 10.52 %. Calcd.: C, 59.11; H, 4.58; N, 10.61%. IR (cm^{-1}): 3375 υ(O–H hydrated water), 1630 υ(C=N), ~1520 υ(–N=N–), 1340 υ(C=C), 1105 υ(C–O–C), 855 (coordinated water) , 691 υ(Cu–O), 546 υ(Cu–N).

2.7. Synthesis of di(aqua)bis{2-[(E)-{[4-(benzyloxy)phenyl]imino}methyl]-4-[(E)-(4-nitrophenyl)diazenyl]phenolato}cobalt(II), $[Co(mb)_2(H_2O)_2].8H_2O$

A methanolic solution (10 mL) of $Co(CH_3COO)_2{\cdot}4H_2O$ (2.60 g, 0.011 mmol) was added gradually to a dichloromethane solution (20 mL) of the ligand (10.00 mg, 0.022 mmol). The solution was stirred for 2 hours and a reddish brown precipitate formed and the product was filtered and washed with ethyl alcohol then diethyl ether and finally dried

in air. Yield, 7.40 mg (59%). Mp: 254 °C. Elemental analyses for $C_{52}H_{58}CoN_8O_{18}$ (1141.99 g/mol): Found: C, 54.74; H, 4.70; N, 9.78 %. Calcd.: C, 54.69; H, 5.12; N, 9.81%. IR (cm^{-1}): 3390 υ(O-H/hydrated water), 1632 υ(C=N), ~1520 υ(–N=N–), 1342 υ(C=C), 1106 υ(C–O–C), 857 (coordinated water), ~650 υ(Co–O), 547 υ(Co–N).

2.8. Synthesis of di(aqua)bis{2-[(E)-{[4-(benzyloxy)phenyl]imino}methyl]-4-[(E)-(4-nitrophenyl)diazenyl]phenolato}zinc(II), $[Zn(mb)_2(H_2O)_2].H_2O$

The red coloured compound was prepared by the addition of $Zn(CH_3COO)_2.2H_20$ (2.40 mg, 0.011 mmol) in methyl alcohol (10 mL) to a refluxing mixture of the ligand (10.00 mg, 0.022 mmol) mbH in dichloromethane (20 mL). The red compound was separated out *via* filtration, washed with cold ethyl alcohol and dried in vacuo. Yield, 6.10 mg (55%). Mp: 286–287 °C. Elemental analyses for $C_{52}H_{44}N_8O_{11}Zn$ (1022.36 g/mol): Found: C, 61.00; H, 3.83; N, 10.83 %. Calcd.: C, 61.09; H, 4.34; N, 10.96 %. IR (cm^{-1}): 3395 υ(O–H hydrated water), 1625 υ(C=N), ~1515 υ(N=N), 1340 υ(C=C), 1103 υ(C–O–C), 845 *(*coordinated water*)*, 698 υ(Zn–O), 545 υ(Zn–N).

2.9. Salmonella/microsome test (Ames)

2.9.1. Bacterial strains

Histidine deficient (his–) tester strains TA98 and TA100 of *Salmonella typhimurium* were provided by L. K. Nakamura (Microbiologist Emeritus, Microbial Properties Research, United States, Department of Agriculture 1815 North University Street Peoria Illinois 61604, USA). The TA98 strain is used to detect the frameshift mutagens and TA100 strain for the detection of base pair substitution mutagens. Each strain used for testing was checked for the presence of strain-specific marker as described by Maron and Ames.[25]

2.9.2. Mutagenicity assay and preparation of S9

The standard plate-incorporation assay was examined with *Salmonella typhimurium* TA98 and TA100 strains in the presence and absence of S9 mix according to Maron and Ames.[25] Mutagenicity tests and preparation of S9 for the compounds were performed according to literature report.[25,26] For the test, the mbH bidentate ligand and its metal complexes were dissolved in DMSO and used of 0.06, 0.12, 0.24, 0.49 and 0.98 mg per plate. Each sample was evaluated with three replicate plates and all tests were performed twice. The fresh S9 mix was used for each mutagenicity assay.

2.9.3. Statistical significance

The significance between control revertants and revertants of treated goups were also compared by using t-test. Dose-response relationships were also evaluated by using regression and correlation (r) test systems.

3. Results and discussion

3.1. Synthesis

2-[(E)-{[4-(benzyloxy)phenyl]imino}methyl]-4-[(E)-(4-nitrophenyl)diazenyl]phenol (mbH) was prepared by the reaction of 2-hydroxy-5-[(4-nitrophenyl)diazenyl]benzaldehyde with 4-benzyloxyanilinehydrochloride in ethyl alcohol. The product of condensation reaction of 2-hydroxy-5-[(4-nitrophenyl)diazenyl]benzaldehyde salt with 4-benzyloxyanilinehydrochloride is depicted in Figure 1. The new azo-azomethine ligand, 2-[(E)-{[4-(benzyloxy)phenyl]imino}methyl]-4-[(E)-(4-nitrophenyl)diazenyl]phenol (mbH), resulted in mononuclear complexes (Figure 2) with Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) as follows:

$$MX_2 + 2\ mbH \xrightarrow[\text{reflux}]{\text{dichloromethane}} [M(mb)_2(H_2O)_2].nH_2O + 2\ HX$$

mbH: 2-[(*E*)-{[4-(benzyloxy)phenyl]imino}methyl]-4-[(*E*)-(4-nitrophenyl)diazenyl]phenol

M = Mn(II) (n = 4); Co(II) (n = 8); Ni(II) (n = 3); Cu(II) (n = 3); Zn(II) (n = 1)

Experimental results of the elemental analyses of the synthesized ligand and its metal chelates are in good agreement with theoretical values. The elemental analyses of the complexes indicate that the metal–ligand ratios are 1:2 in the $[M(mb)_2(H_2O)_2].nH_2O$ [M = Mn(II), n =4; Co(II), n = 8; Ni(II), n = 3; Cu(II), n = 3; or Zn(II), n = 1], metal complexes. The purity of the compounds was checked by TLC using silica gel G as adsorbent. The ligand and its mononuclear complexes are not soluble in water. Single crystals of the new azo-azomethine ligand and its transition metal chelates were not be able to isolate from any organic solvent, thus, no definite structures could be described.

However, structures of the compounds were proposed by the analytical and spectroscopic data as shown in Figures 1 and 2. The analytical and spectroscopic data showed that M(II) ions are six–coordinate, bonded to two nitrogen (C=N), two phenolic

oxygen atoms of two azo-azomethine ligands and two water molecules. Coordination geometry around the metal centers are octahedral. M–N and M–O bonds are expected to be *trans* in configuration due to steric reasons and this *trans* configuration was also observed for the similar complexes reported in the literature.[21-23]

3.2. ^{1}H NMR spectrum of the ligand

For further information about the azo-azomethine ligand the ^{1}H NMR was recorded in $CDCl_3$. NMR shifts of the ligand is shown in Table 1. The ^{1}H NMR spectrum confirms that the ligand is intact in solution. The hydrogen atom of the azomethine group (–CH=N–) was observed at the δ 8.67 ppm as a singlet.[6] The aromatic protons were observed in the range of δ 6.98-8.67 ppm as a multiplet. Benzyl (C19) protons were assigned to a singlet peak at δ 5.05 ppm. A shift at δ 14.29 ppm could be assigned to phenolic proton (O(16)H).[27] Additionally, there is also water protons observed at δ 1.52 ppm. The presence of water in the structure was also confirmed by infrared spectroscopy and elemental analysis.

Insert Figure 2., Table 1.

3.3. FT-IR spectra

In order to study the bonding of the mbH azo-Schiff base to the metal, the infrared spectrum of the mbH was compared with spectra of the corresponding metal chelates. The infrared spectra provided valuable information regarding the nature of the functional groups attached to the metal ion. The main infrared bands and their assignments are given in experimental section. In the spectrum of azo-azomethine ligand (mbH), a strong band at 1637 cm^{-1} is attributed to C=N (azomethine) group.[28] Upon coordination, this band C=N (azomethine) shifted to lower frequency due to shift of lone pair density toward the metal ion indicating coordination of azomethine nitrogen to the metal center.[29-31] The spectrum of the mbH ligand exhibits a broad band at 3448 cm^{-1} due to phenolic and water –OH.[32] The phenolic –OH stretch disappears in the spectra of metal complexes indicating that upon coordination of the ligand to metal centers, the phenolic oxygen atoms are deprotonated. The spectra of the metal chelates

exhibited broad bands at 3448–3375 cm^{-1} that are attributed to OH of the crystal water molecules while the bands observed at approximately 857–845 cm^{-1} assigned to coordinated water molecules .[27,32] A comparison between infrared spectra of mbH and the $[M(mb)_2]$ complexes also show that a band, characteristic of *ν*(C–O) at 1315 cm^{-1}, is shifted to 1345–1325 cm^{-1}, due to C-O-M bond formation. Bands at 2920–2885 cm^{-1} are assigned to CH_2 asymmetric and symmetric stretching vibrations. The azo-Schiff base mbH showed a band at 1342 cm^{-1} for ν(C=C) of aromatic rings while its metal complexes shift to 1376-1340 cm^{-1}. In addition to these, all the metal complexes show two new bands at 698-610 and 547–540 cm^{-1} due to formation of M–O and M–N bonds, further confirming formation of coordination complexes.[33] All the vibrational data suggest that the metal ion bonded to the azo-azomethine ligand through the phenolic oxygen and the imino nitrogen atoms.

3.4. Electronic spectra

The electronic spectra of the mbH ligand and its metal chelates were recorded in DMSO between 200 and 800 nm. The compared dates of the UV–Vis. spectra for the azo-azomethine dye and its metal chelates are shown in Table 2. The UV–Visible spectra of the ligand and its Ni(II) chelate in DMSO solution are shown in Figures 3 and 4.

The absorption of the synthesized ligand (mbH) displays mainly three bands in DMSO solution at room temperature within the studied range. The band at 238 nm were assigned to the π→π* transition of aromatic rings, while the band at 292 nm as shoulder are due to the low energy π→π* transition of the –CH=N– and –N=N– groups.[34,35] The peaks belonging to the π→π* transitions in the spectra of the $[Mn(mb)_2(H_2O)_2].4H_2O$, $[Ni(mb)_2(H_2O)_2].3H_2O$, $[Co(mb)_2(H_2O)_2].8H_2O$, $[Cu(mb)_2(H_2O)_2].3H_2O$ and $[Zn(mb)_2(H_2O)_2].H_2O$ coordination compounds were observed at 234, 238, 240, 237 and 240 nm, respectively. The band at 361 nm was assigned to the n→π* transitions of the –CH=N– and –N=N– azo chromophore groups. The peaks belonging to the these groups in the spectra of the $[Mn(mb)_2(H_2O)_2].4H_2O$, $[Ni(mb)_2(H_2O)_2].3H_2O$, $[Co(mb)_2(H_2O)_2].8H_2O$, $[Cu(mb)_2(H_2O)_2].3H_2O$ and $[Zn(mb)_2(H_2O)_2].H_2O$ complexes appeared at 372, 314, 391, 327 and 410 nm, respectively. The d–d transition bands in the spectra of the Mn(II), Co(II), Ni(II) and Cu(II) chelates were observed at 493–535

nm. Also, the band at 414 nm of Ni(II) and 540 nm of the Zn(II) chelates can be assigned to charge-transfer transitions. The spectroscopic data obtained in this work are agreed well with previous works.[36]

Insert Figures 3 and 4., Table 2.

3.5. X-ray powder diffraction analysis

Growth of single crystals of azo-azomethine compounds from various solvents including DMF, ethyl alcohol, chloroform *etc*. failed, so they were characterized by XRD.[37,38] X–ray powder diffraction analysis of the mbH ligand and its metal complexes was carried out to determine the type of crystal system, lattice parameters and the cell volume. As shown in Figure 5 the XRD patterns indicate crystalline nature for the mbH ligand and its metal complexes. Indexing of the diffraction patterns were performed using HighScore Plus software.For the Mn(II) and Co(II) complexes, for example, their Miller indices (*h k l*) along with observed and calculated 2θ angles, *d* values and relative intensities were given in Tables 3 and 4. From the indexed data the unit cell parameters were also calculated and listed in Table 5. The powder XRD patterns of the compounds are completely different from those of the starting materials, demonstrating the formation of coordination compounds. It is found that mbH ligand and Ni(II), Cu(II), Co(II), Zn(II) complexes have monoclinic structure, while Mn(II) complex has orthorhombic structure. The crystal structures of similar type of samples were reported as monoclinic and orthorhombic.[27,39-41] Moreover, with help of the diffraction data, the mean crystallite sizes of the complexes, *D* were determined according to the Scherrer equation ($D = 0.9\lambda / (\beta \cos\theta)$, where λ is X–ray wavelength (1.5406 Å), θ is Bragg diffraction angle, β is the full width at half maximum of the diffraction peak).[42,43] The average crystallite sizes of the all samples were found to be ~ 38–75 nm and the values are given in Table 5.

Insert Figure 5., Table 3., Table 4., Table 5.

3.6. Cyclic voltammograms

Cyclic voltammograms of the ligand and its complexes were run in DMF and CH_3CN solutions at room temperature using Bu_4NBF_4 as supporting electrolyte at 293 K. All

potentials quoted refer to measurements run at a scan rates (v) of 200, 250 and 500 mVs^{-1} and against an internal ferrocene–ferrocenium standard, unless otherwise stated. In order to investigate the effect of the ligand concentration, the electrochemical studies were performed in the 1×10^{-3} and 1×10^{-4} M solutions, of the ligand and its complexes. The voltammograms were recorded in the range from – 2.0 to 2.0 V vs Ag^{+}/AgCl. The electrochemical data of the ligand and its complexes are summarised in Tables 6 and 7.

Insert Table 6., Table 7.

All complexes show strong cathodic peaks in the range from – 0.5 to 1.0 V. The complexes have two anodic peaks in the 1.4 – 2.0 V range. The anodic and cathodic peaks are irreversible. The complexes show the irreversible cathodic peak potentials in the 1.0 – 1.4 V range.

The $[Co(mb)(H_2O)_2].8H_2O$ complex shows the reversible process (Ipa:Ipc = 1.0) in the 1×10^{-3} M DMF and CH_3CN solutions at the 250 and 500 mV s^{-1} scan rates. Their potential ranges change from 0.35 V to 1.28 V (Epc) and 0.35 V to 1.34 V (Epa). The $[Cu(mb)(H_2O)_2].3H_2O$ complex show the irreversible process (Ipa:Ipc $\neq$ 1.0) in the 1×10^{-3} M CH_3CN solution at the 250 and 500 mV s^{-1} scan rate. Their potential ranges change from -0.25 V to 1.60 V (Epc) and 0.21 V to 1.54 V (Epa). At the 250 mV s^{-1} scan rate, the $[Cu(mb)(H_2O)_2].3H_2O$ complex shows the reversible process (Ipa:Ipc $\approx$ 1.0) at the 1.60 V (Epc) and 1.54 V (Epa). All process at other potentials are irreversible in the 1×10^{-3} M, CH_3CN and DMF solutions. The electrochemical curves of the $[Co(mb)(H_2O)_2].8H_2O$, $[Cu(mb)(H_2O)_2].3H_2O$, $[Mn(mb)(H_2O)_2].4H_2O$, $[Ni(mb)(H_2O)_2].3H_2O$ and $[Zn(mb)(H_2O)_2].H_2O$ complexes at 200, 250 and 500 mV s^{-1} scan rates in the 1×10^{-3} M, DMF solutions are shown in Figures 6a-e.

Insert Figure 6a-e.

The $[Mn(mb)(H_2O)_2].4H_2O$ complex shows the reversible process (Ipa: Ipc = 1.0) in the 1×10^{-4} M CH_3CN solution at the 500 mV s^{-1} scan rates. Their potential ranges change from – 1.35 V to – 0.76 V (*Epc*) and – 1.35 V to 0.61 V (*Epa*). The $[Mn(mb)(H_2O)_2].4H_2O$ complex show the irreversible process (Ipa : Ipc $\neq$ 1.0) in the 1

× 10^{-4} M DMF solution at the 250 and 500 mV s^{-1} scan rate. All process at other potentials are irreversible in the 1 × 10^{-4} M, CH_3CN and DMF solutions.

The Zn(II) complex of the azo-Schiff base ligand mbH shows the reversible process at the – 0.70 V (E_{pc}) and – 0.73 V (E_{pa}) potentials at the 500 mVs^{-1} scan rate in the 1 × 10^{-3} M solution. The $[Zn(mb)_2(H_2O)_2].H_2O$ chelate has reversible process at the -0.70, 1.20 V (E_{pc}) and -0.73, -0.20 and 0.98 V (E_{pa}) potentials at the 500 mVs^{-1} scan rates in the 1 × 10^{-3} M solution. The $[Zn(mb)(H_2O)_2].H_2O$ complex show the irreversible process (Ipa : Ipc ≠1.0) in the 1 × 10^{-3} M and 1 × 10^{-4} M CH_3CN solution at the 250 and 500 mV s^{-1} scan rate. The electrochemical curves of the $[Co(mb)(H_2O)_2].8H_2O$, $[Cu(mb)(H_2O)_2].3H_2O$, $[Mn(mb)(H_2O)_2].4H_2O$, $[Ni(mb)(H_2O)_2].3H_2O$ and $[Zn(mb)(H_2O)_2].H_2O$ complexes at 200, 250 and 500 mV s^{-1} scan rates in the 1 × 10^{-3}M, CH_3CN solutions are shown in Figures 7a-e.

Insert Figures 7a-e.

As the ligand have electron donating benzyloxy group, the cathodic and anodic peak potentials were shifted to the negative regions. However, the ligand has a nitro group. As nitro group has the electron accepting property, the redox potentials in the metal complexes shifted to the positive regions due to the $–NO_2$ groups in the complexes, the reduction and oxidation potentials were shifted to the higher positive regions.[44] The quinoid process would involve self–protonation reactions where the benzyloxy group act as proton donors. Oxidation–reduction peaks of the ligands at the different scan rates shifted to the lower or higher potentials.[40] This process is shown below:

$$M^{II} + e^- \leftrightarrow M^{I}$$

The mbH ligand shown the quinoid forms (Figure 8).

Insert Figure 8.

3.7. Genotoxicity

Azo-azomethine (mbH) ligand was mutagenic on *S. Typhimurium* TA98 strain but not mutagen on *S. Typhimurium* TA100 strain in the presence and absence of S9 mix (Table 8). In addition, mutagenic activity of (mbH) ligand on the TA98 strain increased with

the increasing dose in the presence and absence of S9 mix (Figure 9, r = 0.95924; Figure 10, r = 0.96762).

Insert Figures 9., 10., Table 8.

Similarly, Cu(II), Ni(II) and Zn(II) metal complexes of (mbH) ligand [(4)[$Cu(mb)_2(H_2O)_2$].$3H_2O$, (2)[$Ni(mb)_2(H_2O)_2$].$3H_2O$ and (6)[$Zn(mb)_2(H_2O)_2$].H_2O] were also mutagenic on *S. Typhimurium* TA98 strain but not mutagen on *S. Typhimurium* TA100 strain in the absence or presence of S9 mix. In addition, mutagenic activity of (2)[$Ni(mb)_2(H_2O)_2$].$3H_2O$ on the *S. Typhimurium* TA98 strain increased with the increasing dose in the presence or absence of S9 mix (Figure 9, r = 0.98882; Figure 10, r = 0.95068) and mutagenic activity of (6)[$Zn(mb)_2(H_2O)_2$].H_2O on the *S. Typhimurium* TA98 strain increased with the increasing dose in the presence of S9 mix (Figure 10, r = 0.97699).

Co(II) and Mn(II) metal complexes of (mbH) ligand [(5)[$Co(mb)_2(H_2O)_2$].$8H_2O$, (3)[$Mn(mb)_2(H_2O)_2$].$4H_2O$] were strong mutagenic activity on *S. Typhimurium* TA98 strain but weak mutagenic activity on *S. Typhimurium* TA100 strain in the absence or presence of S9 mix. Besides, mutagenic activity of (5)[$Co(mb)_2(H_2O)_2$].$8H_2O$ and (3)[$Mn(mb)_2(H_2O)_2$].$4H_2O$ on the *S. Typhimurium* TA98 strain increased with the increasing dose in the absence os S9 mix (Figure 9, r = 0.99735; Figure 10, r = 0.9768).

4. Conclusion

In this work an azo chromophore group containing Schiff base ligand, 2-[(E)-{[4-(benzyloxy)phenyl]imino}methyl]-4-[(E)-(4-nitrophenyl)diazenyl]phenol derived from 2-hydroxy-5-[(4-nitrophenyl)diazenyl]benzaldehyde with 4-benzyloxyaniline hydrochloride in ethyl alcohol and its some transition metal complexes have been prepared. The analytical data and the spectroscopic studies suggested that the complexes had general formula of [$M(mb)_2(H_2O)_2$].nH_2O, where M is manganese(II), cobalt(II), nickel(II), copper(II) or zinc(II). According to the UV–Vis. and IR data of the nitrophenylazo linked Schiff base ligand, mbH was coordinated to the metal ion through the azomethine nitrogen (–CH=N–) and phenolic oxygen atom. From the XRD results,

it is found that the mbH ligand and Ni(II), Cu(II), Co(II), Zn(II) complexes have monoclinic structure, while Mn(II) complex has orthorhombic structure. In the electrochemical studies of the ligand and its metal chelates, the reversible and irreversible redox processes were shown. Based on the above results, the structure of the coordination compounds under investigation can be formulated as Figure 2.

According to data obtained from salmonella/microsome test, the mbH ligand and its five transition metal complexes tested and their metabolites induced frameshift mutation (TA98). Generally, the effect of the mbH azo-azomethine ligand and its complexes on TA98 strain was greater than on TA100 strain.

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