

Turkish Journal of Chemistry

http://journals.tubitak.gov.tr/chem/

# Synthesis, spectroscopic characterization, and genotoxicity of a new group of azo-oxime metal chelates

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<b>Received:</b> 29.10.2012	٠	Accepted: 21.03.2013	٠	<b>Published Online:</b> 10.06.2013	٠	Printed: 08.07.2013
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Abstract: A new azo-oxime ligand, 2-[(E)-(hydroxyimino)methyl]-4-[(E)-(4-nitrophenyl)diazenyl]phenol, (pxoxH<sub>2</sub>) (2), was prepared by condensation of hydroxylamminehydrochloride and 2-hydroxy5-[(4-nitrophenyl)diazenyl]benzaldehyde (1) with treatment of a solution of diazonium salt of p-nitroaniline and 2-hydroxybenzaldehyde in ethanol. The 6 coordination compounds,  $[Mn(pxoxH)_2(H_2O)_2].H_2O(3)$ ,  $[Co(pxoxH)_2(H_2O)_2].4H_2O(4)$ ,  $[Ni(pxoxH)_2(H_2O)_2].2H_2O(5)$ ,  $[Cu(pxoxH)_2].H_2O(6)$ ,  $[Zn(pxoxH)_2].6H_2O(7)$ , and  $[Cd(pxoxH)_2(H_2O)_2].H_2O(8)$ , were prepared by reacting Mn(II), Ni(II) Cu(II), Co(II), Zn(II), and Cd(II) ions with the ligand. The structures of the compounds were elucidated from the elemental analysis data and spectroscopic studies. The azo-oxime metal complexes were also characterized by XRD, magnetic moment, molar conductivity, and thermal analyses. Elemental analyses of the chelates suggested that the metal to ligand ratio was 1:2. It was found that the ligand acts as a bidentate bending through the phenolic oxygen and nitrogen atom of the C=N-OH group similar to most oximes. Upon complexation with the ligand, the Cu(II) and Zn(II) ions formed square planar and tetrahedral structures, respectively, and the Mn(II), Ni(II), Co(II), and Cd(II) ions formed octahedral structures with 2 water molecules as axial ligands. Finally, the ligand and its complexes were assessed for their genotoxicity.

Key words: Synthesis, spectroscopy, phenolic oxime, metal complex, X-ray powder diffraction, genotoxicity

## 1. Introduction

Transition metal complexes show good thermal, optical, and chemical properties. Therefore, they are widely used in applications such as optical data storage, photoswitching, nonlinear optics and photochromic materials, dyes, chemical analysis, and pharmaceuticals.<sup>1-6</sup> The synthesis and properties of new transition metal complexes have been widely investigated owing to their possible applications in a variety of fields.

Azo-oximes are a class of compounds possessing both azo and hydroxyimino groups. One of the major characteristics of azo dyes is the presence of one or more azo (-N=N-) groups. These compounds can be regarded as the largest class of industrially synthesized organic dyes due to their versatile application in various fields such as textile dyes, biomedical applications, advanced application in organic synthesis, lasers, liquid crystalline displays, electro-optical devices, and ink-jet printers.<sup>7-9</sup>

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The hydroxyimino groups are good donors and can form stable complexes with transition metal ions. The oximes and their metal complexes have a significant role in the development of inorganic chemistry. They have been widely studied coordination compounds used in a variety of biochemical<sup>10,11</sup> and analytical applications.<sup>12,13</sup> They have also attracted attention due to their possible antimicrobial applications.<sup>14,15</sup> The *ortho*-hydroxy-aryloximes act as excellent bidentate ligands through the nitrogen and oxygen donor atoms. Their metal complexes are well-recognized extractants that find industrial applications in extractive metallurgy and separation science.<sup>16,17</sup>

The assessment of genotoxicity is a mandatory preliminary step in the safety assessment of newly synthesized chemicals including pharmaceuticals, food additives, and industrial substances.  $^{18-20}$  The bacterial Ames test developed by Dr Bruce Ames in 1975<sup>21</sup> is commonly used for possible genotoxic effects of chemical compounds (ability to induce a mutation or cancer). The assay performed in *Salmonella* Typhimurium is less expensive and gives faster results compared to standard tests for carcinogenicity in animals.<sup>22,23</sup> The test strains of *S*. Typhimurium (TA 98 and TA 100) that possess different types of mutations in the histidine operon are used in the test for identifying reverse mutation.

In our previous studies, we investigated the synthesis and characterization of various new monodentate and bidentate ligands.<sup>24–27</sup> In the present study, the synthesis and characterization of Mn(II), Ni(II), Cu(II), Co(II), Zn(II), and Cd(II) complexes of a new azo-oxime ligand (pxoxH<sub>2</sub>) are reported (Figure 1). The X-ray diffraction studies on the manganese(II) and cadmium(II) complexes are described. Both the new oxime ligand linked 4-nitrophenylazo and its metal complexes were also screened for their genotoxicity.



Figure 1. Synthesis reaction of 2-[(E)-(hydroxyimino)methyl]-4-[(E)-(4-nitrophenyl)diazenyl]phenol ligand.

#### 2. Experimental

#### 2.1. Materials

p-Nitroanilin, salicylaldehyde, and hydroxylamminehydrochloride were purchased from Aldrich. MnCl<sub>2</sub>, NiCl<sub>2</sub>.6H<sub>2</sub>O, CuCl<sub>2</sub>.2H<sub>2</sub>O, CoCl<sub>2</sub>.6H<sub>2</sub>O, ZnCl<sub>2</sub>, and CdCl<sub>2</sub> (Merck) were used as purchased with no additional purification. All solvents were reagent grade and used without further purification.

#### 2.2. Physical measurements

The microanalysis of carbon, hydrogen, and nitrogen was performed with a LECO CHNS 932 elemental analyzer. The <sup>1</sup>H NMR spectrum of the azo-oxime ligand was obtained in deuterated DMSO as solvent on a Bruker FT-NMR AC-400 (300 MHz) spectrometer. All chemical shifts were reported in  $\delta$  (ppm) relative to the tetramethylsilane as an internal standard. Infrared spectra (KBr disk) were recorded in the 4000–400 cm<sup>-1</sup> range on a Shimadzu 8300 FT-IR spectrometer. The UV/Vis spectra were obtained on a Shimadzu 160A UV spectrometer. The X-ray powder diffraction analysis was performed by a Philips X'Pert PRO X-ray diffractometer using Cu–K<sub> $\alpha$ </sub> radiation with the wavelength of 0.154 nm at 40 kV and 30 mA. The measurements were obtained by scanning at diffraction angles (2 $\theta$ ) in the range of 10° to 50° at room temperature. The phase identification was performed using X'Pert High Score software using the ICDD-PDF 2 database. Melting points were obtained by the Gouy method using Hg[Co(SCN)<sub>4</sub>] as a calibrant. Molar conductance of the transition metal complexes was determined in DMF at room temperature by using an IQ Scientific Instruments Multimeter. Thermal gravimetric analysis (TGA) measurements were obtained by a PerkinElmer Diamond thermal analyzer.

#### 2.3. Synthesis of 2-hydroxy5-[(4-nitrophenyl)diazenyl]benzaldehyde (px)(1)

The azo-coupled 2-hydroxybenzaldehyde was synthesized using the known coupling methods. <sup>28</sup> 2-Hydroxybenzaldehyde (1.22 g, 10 mmol) was dissolved in distilled water (30 mL) containing 0.7 g (8.8 mmol) of sodium hydroxide and 40 mmol of sodium carbonate over 30 min at 0 °C. The resulting solution was added slowly to the solution of diazonium chloride of 1.38 g (10 mmol) of *p*-nitroanilin in water at 0–5 °C. The reaction mixture was stirred for 1 h at 0 °C and then allowed to warm slowly to room temperature. The product was collected by filtration and washed with 100 mL of NaCl solution (10%) under vacuum. The obtained yellowish orange solid was dried under vacuum at 80 °C overnight.

## 2.4. Synthesis of 2-[(E)-(hydroxyimino)methyl]-4-[(E)-(4-nitrophenyll)diazenyl]phenol (pxoxH<sub>2</sub>) (2)

The azo-oxime compound was prepared according to the following method. NH<sub>2</sub>OH.HCl (0.111 g, 16 mmol) and 2-hydroxy-5-[(*E*)-phenyldiazenyl] benzaldehyde (0.254 g, 10 mmol) were dissolved in 30 mL of EtOH with NaOH (0.2 g, 50 mmol) in EtOH. The solution was refluxed for 1 h and then left at room temperature. After cooling, the azo-oxime ligand was obtained as orange crystals. The crystals were filtered, washed with 20 mL of cold EtOH, and then dried under vacuum at 80 °C overnight. Yield: 2.10 g (73%). Mp: 226–227 °C. Elemental analysis for C<sub>13</sub>H<sub>10</sub>N<sub>4</sub>O<sub>4</sub> (286.24 g/mol): Calcld. (%): C, 54.56; H, 3.50; N, 19.58. Found (%): C, 54.30; H, 3.50; N, 19.26. Selected IR bands (KBr, cm<sup>-1</sup>): 3371 (O-H), 3040 (Ar-C-H), 1619 (C=N), 1516 (C-C), 1489 (N=N), 1104 (N-O). <sup>13</sup>C NMR ( $\delta$ ) 160.5, 155.8, 148.5, 146.7, 146.0, 128, 126, 124.2, 123.4, 120.6, 117.5.

## 2.5. Synthesis of $[Mn(pxoxH)_2(H_2O)_2]$ .H<sub>2</sub>O (3)

A solution of  $MnCl_2$  (0.010 g, 0.07 mmol) in 5 mL of hot EtOH was added to a solution of  $pxoxH_2$  (0.040 g, 0.14 mmol) in 10 mL of hot MeOH. After the addition, the pH of the solution fell to 3.5 and was adjusted with 0.1 N NaOH in EtOH to 4.5–5.0 where the precipitation of the complex started. The mixture was then heated in a water bath for another 2 h to complete the precipitation. The dark red complex obtained was filtered,

washed with cold EtOH, and dried under vacuum. Yield: 0.041 g (50%). Mp: 238–239 °C. Elemental analyses for C<sub>26</sub>H<sub>22</sub>N<sub>8</sub>MnO<sub>10</sub> (663.44 g/mol): Calcld. (%): C, 47.07; H, 3.34; N, 16.89. Found (%): C, 47.87; H, 3.70; N, 16.49. Selected IR bands (KBr, cm<sup>-1</sup>): 3586 and 3370 (O-H), 3103 (Ar-C-H), 1611 (C=N), 1517 (C-C), 1489 (N=N), 1104 (N-O), 860 (M-OH<sub>2</sub>), 508 (Mn-O), 480 (Mn-N).  $\mu_{eff}$  (BM): 5.05. Ao (S cm<sup>2</sup> mol<sup>-1</sup>): 25.06.

## 2.6. Synthesis of $[Co(pxoxH)_2(H_2O)_2].4H_2O$ (4)

A solution of CoCl<sub>2</sub>.6H<sub>2</sub>O (0.029 g, 0.12 mmol) in 20 mL of MeOH was added to a magnetically stirred 15-mL MeOH solution containing the ligand (0.07 g, 0.24 mmol) at 70 °C. The obtained solution was left at room temperature. The cobalt(II) complex was obtained as dark red precipitates. The brown product was filtered, washed with EtOH, and then dried under vacuum. Yield: 0.088 g (60%). Mp: 234–235 °C. Elemental analyses for C<sub>26</sub>H<sub>30</sub>N<sub>8</sub>CoO<sub>14</sub> (739.50 g/mol): Calcld. (%): C, 42.20; H, 4.08; N, 15.15. Found (%): C, 42.30; H, 3.82; N, 15.19. Selected IR bands (KBr, cm<sup>-1</sup>): 3370 (O-H), 3072 (Ar-C-H), 1612 (C=N), 1516 (C-C), 1488 (N=N), 1103 (N-O), 854 (M-OH<sub>2</sub>), 501 (Co-O), 476 (Co-N).  $\mu_{eff}$  (BM): 3.73. Ao (S cm<sup>2</sup> mol<sup>-1</sup>): 12.75.

## 2.7. Synthesis of $[Ni(pxoxH)_2(H_2O)_2].2H_2O$ (5)

A methanolic solution (30 mL) of NiCl<sub>2</sub>.6H<sub>2</sub>O (0.042 g, 0.174 mmol) was added to 50 mL of a clear solution of pxoxH<sub>2</sub> (0.1 g, 0.35 mmol). The resulting mixture was refluxed for 1 h in a water bath. The volume was reduced by half via slow evaporation. After cooling, the precipitated reddish-brown crystals were filtered, washed several times with EtOH and MeOH, and dried under vacuum. Yield: 0.121 g (58%). Mp: 258–259 °C. Elemental analysis for C<sub>26</sub>H<sub>26</sub>N<sub>8</sub>NiO<sub>12</sub> (703.23 g/mol): Calcld. (%): C, 44.40; H, 3.72; N, 15.93. Found (%): C, 44.42; H, 3.67; N, 15.80. Selected IR (KBr, cm<sup>-1</sup>): 3367 (O-H), 3090 (Ar-C-H), 1609 (C=N), 1510 (C-C), 1488 (N=N), 1103 (N-O), 854 (M-OH<sub>2</sub>), 503 (Ni-O), 475 (Ni-N).  $\mu_{eff}$  (BM): 2.62. Ao (S cm<sup>2</sup> mol<sup>-1</sup>): 22.20.

## 2.8. Synthesis of $[Cu(pxoxH)_2]$ .H<sub>2</sub>O (6)

Copper(II)chloride hexahydrate (0.024 g, 0.174 mmol) dissolved in MeOH (15 mL) was added to a hot solution of the azo-oxime ligand (0.1 g, 0.35 mmol)) dissolved in MeOH (20 mL). The resulting solution was stirred and heated on a hot plate at 70–80 °C for 1 h. The volume of the obtained solution was reduced to one-half by evaporation. One day later, the brown solid of the complex formed was filtered, the solid was washed with cold EtOH, and finally it was dried under vacuum. Yield: 0.1159 g (55%). Mp: >300 °C. Elemental analysis for  $C_{26}H_{20}N_8CuO_9$  (657.04 g/mol): Calcld. (%): C, 47.53; H, 3.07; N, 17.05. Found (%): C, 47.34; H, 2.99; N, 17.87. Selected IR bands (KBr, cm<sup>-1</sup>): 3399 and 3242 (O-H), 3060 (Ar-C-H), 1613 (C=N), 1522 (C-C), 1478 (N=N), 1110 (N-O), 498 (Cu-O), 480 (Cu-N).  $\mu_{eff}$  (BM): 1.89. Ao (S cm<sup>2</sup> mol<sup>-1</sup>): 13.54.

## 2.9. Synthesis of $[Zn(pxoxH)_2].6H_2O$ (7)

Zinc(II) chloride (0.0167 g, 0.122 mmol) was dissolved in 10 mL of EtOH. To this solution, a 30 mL of MeOH solution containing  $pxoxH_2$  (2) (0.07 g, 0.25 mmol) was added. The mixture was stirred for 2 h at room temperature and refluxed for another 2 h. The precipitated black compound was filtered, washed with cold EtOH, and dried under vacuum. Yield: 0.0885 g (60%). Mp: 201–202 °C. Elemental analyses for  $C_{26}H_{30}N_8ZnO_{14}$  (745.96 g/mol): Calcld. (%): C, 41.86; H, 4.05; N, 15.02. Found (%): C, 41.35; H, 3.65; N,

14.53. Selected IR bands (KBr, cm<sup>-1</sup>): 3475 and 3368 (O-H), 3103 (Ar-C-H), 1612 (C=N), 1517 (C-C), 1489 (N=N), 1104 (N-O), 510 (Zn-O), 482 (Zn-N).  $\mu_{eff}$  (BM): Dia. Ao (S cm<sup>2</sup> mol<sup>-1</sup>): 16.13.

## 2.10. Synthesis of $[Cd(pxoxH)_2(H_2O)_2]$ .H<sub>2</sub>O (8)

CdCl<sub>2</sub> (0.0134 g, 0.07 mmol) in 10 mL of MeOH solution was slowly added to a constantly stirring solution of pxoxH<sub>2</sub> (**2**) (0.042 g (0.15 mmol) in 20 mL of MeOH. The color of the solution turned dark brown. The reaction mixture was kept stirring at 70–80 °C until the precipitation was completed. Then it was brought to room temperature and the brown crystalline complex was precipitated. The product was filtered, washed with water and EtOH, and finally dried under vacuum. The obtained compound was soluble in DMF and DMSO. Yield: 0.045 g (47%). Mp: 248–249 °C. Elemental analyses for C<sub>26</sub>H<sub>20</sub>N<sub>8</sub>CdO<sub>9</sub> (702.90 g/mol): Calcld. (%): C, 44.42; H, 2.86; N, 15.94. Found (%): C, 44.06; H, 3.12; N, 15.12. Selected IR bands (KBr, cm<sup>-1</sup>): 3453 and 3366 (O-H), 3073 (Ar-C-H), 1612 (C=N), 1515 (C-C), 1488 (-N=N-), 1106 (N-O), 853 (M-OH<sub>2</sub>), 502 (Cd-O), 474 (Cd-N).  $\mu_{eff}$  (BM): Dia.  $\Lambda o$  (S cm<sup>2</sup> mol<sup>-1</sup>): 17.63.

## 2.11. Ames test

The reagents including medium, buffers, and the S9-mix used in the Ames test were prepared as described in the study by Kayraldiz et al.<sup>29</sup> Histidine-deficient S. Typhimurium strains TA98 and TA100 were provided by JL Swezey (Microbial Genomics and Bioprocessing Research Unit, North University, Illinois, USA). Dimethyl sulfoxide (DMSO) suspensions of 2-aminofluerene (2-AF) and 4-nitro-o-phenylenediamine (NPD) as well as sodium azide (dissolved in distilled water) were used as positive controls. The test reagents were suspended in DMSO (px, pxoxH<sub>2</sub>, [Zn(pxoxH)<sub>2</sub>].6H<sub>2</sub>O, [Co(pxoxH)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>].4H<sub>2</sub>O, [Mn(pxoxH)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>].H<sub>2</sub>O, [Cd(pxoxH)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>].H<sub>2</sub>O) with the final concentrations of 1.25, 0.75, 0.50, and 0.25 mg/plate for the mutagenicity test. The final concentrations, i.e. 0.62, 0.49, 0.37, 0.24, and 0.12 mg/plate of [Ni(pxoxH)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>].2H<sub>2</sub>O and 0.31, 0.24, 0.18, 0.12, and 0.06 mg/plate of [Cu(pxoxH)<sub>2</sub>].H<sub>2</sub>O, suspended in DMSO were used for the mutagenicity test.

The standard procedure for the plate-incorporation assay was carried out using TA98 and TA100 strains for determination of the frame-shift mutations and base-pair substitution mutations, respectively. Using both of the strains, the Ames test was performed with metabolic activation (+S9 mix) to obtain a first approximation of mammalian metabolism, and without metabolic activation (-S9 mix). The assay was carried out according to Maron and Ames.<sup>30</sup> TA98 and TA100 strains were exposed to a range of concentrations of each test compound in a soft agar overlay. The number of revertants observed in each concentration of the test substances and the spontaneous control was scored. The observed differences between the test compounds and control were analyzed by t-test (Minitab V14). Regression and correlation tests were used for dose–response relationships.

#### 3. Results and discussion

## 3.1. Synthesis and solubility of the metal chelates

The azo-oxime ligand 2-[(E)-(hydroxyimino)methyl]-4-[(E)-(4-nitrophenyll)diazenyl]phenol (pxoxH<sub>2</sub>) (**2**) was prepared by reacting 2-hydroxy-5-[(4-nitrophenyl)diazenyl]benzaldehyde (**1**) with hydroxylamminehydrochloride (Figure 1). The new azo-oxime ligand, 2-[(E)-(hydroxyimino)methyl]-4-[(E)-(4-nitrophenyll)diazenyl]phenol (pxoxH<sub>2</sub>), formed mononuclear complexes (Figure 2) with Ni(II), Co(II), Cu(II), Zn(II), and Cd(II) as follows:  $2 \text{ pxoxH}_2+ \text{ MX}_2 \rightarrow [\text{M}(\text{pxoxH})_2.(\text{H}_2\text{ O})_m].\text{nH}_2\text{ O} + 2\text{HX}$ 

pxoxH<sub>2</sub>: 2-[(E)-(hydroxyimino)methyl]-4-[(E)-(4-nitrophenyll)diazenyl]phenol.

$$M = Mn(II), m = 2, n = 1); Ni(II), m = 2, n = 2); Cu(II), m = 0, n = 2);$$

Co(II), m = 2, n = 4); Cd(II), m = 2, n = 2); Zn(II), m = 0, n = 6) and X = Cl.



$$\begin{split} M &= Mn(II)(OH_2)_2(n=1); Ni(II)(OH_2)_2)(n=2); Cu(II) (n=2); Co(II)(OH_2)_2 (n=4); \\ Cd(II)(OH_2)_2(n=2); Zn(II)(n=6). \end{split}$$
 Figure 2. The proposed structure of the metal complexes.

The structures of the synthesized compounds were elucidated using a number of analytical methods and spectroscopic techniques. The level of impurity in the product was checked with thin layer chromatography (TLC). All of the metal complexes,  $[Mn(pxoxH)_2(H_2O)_2].H_2O$  (3),  $[Co(pxoxH)_2(H_2O)_2].4H_2O$  (4),  $[Ni(pxoxH)_2(H_2O)_2].2H_2O$  (5),  $[Cu(pxoxH)_2].H_2O$  (6),  $[Zn(pxoxH)_2].6H_2O$  (7), and  $[Cd(pxoxH)_2(H_2O)_2].$ 

 $H_2O(8)$ , are stable in air, soluble in DMSO, and insoluble in common organic solvents. The single crystals of the compounds could not be isolated from any organic solution; thus no definite structures could be described. However, the analytical and spectroscopic data enable us to predict possible structures as shown in Figures 1 and 2.

The results of the elemental analyses of the azo-oxime ligand and its metal chelates are in good agreement with the theoretical expectations. The elemental analyses of the coordination compounds indicate that the metal–ligand ratios are 1:2 in all the metal complexes. The molar conductivities of the metal chelates in DMF at 25 °C are in the range of 12.75–25.06  $\Omega^{-1}$  cm<sup>2</sup> mol<sup>-1</sup>, indicating nonelectrolytes.<sup>31</sup>

## **3.2.** <sup>1</sup>H and <sup>13</sup>C NMR spectra

The <sup>1</sup>H NMR data were obtained from the pxoxH<sub>2</sub> compound in DMSO-d<sub>6</sub> and peak assignments are provided in Table 1. The <sup>1</sup>H NMR spectrum of pxoxH<sub>2</sub> (**2**) in DMSO-d<sub>6</sub> exhibited 2 singlet peaks at 11.55 and 11.15 ppm, which were attributed to -OH (N<sub>9</sub>-) and -OH (C<sub>7</sub>-), respectively. The -OH (C<sub>10</sub>-) and -OH (C<sub>7</sub>-) signals disappeared in D<sub>2</sub>O, indicating an acidic nature. The <sup>1</sup>H NMR spectrum of the azo-oxime ligand is shown in Figure 3. The spectrum of the pxoxH<sub>2</sub> (**2**) showed a singlet peak at 10.38 ppm due to the presence of hydrogen of the azomethine group (C<sub>8</sub>H=N). The peaks observed in the range of 7.14–7.25 and 7.93–7.94 ppm in <sup>1</sup>H NMR spectrum of pxoxH<sub>2</sub> (**2**) were attributed to the aromatic protons.<sup>26–28</sup> The <sup>13</sup>C NMR data were obtained from the pxoxH<sub>2</sub> compound in DMSO-d<sub>6</sub>. The <sup>13</sup>C NMR spectrum displayed characteristic signals at 160.5

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and 155.8 ppm due to the Ar-C-OH and -C-N=N of the ligand, respectively. The peaks at 148.5 and 146.7 ppm were attributed to azomethine and C-NO<sub>2</sub> carbons of the ligand, respectively. On the other hand, the spectrum of the ligand showed peaks in the region  $\delta$  117.5–146.0 ppm, due to the presence of aromatic carbons.

$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	OH 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9
Chemical shifts, $\delta_{TMS}$ (ppm)	$Assignments^{a}$
11.55	[s, 1H] (10)
11.15	[s, 1H] (7)
10.38	[s, 1H](8)
7.94	[d, 2H] (15, 17)
7.93	[d, 2H] (14, 18)
7.25	[s, 1H] (3)
7.22	[d, 1H] (5)

[d, 1H] (6)

Table 1. <sup>1</sup>H NMR data of the ligand  $pxoxH_2$  in DMSO-d<sub>6</sub>.



7.14



Figure 3. <sup>1</sup>H NMR spectrum of the ligand (i: <sup>1</sup>H NMR spectrum; ii: deuterated-NMR).

#### 3.3. Magnetic and electronic spectral studies

The UV/Vis spectra of phenylazo-linked azo-oxime ligand and its metal complexes were measured in DMF at room temperature, and the absorbance maxima are presented in Table 2. The pxoxH<sub>2</sub> ligand (2) was characterized mainly by 4 absorption bands in the region of 36,363-24,691 cm<sup>-1</sup>. The bands at 24,691 and

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28,571 cm<sup>-1</sup> were assigned to  $n \to \pi^*$  transitions in the azo and hydroxyimine groups in the phenyl azolinked hydroxyimino ligand, pxoxH<sub>2</sub>. The third and fourth bands, located at 33,333 and 36,363 cm<sup>-1</sup>, can be assigned to the moderate energy  $\pi \to \pi^*$  transition of the -N=N- and -CH=N- groups or aromatic rings. The UV-visible spectrum of the pxoxH<sub>2</sub> azo-oxime ligand in DMF solution is shown in Figure 4.

No. Compound		Absorbance, $\lambda_{max}$ (	Band	
		$\pi \to \pi^*,  n \to \pi^* a$	nd charge	assignment
		transfer transitions	$d \rightarrow d$ transitions	assignment
1		315, 360, (31,746),		
L	px	(27,777)	_	—
		275, 300, 350, 405,		
2	$pxoxH_2$	(36,363), (33,333),	_	_
		(28,571), (24,691)		
				$^{6}\mathrm{A}_{1g} \rightarrow ^{4}\mathrm{T}_{1g}$
		275, 300, 350, 400,	470, 515, 590, 630,	$^{6}A_{1g} \rightarrow {}^{4}Eg$
3	$[\operatorname{Mn}(\operatorname{pxox}H)_2(H_2O)_2].H_2O$	(36, 363), (33, 333),	(21,276), (19,417),	$^{6}\mathrm{A}_{1g} \rightarrow ^{4}\mathrm{Eg}$
		(28,571), (25,000)	(16,949), (15,872)	$^{6}A_{1g} \rightarrow {}^{4}T_{1g}$
		275, 300, 350, 405, 445,	485, 535, 585,	${}^{4}\mathrm{T}_{1g} \rightarrow {}^{4}\mathrm{T}_{1g}$
4	$[Co(pxoxH)_2(H_2O)_2].4H_2O$	(36, 363), (33, 333),	(20,619), (18,691),	${}^{4}\mathrm{T}_{1g} \rightarrow {}^{4}\mathrm{A}_{2g}$
		(28,571), (24,691), (22,471)	(17,094)	${}^{4}\mathrm{T}_{1g} \rightarrow {}^{4}\mathrm{T}_{2g}$
				$^{3}A_{2g} \rightarrow ~^{3}T_{1g}$
5	$[Ni(pxoxH)_2(H_2O)_2].2H_2O$	290, 345, 360, (34, 482),	420, 485, 550,	$^{3}A_{2g} \rightarrow ~^{3}T_{1g}$
		(28,985), (27,777)	(18,181)	$^{3}A_{2g} \rightarrow ~^{3}T_{2g}$
G		290, 345, 420, (34, 482),	550, 590, (18, 181),	$^{2}\mathrm{B}_{1g} \rightarrow ^{2}\mathrm{Eg}$
0	$[Cu(pxoxn)_2].n_2O$	(28,985), (23,809)	(16,949)	$^{2}\mathrm{B}_{1g} \rightarrow ^{2}\mathrm{B}_{2g}$
		275, 300, 350, 405, 470,		
7	$[Zn(pxoxH)_2].6H_2O$	(36, 363), (33, 333),	-	-
		(28,571), (24,691), (21,276)		
		275, 300, 350,		
8	$[Cd(pxoxH)_2(H_2O)_2].H_2O$	485, (36, 363), (33, 333),	-	-
		(28,571), (24,691)		

Table 2. Electronic spectral data for azo-oxime ligand and its metal complexes in DMF.

The UV/Vis spectra can often provide quick and reliable information about the ligand arrangement in metal complexes. The manganese(II) complex (**3**) displayed weak absorption bands in the range of 15,872–21,276 cm<sup>-1</sup>. In the high-spin octahedrally coordinated Mn(II) complex, the lowest configuration  $(t_{2g})^3$  (eg)<sup>2</sup> gives rise to ground state  ${}^{6}A_{1g}$ . Since this is the only sextet level present, all the absorption bands must therefore be spin-forbidden transitions. The UV/Vis spectrum of the Mn(II) complex displayed weak absorption bands at 15,872, 16,949, 19,417, and 21,276 cm<sup>-1</sup>, which were characteristic of the octahedral geometry of this complex. These bands may be assigned to  ${}^{6}A_{1g} \rightarrow {}^{4}T_{1g}$ ,  ${}^{6}A_{1g} \rightarrow {}^{4}E_{g}$ ,  ${}^{6}A_{1g} \rightarrow {}^{4}T_{1g}$ , and  ${}^{6}A_{1g} \rightarrow {}^{4}E_{g}$  transitions, respectively.<sup>32</sup> The magnetic moment value for Mn(II) complex was 5.05 B.M., which is in the expected range of octahedral geometry around the central metal ion.<sup>33,34</sup> The electronic spectrum of the [Ni(pxoxH)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>].2H<sub>2</sub>O (**5**) complex showed 2 well-defined bands assignable to  ${}^{3}A_{2g} \rightarrow {}^{3}T_{2g}$  and  ${}^{3}A_{2g} \rightarrow {}^{3}T_{1g}$  transitions in an octahedral structure, respectively. The third d–d transition band, which may be obscured by the more intense charge transfer band, was calculated theoretically and found to be at 23,809 cm<sup>-1</sup>.<sup>35</sup> The observed magnetic moment of the Ni(II) complex was 2.62 B.M. The UV/Vis spectrum of

copper(II) complex showed 2 absorption bands at 16,949 and 18,181 cm<sup>-1</sup> associated to  ${}^{2}B_{1g} \rightarrow {}^{2}B_{2g}$  and  ${}^{2}B_{1g} \rightarrow {}^{2}E_{g}$  transitions, respectively, indicating the possibility of having a square planar geometry of the metal complex.<sup>36</sup> The spectrum of Cu(II) complex in dimethylformamide solution is given in Figure 5. The magnetic moment of Cu(II) complex was 1.89 B.M., indicating the presence of one unpaired electron.<sup>37</sup>



Figure 4. UV-visible spectrum of the  $pxoxH_2$  ligand in DMF.

Figure 5. UV-visible spectrum of  $[Cu(pxoxH)_2]$ .H<sub>2</sub>O complex in DMF.

The cobalt(II) complex exhibited 3 bands, which are expected from an octahedral structure.<sup>35</sup> The UV/Vis spectrum of the Co(II) complex displayed 2 well-defined spectral bands at 17,094 cm<sup>-1</sup> and 20,619 cm<sup>-1</sup> along with a shoulder at 18,691 cm<sup>-1</sup>. These spectral bands may have corresponded to  ${}^{4}T_{1g} \rightarrow {}^{4}T_{2g}$ ,  ${}^{4}T_{1g} \rightarrow {}^{4}A_{2g}$ , and  ${}^{4}T_{1g} \rightarrow {}^{4}T_{1g}$  transitions, respectively, which are characteristic of 6-coordinated geometry around cobalt(II). The measured magnetic moment value (3.73 B.M.) of the Co(II) complex was lower than the spin-only value (3.87 B.M.) and also lower than the values reported for the complexes having octahedral structures.<sup>38</sup>

The electronic absorption spectrum of Zn(II) complex showed 5 bands at 21,276, 24,691, 28,571, 33,333, and 36,363 cm<sup>-1</sup>, which were assigned to the charge transfer,  $n \rightarrow \pi^*$  and  $\pi \rightarrow \pi^*$  transitions, respectively. The bands at 24,691, 28,571, 33,333, and 36,363 cm<sup>-1</sup> in the UV-visible spectrum of the [Cd(pxoxH)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>].H<sub>2</sub>O complex (8) can be related to  $n \rightarrow \pi^*$  (-N=N-),  $n \rightarrow \pi^*$  (-CH=N-),  $\pi \rightarrow \pi^*$  (-N=N-), and  $\pi \rightarrow \pi^*$  (-CH=N-) or aromatic ring transitions, respectively. The spectroscopic data are given in Table 2.

#### 3.4. Infrared spectra

The IR measurements of the pxoxH<sub>2</sub> azo-oxime ligand (2) and its metal complexes were obtained in the range 4000–400 cm<sup>-1</sup>. The characteristic IR bands of the pxoxH<sub>2</sub> ligand and its metal chelates were explained in the experimental section. Generally, the oxime compounds can be characterized by 3 IR absorption bands at 3700–3200 cm<sup>-1</sup> (O–H str), 1615–1650 cm<sup>-1</sup> (C=N str), and 1100–950 cm<sup>-1</sup> (N–O str).<sup>15</sup>

The strong and broad band appearing at  $3371 \text{ cm}^{-1}$  in the spectrum of the azo-oxime ligand was assigned to the vibrations of the hydroxyl groups belonging to the hydroxylmino (-C=N-OH) and phenolic groups (Ar-OH) in the structure.<sup>39</sup> The vibration spectrum of the bidentate ligand is shown in Figure 6. Although we know that the coordinated water and the lattice water are present in the crystal structure and can give vibrational bands at different regions in the infrared spectrum, the hydroxyl vibration bands coming from the hydroxylmino group and coordinated or latticed water in the structure were observed as combined strong and broad bands between 3586 and 3242 cm<sup>-1</sup>. The infrared spectra of the Mn(II), Ni(II), Co(II), and Zn(II) metal complexes exhibited a broad band at 3586–3242 cm<sup>-1</sup>, which was attributed to v(OH) associated water molecules, while the band observed at approximately 860–853 cm<sup>-1</sup> was assigned to coordinated water molecules.<sup>40</sup>

The absence of a sharp band at around 1657 cm<sup>-1</sup>, which is related to the azo-aldehyde (azo-coupled 2-hydroxybenzaldehyde) (1), supports the proposed structure. However, the band that appeared at 1619 cm<sup>-1</sup> on the spectrum of the ligand belongs to the azomethine moiety. Thus, we can conclude that the proposed structure is in agreement with the spectral data. It is also common knowledge that the  $\nu$  (C=N) stretching vibrations are affected by complexation and are positioned at a frequency significantly different than the frequency of ligand. The coordination of the azo-oxime ligand to the metal center through the nitrogen atom is expected to reduce the electron density in the azomethine link and shift to lower  $\nu$  (C=N) absorption frequency. In the synthesized metal complexes, we observed the  $\nu$  (C=N) stretching shifted to the lower frequencies and appeared between 1613 and 1609 cm<sup>-1</sup>, indicating the coordination of the azomethine nitrogen to the metal ions. The IR spectrum of the copper(II) complex is given in Figure 7.



Figure 6. IR spectrum of the  $pxoxH_2$  ligand (KBr,  $cm^{-1}$ ).

Figure 7. IR spectrum of  $[Cu(pxoxH)_2]$ .H<sub>2</sub>O complex (KBr, cm<sup>-1</sup>).

The stretching vibration bands belonging to  $\nu$  (Ar-C-H),  $\nu$  (C=N),  $\nu$  (C-C),  $\nu$  (N=N), and  $\nu$  (N-O) functional groups appeared in the spectrum of the ligand at 3040, 1619, 1516, 1489, and 1104 cm<sup>-1</sup> frequencies, respectively.

In the metal complexes, the aromatic C-H stretching bands were observed between 3103 and 3072 cm<sup>-1</sup>. The vibration band of the azo chromogen group both in the ligand and the complexes was seen at around 1484 cm<sup>-1</sup>. Although the azo chromogen group has a donor character and can form coordination compounds, the constant value of its vibration both in the ligand and the complexes shows that there is no coordination between the azo chromogen group and the metal ions. On the other hand, there were 2 new bands, which were not seen in the spectrum of the ligand, appearing also in the spectra of the complexes; the first one was the M-O stretching bands seen at 510–498 cm<sup>-1</sup>, which showed that the ligand was coordinated with the metals through the phenolic hydroxyls, and the second weak band was M-N vibrations seen between 482 and 474 cm<sup>-1</sup>. These values are in good agreement with the values reported in the literature.<sup>15,26b</sup>

## 3.5. X-ray powder diffraction analysis

We attempted to prepare single crystals of the ligand and metal complexes from different solvents, but were unsuccessful. However, the crystalline nature of Cd(II) and Mn(II) metal complexes can be readily evidenced from their X-ray powder patterns. The X-ray powder diffractograms of the  $[Cd(pxoxH)_2(H_2O)_2]$ .H<sub>2</sub>O and  $[Mn(pxoxH)_2(H_2O)_2]$ .H<sub>2</sub>O metal complexes were recorded using Cu–K<sub> $\alpha$ </sub> as the source in the 2 $\theta$  range 10–50°. The X-ray powder diffraction patterns of the Cd(II) and Mn(II) complexes are given in Figure 8.



Figure 8. X-ray powder diffraction patterns for the metal complexes; (a)  $[Cd(pxoxH)_2(H_2O)_2]$ .  $H_2O$ , (b)  $[Mn(pxoxH)_2(H_2O)_2]$ .  $H_2O$ .

The observed and calculated diffraction data of the Cd(II) and Mn(II) complexes are given in Tables 3 and 4, respectively. The XRD spectrum shows 13 reflections for Cd(II) complex with maxima at  $2\theta = 25.98^{\circ}$ and d = 3.42 Å, and 8 reflections for Mn(II) complex with maxima at  $2\theta = 25.91^{\circ}$  and d = 3.43 Å. All of the main peaks were indexed and their d-spacing and  $\sin^2\theta$  values were compared with the calculated ones. The comparison of the values reveals that there is good agreement between calculated and observed values of d-spacing and  $\sin^2\theta$ . The parameters of unit cell for each sample were calculated by trial and error methods as follows: the observed values were well fitted with the monoclinic system with the cell parameters, for Cd(II) complex a = 17.20094 Å, b = 7.43588 Å, c = 7.85396 Å,  $\beta = 110.58710^{\circ}$ , and cell volume V = 940.40070Å<sup>3</sup>; for Mn(II) complex a = 6.74792 Å, b = 8.94387 Å, c = 6.29666 Å,  $\beta = 127.46370^{\circ}$ , and cell volume V = 301.63610 Å<sup>3</sup>.

#### 3.6. Thermal gravimetric analysis

The TGA data agree with the formula suggested from the elemental analyses. The thermal stabilities were investigated by TGA with a heating rate of 10 °C min<sup>-1</sup> in N<sub>2</sub> from 50 to 140 °C. The Mn(II), Co(II), Ni(II), Cu(II), Zn(II), and Cd(II) complexes can suffer from the loss of H<sub>2</sub>O in the 50–140 °C range. The complexes contain 1, 4, 2, 1, 6, and 1 moles of water of crystallization per complex molecule, respectively.

#### 3.7. Genotoxicity study

The data obtained from the Ames mutagenicity test system are presented in Table 5. The px aldehyde-linked azo chromogene was weakly mutagenic on S. Typhimurium TA98 strains in the absence of s9 mix. On the

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other hand, the pxoxH<sub>2</sub> ligand (2) was strongly mutagenic on S. Typhimurium TA98 strains with and without s9 mix but it was weakly mutagenic on S. Typhimurium TA100 strains only in the absence of s9 mix. In the absence and presence of s9 mix, mutagenic activity of the pxoxH<sub>2</sub> azo-oxime compound on the TA98 strain was observed to be dose-dependent (r = 0.954, Figure 9, r = 0.920, Figure 10, respectively).

Peak no.	d-sp. (obs.)	d-sp. (cal.)	Rel. int.	$Sin^2\theta$	$\mathrm{Sin}^2\theta$	hkl	
	[Å]	[Å]	(%)	(obs.)	(cal.)	пкі	
1	7.367228	7.352391	34.25	0.01093	0.01098	001	
2	5.928858	5.944844	64.55	0.01688	0.01679	101	
3	4.672563	4.672395	29.78	0.02718	0.02718	201	
4	3.665163	3.662953	23.32	0.04417	0.04422	411	
5	3.425934	3.425633	100	0.05055	0.05056	501	
6	3.32207	3.317855	41.3	0.05377	0.05390	021	
7	3.004449	3.001352	14.31	0.06573	0.06587	502	
8	2.631851	2.632069	31.99	0.08566	0.08565	302	
9	2.432852	2.434248	23.9	0.010025	0.010014	520	
10	2.35744	2.35826	15.21	0.010677	0.010669	702	
11	1.959023	1.959245	10.78	0.015461	0.015458	123	
12	1.903099	1.903204	16.47	0.016383	0.016381	104	
13	1.876439	1.876077	12.75	0.016852	0.016858	602	

Table 3. Observed and calculated XRD data of the [Cd(pxoxH)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>].H<sub>2</sub>O metal complex.

Table 4. Observed and calculated XRD data of the [Mn(pxoxH)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>].H<sub>2</sub>O metal complex.

Pool: no	d-sp. (obs.)	d-sp. (cal.)	Rel. int.	$\mathrm{Sin}^2\theta$	$\mathrm{Sin}^2\theta$	hŀl	
I eak no.	[Å]	[Å]	(%)	(obs.)	(cal.)	11.7.1	
1	5.811424	5.811424	62.33	0.01757	0.01750	200	
2	4.462712	4.461999	13.73	0.02979	0.02980	210	
3	3.435318	3.431299	100	0.05028	0.05040	011	
4	3.328647	3.327184	39.4	0.05355	0.05360	120	
5	2.983147	2.982109	15.38	0.06668	0.06672	220	
6	2.929839	2.929626	41.98	0.06912	0.06913	111	
7	2.770278	2.770098	6.13	0.07732	0.07733	121	
8	2.604771	2.606827	12.23	0.08745	0.08732	021	

The nickel(II) metal chelate of the azo-oxime ligand  $[Ni(pxoxH)_2(H_2O)_2].2H_2O$  was strongly mutagenic on S. Typhimurium TA98 strains in the presence and absence of s9 mix. It was weakly mutagenic on S. Typhimurium TA100 strains in the presence and absence of s9 mix.

While the  $[Zn(pxoxH)_2].6H_2O$  and  $[Co(pxoxH)_2(H_2O)_2].4H_2O$  metal complexes were weakly mutagenic on S. Typhimurium TA98 strain in the presence and absence of s9 mix, they were not mutagenic on S. Typhimurium TA100 strain in the presence and absence of s9 mix.

 $[Cu(pxoxH)_2]$ .H<sub>2</sub>O was not mutagenic on S. Typhimurium TA98 and TA100 strains in the presence and absence of s9 mix.

Tost substances	Cont.	TA 98		TA 100		
Test substances	mg/plt.	- S9	+ S9	- S9	+ S9	
Spontaneous control	-	$12.50 \pm 1.93$	$14.67 \pm 2.12$	$103 \pm 9.57$	$135.17 \pm 6.04$	
NPD		$3510 \pm 247$				
2-AF			$836.2 \pm 45.70$		$3664 \pm 337$	
SA				$743.2 \pm 38.2$		
	1.25	$15.33 \pm 1.05^*$	$22.00 \pm 3.16$	$121.80 \pm 10.3$	$121.8 \pm 10.30$	
	1	$18.83 \pm 2.32^*$	$19.00 \pm 2.37$	$106.00 \pm 6.70$	$105.83 \pm 6.58^{**}$	
Px	0.75	$20.67 \pm 2.76^*$	$20.67 \pm 2.76$	$119.00 \pm 5.20^{*}$	$117.00 \pm 5.34^*$	
	0.50	$15.66 \pm 0.76^*$	$15.66 \pm 0.76$	$106.17 \pm 7.17$	$116.50 \pm 7.04^*$	
	0.25	$20.50 \pm 2.40^*$	$23.50 \pm 2.40^*$	$118.83 \pm 5.50^*$	$118.17 \pm 5.43^*$	
	1.25	$122.83 \pm 6.17^{***}$	$112.33 \pm 8.20^{***}$	$175.20 \pm 23.20^{*}$	$132.00 \pm 13.80$	
	1	$99.33 \pm 9.27^{***}$	$102.50 \pm 9.62^{***}$	$143.20 \pm 20.80$	$138.80 \pm 19.40$	
$pxoxH_2$	0.75	$95.50 \pm 7.46^{***}$	$90.00 \pm 5.38^{***}$	$105.67 \pm 8.97$	$103.67 \pm 9.39^*$	
	0.50	$77.00 \pm 5.26^{***}$	$76.83 \pm 5.19^{***}$	$82.83 \pm 3.08^{***}$	$94.20 \pm 10.40^{**}$	
	0.25	$61.17 \pm 4.65^{***}$	$71.17 \pm 8.92^{***}$	$73.00 \pm 7.87^*$	$70.83 \pm 6.18^{***}$	
	0.62	$72.00 \pm 8.56^{***}$	$65.70 \pm 10.30^{***}$	$300.50 \pm 39.70^{***}$	$271.50 \pm 53.80^{**}$	
	0.49	$77.67 \pm 9.91^{***}$	$80.20 \pm 10.50^{***}$	$166.30 \pm 40.50$	$201.80 \pm 49.30^*$	
$[\mathrm{Ni}(\mathrm{pxoxH})_2(\mathrm{H}_2\mathrm{O})_2].2\mathrm{H}_2\mathrm{O}$	0.37	$57.33 \pm 9.33^{***}$	$65.70 \pm 13.20^*$	$132.20 \pm 19.40$	$131.30 \pm 19.20$	
	0.24	$52.67 \pm 7.58^{***}$	$56.50 \pm 6.73^{***}$	$106.00 \pm 8.40$	$107.50 \pm 8.48^*$	
	0.12	$38.83 \pm 8.90^*$	$42.00 \pm 6.51^{***}$	$97.83 \pm 5.59$	$100.83 \pm 5.04^{***}$	
	1.25	$26.33 \pm 3.40^*$	$26.50 \pm 2.01^{**}$	$123.30 \pm 16.10$	$109.33 \pm 7.52^*$	
	1	$27.33 \pm 2.60^{**}$	$26.50 \pm 2.43^*$	$102.00 \pm 9.74$	$103.33 \pm 8.22^*$	
$[Zn(pxoxH)_2].6H_2O$	0.75	$19.67 \pm 2.04^*$	$22.67 \pm 2.85^*$	$111.83 \pm 7.06$	$105.00 \pm 9.07^*$	
	0.50	$20.33 \pm 1.73^*$	$19.50 \pm 2.19$	$88.33 \pm 3.93^*$	$91.83 \pm 5.89^{***}$	
	0.25	$19.83 \pm 2.50^*$	$20.83 \pm 2.63$	$83.67 \pm 8.96$	$84.33 \pm 8.55^{***}$	
	0.31	$20.33 \pm 3.32$	$15.67 \pm 2.58$	$58.67 \pm 6.49^{***}$	$52.83 \pm 5.80^{***}$	
	0.24	$16.17 \pm 2.76$	$15.17 \pm 2.82$	$47.33 \pm 6.56^{***}$	$49.17 \pm 7.05^{***}$	
$[Cu(pxoxH)_2].H_2O$	0.18	$10.00 \pm 2.13$	$13.33 \pm 3.36$	$36.50 \pm 4.94^{***}$	$33.83 \pm 4.80^{***}$	
	0.12	$8.67 \pm 1.20^{*}$	$7.67 \pm 1.45^{***}$	$29.67 \pm 5.51^{***}$	$28.50 \pm 2.72^{***}$	
	0.06	$7.00 \pm 1.24^{***}$	$7.67 \pm 1.33^{***}$	$37.30 \pm 15.50^{***}$	$37.20 \pm 15.50^{***}$	
	1.25	$29.17 \pm 3.63^{***}$	$26.33 \pm 1.98^{***}$	$138.70 \pm 34.10$	$132.50 \pm 14.50$	
	1	$31.17 \pm 3.46^{***}$	$29.33 \pm 3.65^{**}$	$131.80 \pm 17.70$	$126.00 \pm 14.30$	
$[\mathrm{Co}(\mathrm{pxoxH})_2(\mathrm{H}_2\mathrm{O})_2].4\mathrm{H}_2\mathrm{O}$	0.75	$20.00 \pm 2.44^*$	$24.50 \pm 3.13^*$	$124.70 \pm 12.20$	$110.17 \pm 7.82^*$	
	0.50	$19.67 \pm 3.01$	$18.33 \pm 3.00$	$89.00 \pm 4.02^*$	$98.33 \pm 9.75^*$	
	0.25	$16.67 \pm 1.94$	$18.67 \pm 2.93$	$83.33 \pm 8.92$	$84.33 \pm 8.49^{***}$	
	1.25	$36.00 \pm 3.53^{***}$	$32.67 \pm 4.27^{***}$	$135.30 \pm 10.80^{*}$	$114.17 \pm 9.63$	
	1	$29.67 \pm 3.73^{***}$	$27.17 \pm 2.41^{***}$	$108.70 \pm 14.70$	$103.33 \pm 8.22^*$	
$[\mathrm{Mn}(\mathrm{pxoxH})_2(\mathrm{H}_2\mathrm{O})_2].\mathrm{H}_2\mathrm{O}$	0.75	$29.17 \pm 4.04^{***}$	$26.00 \pm 2.82^*$	$93.80 \pm 10.80$	$93.30 \pm 11.10^*$	
	0.50	$23.83 \pm 1.62^{***}$	$23.83 \pm 1.66^{***}$	$80.00 \pm 6.32^*$	$92.30 \pm 12.20^*$	
	0.25	$19.00 \pm 4.05$	$20.00 \pm 4.09$	$72.67 \pm 9.16^*$	$81.20 \pm 14.50^*$	
	1.25	$196.50 \pm 13.30^{***}$	$172.00 \pm 17.90^{***}$	$549.20 \pm 83.30^{***}$	$451.20 \pm 95.00^{***}$	
	1	$149.50 \pm 19.30^{***}$	$145.50 \pm 14.30^{***}$	$417.70 \pm 77.80^{***}$	$450.50 \pm 92.60^{***}$	
$[Cd(pxoxH)_2(H_2O)_2].H_2O$	0.75	$93.70 \pm 11.70^{***}$	$130.20 \pm 19.80^{***}$	$328.30 \pm 36.20^{***}$	$253.00 \pm 34.60^{***}$	
	0.50	$77.33 \pm 6.18^{***}$	$83.00 \pm 12.70^{***}$	$198.70 \pm 26.10^{**}$	$197.80 \pm 26.60^{*}$	
	0.25	$59.17 \pm 6.01^{***}$	$64.83 \pm 6.52^{***}$	$130.80 \pm 18.00$	$127.50 \pm 14.00$	

Table 5. The mutagenicity of test substances in *Salmonella* Typhimurium TA98 and TA100 strains in the absence or presence of s9 mix.

\*: P < 0.05; \*\*: P < 0.01; \*\*\*: P < 0.001

NPD: 4-nitro-o-phenylenediamine, 2AF: 2-aminoflourene, SA: sodium azide

 $[Mn(pxoxH)_2(H_2O)_2]$ .  $H_2O$  was strongly mutagenic on S. Typhimurium TA98 strain in the presence and absence of s9 mix but was not mutagenic in the presence and absence of s9 mix for the TA100 strain of S.

Typhimurium. In addition, the mutagenic activity of  $[Mn(pxoxH)_2(H_2O)_2]$ . H<sub>2</sub>O on S. Typhimurium TA98 strain was observed to be dose-dependent in the presence and absence of s9 mix (r = 0.983, Figure 11, r = 0.979, Figure 12, respectively).



Figure 9. Dose-dependent increase in the mutagenic activity of  $(pxoxH_2)$  (2) ligand on TA98 strain in the absence of s9 mix.



Figure 10. Dose-dependent increase in the mutagenic activity of  $(pxoxH_2)$  (2) ligand on TA98 strain in the presence of s9 mix.



Figure 11. Dose-dependent increase in the mutagenic activity of  $[Mn(pxoxH)_2(H_2O)_2]$ .H<sub>2</sub>O complex on TA98 strain in the absence of s9 mix.



Figure 12. Dose-dependent increase in the mutagenic activity of  $[Mn(pxoxH)_2(H_2O)_2]$ .H<sub>2</sub>O on TA98 strain in the presence of s9 mix.

Based on the data obtained from the Ames test, all chemicals tested and their various metabolites induced the frame-shift mutation except  $[Cu(pxoxH)_2].H_2O$  (TA98). In addition,  $[Ni(pxoxH)_2(H_2O)_2].2H_2O$  and  $[Cd(pxoxH)_2(H_2O)_2].H_2O$  and also their metabolites induced the base-pair substitutions (TA100). The pxoxH<sub>2</sub> ligand (2) induced the base-pair substitution mutation but not its metabolites. On the other hand,  $[Cu(pxoxH)_2].H_2O$  and its metabolites did not induce the base-pair substitution mutation.

The  $[Cd(pxoxH)_2(H_2O)_2].H_2O$  complex was strongly mutagenic on S. Typhimurium TA98 and TA100 strains in the presence and absence of s9 mix. The mutagenic activity of  $[Cd(pxoxH)_2(H_2O)_2].H_2O$  ligand on TA98 (r = 0.981, Figure 13, r = 0.987, Figure 14, respectively) and TA100 (in absence of s9 mix) (r = 0.982, Figure 15) was observed to be dose-dependent.





Figure 13. Dose-dependent increase in the mutagenic activity of  $[Cd(pxoxH)_2(H_2O)_2]$ .H<sub>2</sub>O on TA98 strain in the absence of s9 mix.

Figure 14. Dose-dependent increase in the mutagenic activity of  $[Cd(pxoxH)_2(H_2O)_2].H_2O$  ligand on TA98 strain in the presence of s9 mix.



Figure 15. Dose-dependent increase in the mutagenic activity of  $[Cd(pxoxH)_2(H_2O)_2]$ .  $H_2O$  on TA100 strain in the absence of s9 mix.

## 4. Conclusion

In this work, a new azo-oxime ligand, 2-[(E)-(hydroxyimino)methyl]-4-[(E)-(4-nitrophenyl))diazenyl]phenol (pxoxH<sub>2</sub>) (2) (Figure 1) derived from 2-hydroxy5-[(4-nitrophenyl)diazenyl]benzaldehyde (1) and hydroxylamminehydrochloride and some of its transition metal complexes was prepared. The analytical data and the spectroscopic studies suggested that the complexes had the general formula  $[M(pxoxH)_2(H_2O)_m].nH_2O$ , where M is manganese(II), nickel(II), copper(II), cobalt(II), zinc(II), or cadmium(II). Based on the UV/Vis and IR data of the 4-nitrophenylazo linked oxime ligand, pxoxH<sub>2</sub> was coordinated to the metal ion through the hydroxyimino nitrogen and phenolic oxygen atoms. Azo-oxime derivative pxoxH<sub>2</sub> probably forms a square planar complex with Cu(II), a tetrahedral complex with Zn(II) ion, and octahedral complexes with Mn(II), Ni(II), Co(II), and Cd(II) with a metal:ligand ratio of 1:2. Based on the above spectral and analytical data, the proposed structures of the Mn(II), Ni(II), Cu(II), Co(II), Zn(II), and Cd(II) coordination compounds can be formulated as in Figure 2. The genotoxicity study indicates that these compounds show weak genotoxicity and the toxicity may increase with increased dosage.

## Acknowledgment

The authors thank the Research Fund of Kahramanmaraş Sütçü İmam University for its financial support (Project no: 2010/3-15M).

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