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Synthesis, characterization, and *in vitro* anti-neoplastic activity of novel *vic*-dioximes bearing thiosemicarbazone side groups and their mononuclear complexes

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ABSTRACT

Two novel vicinal dioxime ligands containing thiosemicarbazone units, (*2E*)-2-[4-(diethylamino)benzylidene]-*N*-[(*1Z,2E*)-*N*-hydroxy-2-(hydroxyimino)ethanimidoyl]hydrazine carbothioamide ($\mathbf{L^1H_2}$) and (*2E*)-2-[4-(dimethylamino)benzylidene]-*N*-[(*1Z,2E*)-*N*-hydroxy-2-(hydroxyimino)ethanimidoyl]hydrazinecarbothioamide ($\mathbf{L^2H_2}$), were synthesized. Using the HL-60 human leukemia cell line, the *in vitro* anti-neoplastic activity of these thiosemicarbazone-oxime derivatives was evaluated. Mononuclear nickel(II), copper(II), and cobalt(II) complexes with a metal:ligand ratio of 1:2 for both the $\mathbf{L^1H_2}$ and $\mathbf{L^2H_2}$ ligands were also synthesized. To characterize these compounds, Fourier transform-infrared spectroscopy (FT-IR), mass spectrometry (MS), magnetic susceptibility measurements, ^{1}H and ^{13}C nuclear magnetic resonance (NMR), ultraviolet–visible (UV–Vis) absorption spectroscopy, heteronuclear multiple-bond correlation (HMQC), and elemental analysis were performed. For $\mathbf{L^1H_2}$, $\mathbf{L^2H_2}$, and each of their derivatives, antiproliferative effects against HL-60 cells were exhibited and the associated I_pC_{50} values ranged from 5 μM to 20 μM. Furthermore, $\mathbf{L^1H_2}$ and its derivatives inhibited the proliferation of HL-60 cells more effectively than $\mathbf{L^2H_2}$, and 5 μM [Cu(L^1H)$_2$] exhibited the strongest antiproliferative activity.

1. Introduction

Vic-dioximes are coordination compounds which have been well studied over the past few decades. Correspondingly, *vic*-dioximes have been applied to analytical, biological, pigment, and medicinal chemistries. In particular, 1,2-dioximes have been found to play an important role in coordination chemistry, mainly due to their stable MN_4 core [1–4]. The exceptional stability and unique electronic properties of these complexes have also been attributed to the planar structure of 1,2-dioximes that is stabilized by hydrogen bonding interactions [5–8].

The chemistry of transition metal complexes of thiosemicarbazone ligands has also been of interest, primarily due to their bioinorganic relevance [9–11]. Thiosemicarbazones usually bind to a metal ion as bidentate N,S-donor ligands via dissociation of the hydrazinic proton, thereby forming five-membered chelate rings [9,12]. The potential biological benefits of this class of complexes has been found to include antibacterial, antimalarial, antiviral, and antitumour activities [12–14]. For example, it has been reported that thiosemicarbazones are potent antitumour agents that inhibit the enzyme, topoisomerase II(Topo-II). Moreover, in recent studies, di-2-pyridylketonethiosemicarbazone(DpT) and 2-benzoylpyridinethiosemicarbazone(BpT) ligands were found to exhibit selective antitumour activity both *in vitro* and *in vivo* [15]. The 3,5-diacetyl-1,2,4-triazolbis(4,4-dimethylthiosemicarbazone) ligand, H(3)L(1), and its dinuclear platinum complex, [Pt(mu-HL(1))](2), also exhibited high antiproliferative activity against the human non-small cell lung cancer line, NCI-H460 [16].

While there have been many studies of thiosemicarbazones, as well as studies of mono- and di-oximes, studies of *vic*-dioxime derivatives with thiosemicarbazone side groups have not been reported. Therefore, in this work, *vic*-dioximes containing thiosemicarbazone units ($\mathbf{L^1H_2}$ and $\mathbf{L^2H_2}$), and novel mononuclear Ni(II), Cu(II), and Co(II) complexes of $\mathbf{L^1H_2}$ and $\mathbf{L^2H_2}$, were synthesized and structurally characterized. In addition, the cytotoxic activity of these complexes and their derivatives were assayed using the human promyelocytic leukemia cell line, HL60.

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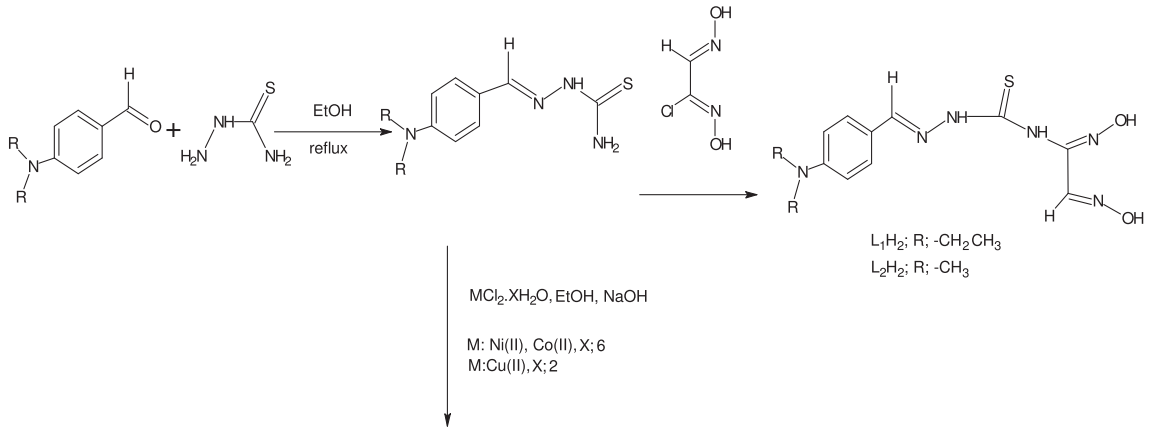

Scheme 1. Synthesis of the ligands (L^1H_2 and L^2H_2).

2. Results and discussion

2.1. Ligand synthesis

(*2E*)-2-[4-(diethylamino)benzylidene]hydrazinecarbothioamide (Scheme 1, **1a**) and (*2E*)-2-[4-(dimethylamino)benzylidene]hydrazinecarbothioamide (Scheme 1, **1b**) were obtained from a condensation reaction of 4-(diethylamino)benzaldehyde and 4-(dimethylamino)benzaldehyde with thiosemicarbazide in the presence of alcohol [11]. (*2E*)-2-[4-(diethylamino)benzylidene]-*N*-[(*1Z,2E*)-*N*-hydroxy-2-(hydroxyimino)ethanimidoyl]hydrazinecarbothioamide (**L^1H_2**) and (*2E*)-2-[4-(dimethylamino)benzylidene]-*N*-[(*1Z,2E*)-*N*-hydroxy-2-(hydroxyimino)ethan imidoyl]hydrazinecarbothioamide (**L^2H_2**) were subsequently prepared from **1a** and **1b** with *anti*-chloroglyoxime (**1c**) [17] in the presence of alcohol. Ni(II), Cu(II), and Co(II) complexes of **L^1H_2** and **L^2H_2** were also prepared in ethanol using $MCl_2.xH_2O$ as the metal salt. To characterize the structures of the metal complexes formed, Fourier transform-infrared spectroscopy (FT-IR), mass spectrometry (MS), magnetic susceptibility measurements, ^1H and ^{13}C nuclear magnetic resonance (NMR), ultraviolet–visible (UV–Vis) absorption spectroscopy, heteronuclear multiple-bond correlation (HMQC), and elemental analysis were performed. Although there were also attempts to grow X-ray quality single crystals, these attempts were unsuccessful.

2.2. FT-IR spectra, MS, and magnetic susceptibility

The FT-IR spectrum of **L^1H_2** and **L^2H_2** included —NH and —OH peaks, as well as —C=N peaks for both the oxime and thiosemicarbazone groups, and peaks representing the -NO stretching vibrations (Table 2). For **L^1H_2**, these peaks occurred at 3458 cm^{-1}, 3289 cm^{-1}, 1648 cm^{-1}, 1689 cm^{-1}, and 951 cm^{-1}, respectively. For **L^2H_2**, these peaks occurred at 3461 cm^{-1}, 3287 cm^{-1}, 1646 cm^{-1}, 1686 cm^{-1}, and 948 cm^{-1}, respectively [16–24]. The C=N and OH stretching vibration data for both ligands were also consistent with those previously reported for *vic*-dioxime and thiosemicarbazone derivatives [16–24]. The absence of bands between 2700 $cm^{-1}$and 2500 cm^{-1} excluded the possibility of a thione-thiol tautomerism (e.g., H—N—C=S, —C=N—SH) [20]. However, the strong ν(C=S) absorption bands that were observed around 813–812 cm^{-1} indicate that both ligands achieved a thion tautomeric form in the solid state (Fig. 1). Moreover, based on the fast atom bombardment (FAB) mass spectrometry data obtained, with values of 335 $[M-1]^+$ for **L^1H_2** and 309 $[M+1]^+$ for **L^2H_2**, the structures predicted for both ligands were confirmed.

FT-IR spectra for complexes with the general formula: $[M(LH)_2]$, [M: Ni(II), Cu(II), and Co(II)·$2H_2O$], exhibited C=N_{oxime} absorptions at 1640–1632 cm^{-1}. Upon complexation, absorptions at 1648–1646 cm^{-1} for the individual ligands were shifted to lower wave numbers, and the intensity of the bands were also reduced. Taken

Table 1
Physical properties and elemental analyses of the ligands and complexes.

Compounds formula	M.p. (d)[b] (°C)	Yield (%)	Color	μ_{eff} (BM)[a]	Calculated (Found)% of C	H	N	S
L^1H_2	195	70	Yellow	–	49.98 (49.94)	5.99 (6.02)	24.98 (24.95)	9.53 (9.48)
$[Ni(L^1H_2)]$	>300	52	Red	Dia.	46.10 (46.23)	5.25 (5.15)	23.04 (23.34)	8.79 (8.91)
$[Cu(L^1H_2)]$	>300	40	Brown	1.70	45.80 (45.68)	5.22 (5.35)	22.89 (23.14)	8.73 (8.56)
$[Co(L^1H_2)(H_2O)_2]$	>300	45	Brown	3.77	43.92 (43.46)	5.53 (5.34)	21.95 (21.67)	8.37 (8.78)
L^2H_2	196	72	Yellow	–	46.74 (46.70)	5.23 (5.45)	27.25 (27.50)	10.40 (10.56)
$[Ni(L^2H_2)]$	>300	60	Red	–	42.81 (42.67)	4.49 (4.98)	24.96 (24.45)	9.52 (9.30)
$[Cu(L^2H_2)]$	>300	45	Brown	1.70	42.50 (42.33)	4.46 (4.68)	24.78 (24.54)	9.46 (10.03)
$[Co(L^2H_2)(H_2O)_2]$	>300	50	Brown	3.75	40.62 (40.97)	4.83 (4.65)	23.68 (23.54)	9.04 (9.51)

[a] μ_{eff}: magnetic moment, Dia.: diamagnetic.
[b] d: decomposition.

Table 2
Characteristic IR bands of the ligands and their metal complexes (cm^{-1}, KBr) (1) L^1H_2 (2) $[Ni(L^1H)_2]$ (3) $[Cu(L^1H)_2]$ (4) $[Co(L^1H)_2(H_2O)_2]$ (5) L^2H_2 (6) $[Ni(L^2H)_2]$ (7) $[Cu(L^2H)_2]$ (8) $[Co(L^2H)_2(H_2O)_2]$.

Compound	NH	—OH/H_2O	C=S	O—H···O	C=N[a]	C=N[b]	N—O
1	3458 b	3289 b	813 m	–	1689 s	1648 s	951 m
2	3410 b	_	813 m	1788 w	1686 s	1639 s	953 m
3	3458 b	_	812 m	1740 w	1689 s	1632 s	945 m
4	3458 b	3285 b	813 m	1765 w	1689 s	1640 s	953 m
5	3461b	3287 b	812 m	_	1686 s	1646 s	948 m
6	3465 b	_	810 m	1863 w	1688 s	1639 s	947 m
7	3462 b	-	811 m	1871 w	1686 s	1639 s	945 m
8	3462 b	3289 b	811 m	1797 w	1686 s	1640 s	948 m

b: board s: Strong, m: medium, w: weak.
[a] ν(C=N): thiosemicarbazone moiety.
[b] ν(C=N): oxime moiety.

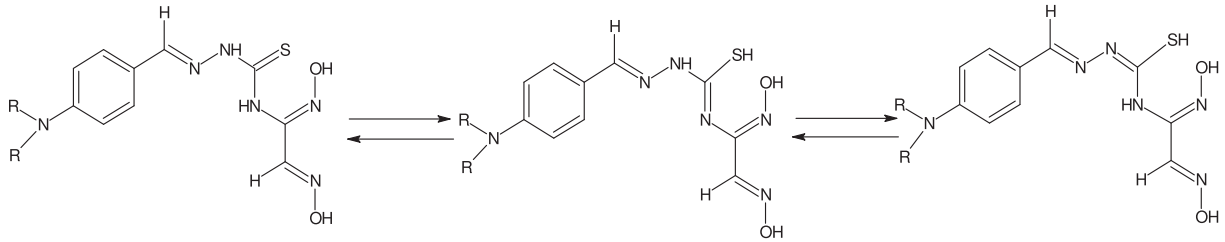

Fig. 1. Thion (I) and thiol (II and III) forms of the ligands (R: $—CH_2CH_3$ for L^1H_2, R: $—CH_3$ for L^2H_2).

together, these data suggest that $\mathbf{L^1H_2}$ and $\mathbf{L^2H_2}$ are N,N′ coordinated with the M(II) ion to form the structures proposed in Figs. 2a–2b. Moreover, these data suggest that complexation only involves the C=N bond of the *vic*-dioximes groups, with N,N′ coordinated with the M(II) ion. The disappearance of functional groups relating to δ OH for the Ni(II), Cu(II), and Co(II) complexes at 3289–3287 cm^{-1} further indicates that intramolecular hydrogen bonds form upon complexation, resulting in a peak between 1797 cm^{-1} and 1740 cm^{-1}.

Coordination of sulfur with the metal ion present would result in the displacement of electrons towards the latter, thereby weakening the (C=S) bond and decreasing the (C=S) stretching

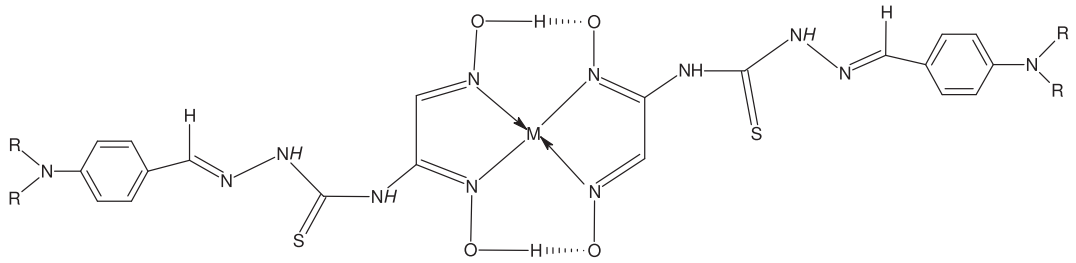

Fig. 2a. *trans* The structure of metal complexes.

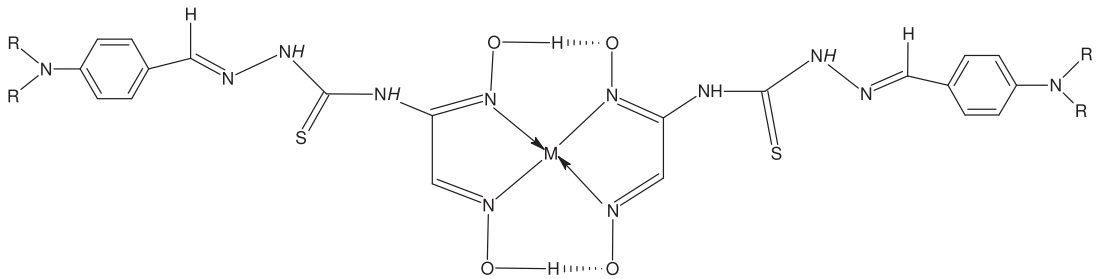

Fig. 2b. *cis* The structure of metal complexes.

vibrations [19]. However, upon complexation, the 813–812 cm^{-1} frequencies for the ligands do not shift to lower wave numbers, and the intensity of the bands do not decrease. Thus, it appears that a metal-sulfur bond is not formed.

FAB mass spectral data for the compounds: **[Ni(L^1H)$_2$]**, **[Cu(L^1H)$_2$]**, **[Co(L^1H)$_2$(H_2O)$_2$]**, **[Ni(L^2H)$_2$]**, **[Cu(L^2H)$_2$]**, and **[Co(L^2H)$_2$(H_2O)$_2$]**, included values of 730 $[M+1]^+$, 735 $[M+1]^+$, 765 $[M]^+$, 674 $[M+1]^+$, 679 $[M+1]^+$, and 709 $[M]^+$, respectively. In addition, the MS-determined metal:ligand ratio for these compounds was 1:2 for the Ni(II), Cu(II) and Co(II) complexes. The elemental analysis data further indicate that the desired compounds were synthesized (Table 1). Thus, these data confrim that the complexes have a metal:ligand ratio of 1:2 and maintain square planar or octahedral geometries (Figs. 2a–2b).

The magnetic moment measurements obtained at room temperature indicate that the Co(II) complexes are paramagnetic and have magnetic susceptibility values of 3.77 Bohr magneton (BM) for **L^1H_2** and 3.75 BM for **L^2H_2**. These values are within the range predicted for high spin octahedral cobalt(II) complexes (the three-spin value is 3.87 BM) [24]. For **[Co(L^1H)$_2$(H_2O)$_2$]** and **[Co(L^2H)$_2$(H_2O)$_2$]**, coordinated H_2O molecules were identified based on the detection of a broad OH absorption peak around 3289–3287 cm^{-1} that exhibited a constant intensity 24 h after these ligands were heated above 110 °C [25]. There was also no difference in the IR spectra of all of the synthesized compounds (**L^1H_2** and **L^2H_2**, and their Ni(II), Cu(II), and Co(II) metal complexes) following this heating period and the IR spectra previously obtained at room temperature. These results demonstrate the stability of the synthesized complexes.

The Cu(II) complexes were found to be diamagnetic, and to have an μ_{eff} value of 1.70 for **L^1H_2** and a 1.70 BM value for **L^2H_2**. These values are consistent with a spin value of 1.73 BM, despite being relatively low values for the magnetic moments. It is possible that the ligands contribute some diamagnetism, thereby leading to a decrease in the total paramagnetism of the complexes [25].

2.3. NMR spectra

In the ^1H NMR spectrum, two low intensity proton resonance singlets were detected at 11.11 ppm and 9.86 ppm for **L^1H_2**, and at 11.14 ppm and 9.90 ppm for **L^2H_2**. For both ligands, these two D_2O-exchangeable singlets correspond to two non-equivalent —OH protons. These data indicate that the —OH groups are in an anti-configuration relative to each other [26]. The chemical shifts which represent the —NH protons were observed at 7.90 ppm and 7.69 ppm as a singlet for **L^1H_2**, and at 7.92 ppm and 7.71 ppm as a singlet for **L^2H_2**. Moreover, these singlets disappeared with D_2O exchange. Chemical shifts for the CH=NOH protons were also observed at 6.26 ppm for **L^1H_2** and at 6.30 ppm for **L^2H_2**, both as singlets [27]. Further evidence of the thion form of these ligands was supported by the appearance of NH signals around 7.92–7.69 ppm.

Carbon resonances for the oxime groups of **L^1H_2** and **L^2H_2** were observed at 157.63 ppm and 129.63 ppm, and at 151.56 ppm and 129.28 ppm, respectively. The detection of these non-equivalent carbon atoms, particularly when they were associated with hydroxyimino carbon atoms, confirmed the anti-structures of **L^1H_2** and **L^2H_2** [28,29]. (NMR spectra data are summarized in Table 3).

2.4. UV–Vis spectra

Electronic spectra of ligands, **L^1H_2** and **L^2H_2**, and their Ni(II), Cu(II), and Co(II) metal complexes, were recorded in the 200–800 nm range in DMSO. Each ligand and complex exhibited between two and seven intense absorption bands in both the visible and ultraviolet regions (e.g., 240–587 nm). This wide distribution of bands may be due to both the $\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$, and the d–d transitions involving the C=N bond, as well as a charge transfer transition that could arise from p electron interactions between the metal and ligand. The latter could involve either a metal-to-ligand or ligand-to-metal electron transfer [30]. The absorption bands present below 262 nm were practically identical for the ligands and their complexes, and this can be attributed to the $\pi \rightarrow \pi^*$ transitions that can occur in the aromatic ring or azomethine (—C=N) groups. Moreover, the absorption bands observed between 397 nm and 320 nm are most likely due to the $n \rightarrow \pi^*$ transition of the imine group of the ligands and complexes [31]. The absorption bands between 404 nm and 435 nm are attributed to a $M \rightarrow L$ charge transfer (MLCT) or a $L \rightarrow M$ charge transfer (LMCT) and $^1A_{1g} \rightarrow {}^1B_{1g}$ transitions [32], respectively. For the Cu(II) and Co(II) complexes, the broad bands ranging from 589 to 590 nm were identified as $^2Eg \rightarrow {}^2T_{2g}$ transitions, which are characteristic of octahedral or square planar geometries [33]. Lastly, weak d–d transitions of the square planar Ni(II) complexes were observed between 435 nm and 431 nm [29a].

2.5. Antiproliferative activity

To evaluate the antiproliferative activity of **L^1H_2** and **L^2H_2**, and their Ni(II), Cu(II), and Co(II) metal complexes, the human promyelocytic leukemia cell line, HL-60, was selected. This cell line has previously been used in studies of myeloid differentiation and the effects of various physiologic, pharmacologic, and viral elements on this process [34]. In the present study, proliferation assays were performed at 37 °C for 72 h, and all of the synthesized compounds exhibited thermal stability during these experiments. The I_pC_{50} values determined for **L^1H_2** and **L^2H_2** and their complexes were found to vary from 5 μM to 20 μM (Figs. 3 and 4). Moreover,

Table 3
^1H NMR and ^{13}C NMR spectra of the ligands[a,b] in DMSO-d_6 in δ (ppm).

^1H NMR spectra of the ligands							
	—OH[c]	NH[c]	Ar—H	CH=NOH	CH=NNH	CH_3	$—CH_2$
L^1H_2	11.11–9.86 s, 2H	7.90–7.69 s, 2H	7.43 d, 2H 6.60 d, 2H	6.26 s, 1H	7.53 s, 1H	1.07 t, 6H	3.35 q,4H
L^2H_2	11.14–9.90 s, 2H	7.92–7.71 s, 2H	7.49 d, 2H 6.68 d, 2H	6.30 s, 1H	7.57 s, 1H	2.91 s, 6H	
^{13}C NMR spectra of the ligands							
	C=S	HNC=NOH	HC=NOH	HC=NNH	Ar—C	$—CH_3$	$—CH_2$
L^1H_2	164.21	157.63	129.61	148.82	128.75–111.69	13.11	44.36
L^2H_2	157.60	151.56	129.28	141.00	128.38–109.09	39.60	–

[a] Chemical shifts (δ) are reported in ppm relative to $SiMe_4$ at 30 °C, s: singlet, d: doublet.
[b] In DMSO-d_6.
[c] Disappears on D_2O exchange.

L^1H_2 and its complexes inhibited the proliferation of HL-60 cells proliferation more effectively than L^2H_2, and the strongest antiproliferative activity was exhibited by the Cu complex of L^1H_2 at 5 μM (Fig. 3).

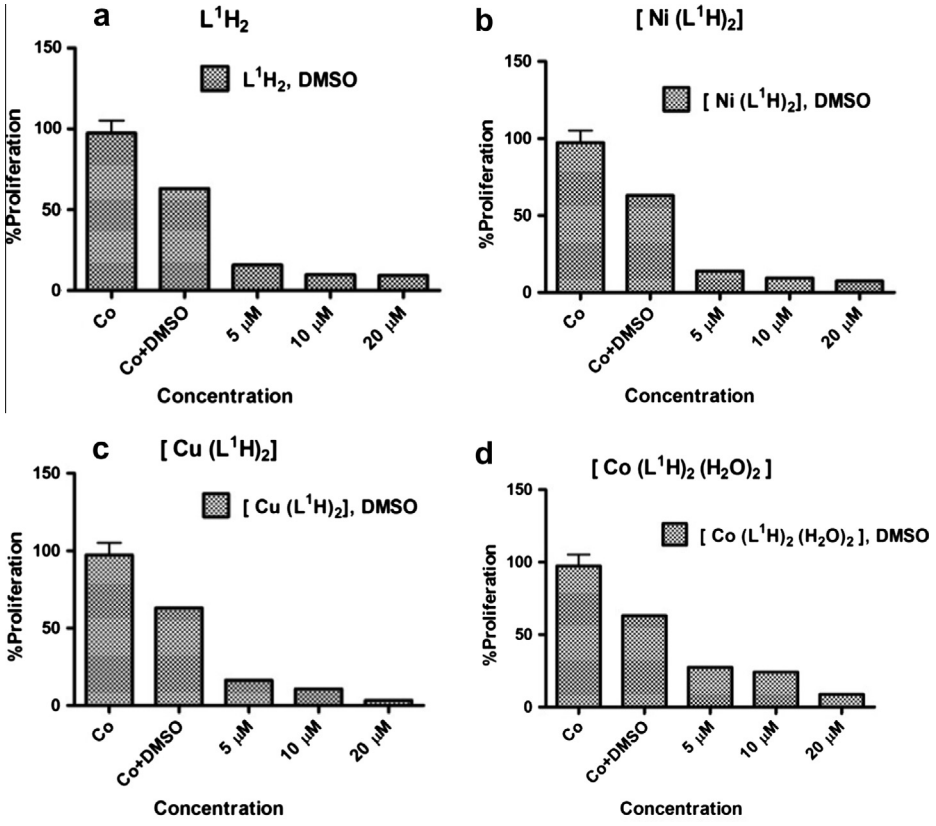

Fig. 3. Antiproliferative effect of L^1H_2 and its complexes.

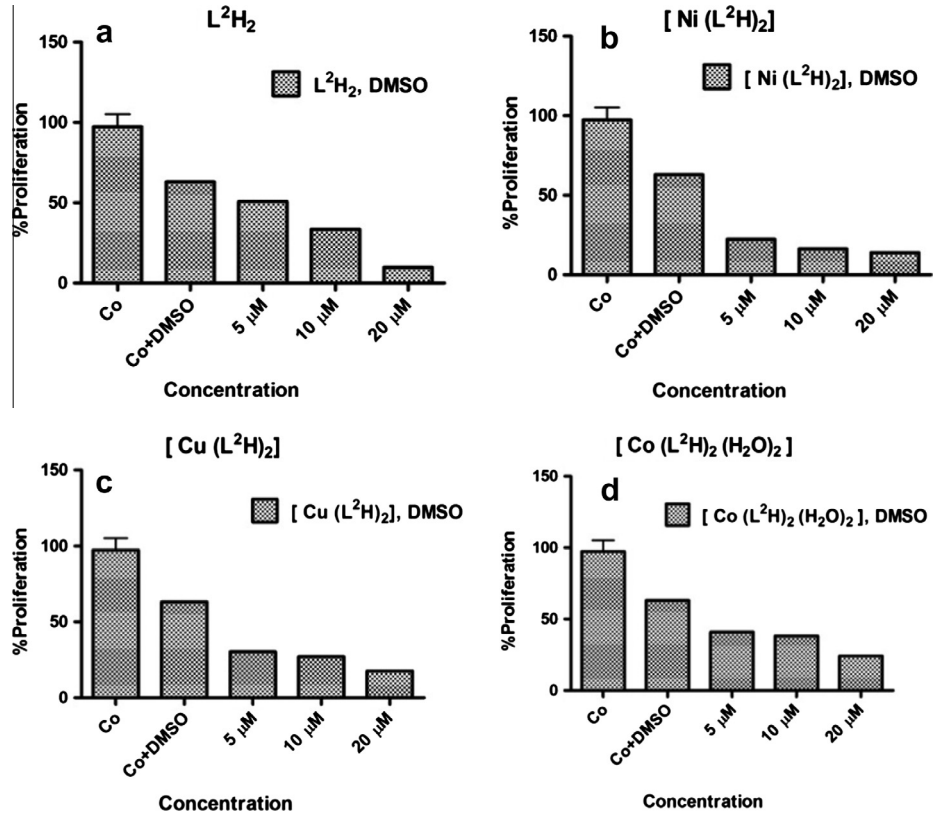

Fig. 4. Antiproliferative effect of L^2H_2 and its complexes.

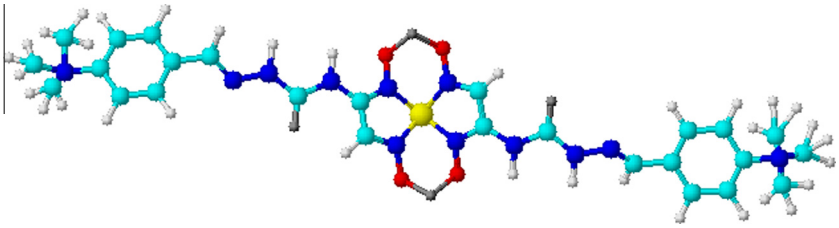

Fig. 5. Conformation of *trans* structure of Ni(II), Cu(II), and Co(II) complexes for $\mathbf{L^2H_2}$.

Inorganic chemistry is a discipline that is increasingly finding applications in the field of medicine, with a subset of inorganic compounds exhibiting anti-tumour or anti-cancer activities. Correspondingly, the novel synthesis of various metal complexes has led to the development of new pharmaceuticals. In particular, several thiosemicarbazones have been found to exhibit antitumour activity against human cancer cells of the breast, bladder, lung, cervix, liver, and skin. Antitumour activity against hematologic malignancies, laryngeal epithelial cells, and leukemia cells has also been observed. Regarding the latter, platinum (II) complexes with thiosemicarbazones derived from 2-formyl and 2-acetylpyridine and Mn(II), Co(II), and Zn(II) complexes with heterocyclic substituted thiosemicarbazones have been found to be very effective chemotherapeutic antileukemic agents [35,36].

These antiproliferative data are consistent with the results of previous studies that have shown some thiosemicarbazone complexes to be more effective than commonly used anti-cancer agents. For example, when zinc(II) complexes of 2-acetylpyridine1-(4-fluorophenyl)-piperazinylthiosemicarbazone were tested against four cancer cell lines, the antiproliferative activity of these complexes was found to be considerably stronger than that of cisplatin, a commonly used chemotherapy agent [37]. The structures of these compounds have also been shown to contribute to the antitumour activity observed as demonstrated in studies of (−)-*alpha*-bisabolol-based thiosemicarbazones and various human tumour cell lines [38].

Thiosemicarbazone platinum complexes have also exhibited antitumour activity against various human cancer cell lines [35,15]. Similarly, thiosemicarbazone derivatives have exhibited very low toxicity against kidney cells compared to cisplatin [39], and palladium(II) complexes of thiosemicarbazones have been found to be effective against human cancer cell lines as well [40,41]. Terminal dimethylation of thiosemicarbazones also plays an important role, and has been shown to enhance the anti-cancer activity of these compounds [42].

Currently, cancer is the second leading cause of death in the United States. In 2013, it is predicted that over 1.5 million new cases of cancer will be diagnosed, and over half a million Americans will die of cancer [43]. Correspondingly, there continues to be a need for new anti-cancer drugs to reduce cancer mortality rates, to avoid the high costs of therapy, and to improve the quality of life of affected patients. The results of the present study demonstrate that two new oxime-thiosemicarbazones and their derivatives represent potential anti-cancer agents. Therefore, further studies are needed to elucidate the specific mechanisms of action mediated by these compounds.

3. Conclusions

In this study, novel *vic*-dioxime ligands ($\mathbf{L^1H_2}$ and $\mathbf{L^2H_2}$) containing thiosemicarbazone groups and novel mononuclear complexes [Ni(II), Cu(II), and Co(II)] were synthesized. In addition, the antiproliferative effects of these compounds on neoplastic cells were demonstrated using human HL-60 promyelocytic leukemia cells.

Both oxime moieties were found to have an E configuration, and the Ni(II), Cu(II), and Co(II) complexes form intramolecular hydrogen bonds. In addition, both ligands can form mononuclear complexes $[(LH)_2M]$ with a metal-to-ligand ratio of 1:2 with M = Co(II)$(H_2O)_2$, Ni(II), and Cu(II). The Co(II) complexes were found to be octahedral with water molecules as axial ligands, and the Ni(II) and Cu(II) complexes formed a square planar geometry.

$\mathbf{L^1H_2}$ and $\mathbf{L^2H_2}$ and their metal complexes were identified as potent anti-cancer agents based on their antiproliferative activity against HL-60 cells, with I_pC_{50} values ranging from 5 μM to 20 μM. $\mathbf{L^1H_2}$ and its derivatives inhibited the proliferation of HL-60 cells more effectively than $\mathbf{L^2H_2}$, and the strongest antiproliferative activity was exhibited by 5 μM **[Cu($\mathbf{L^1H)_2}$]**.

4. Experimental

4.1. Materials and measurements

All reagents used were purchased from Merck. ^{1}H NMR–^{13}C NMR spectra (Bruker 400 MHz), I.R spectra (Varian 900), melting points (Buchi SPM-20) and pH measurements (Orion Expandable Ion Analyzer EA 940) were used to elucidate the structures of the products. The magnetic moments of the complexes were measured by the Gouy method with a Newport type D-104 instrument magnet power supply. Mass spectrometry (MS) spectra were recorded on a Bruker LC/MS/MS-8030 Triple Quadrupole Mass Spectrometer. (2*E*)-2-[4-(diethylamino)benzylidene]hydrazinecarbothioamide (Scheme 1, **1a**) and (2*E*)-2-[4-(dimethylamino)benzylidene]hydrazinecarbothioamide (Scheme 1, **1b**) [11] were prepared by literature methods, as was *anti*-chloroglyoxime (Scheme 1, **1c**) [17].

4.2. Synthesis of $\mathbf{L^1H_2}$ and $\mathbf{L^2H_2}$

A solution of (2*E*)-2-[4-(diethylamino)benzylidene]hydrazinecarbothioamide (**1a**) (1 mmol) or a solution of (2*E*)-2-[4-(dimethylamino)benzylidene]hydrazinecarbothioamide (**1b**) in absolute ethanol 30 mL was added dropwise to a solution of *anti*-chloroglyoxime (**1c**) (1 mmol) in absolute ethanol 10 mL for a 30 min period. The reaction mixture was stirred overnight at room temparature. After cooling to 0 °C the pH of the mixture was raised to 5.0–5.5 with treatment with $NaHCO_3$ dissolved in 5 mL distilled water, and stirring was continued for one hour. The solution was poured into 100 mL cold water with stirrring. After the end of the period, yellow precipitated solid was filtered, washed thoroughly with distilled water and dried. The chemical reaction and molecular structure are shown in Scheme 1.

4.3. Synthesis of the Ni(II), Cu(II) and Co(II) complexes of ligands

A solution of a metal salt (1 mmol of $NiCl_2{\cdot}6H_2O$, $CoCl_2{\cdot}6H_2O$ and $CuCl_2{\cdot}2H_2O$) in 20 mL of water were added to 2 mmol of the ligand solution (0.672 g L^1H_2 or 0.616 g L^2H_2 in 30 mL of ethanol) with stirring. An initial sharp decrease in the pH of the solution from 5.5 to about 3–3.5 is observed. After raising the pH to 5.0–5.5 using 1% aqueous NaOH solution, the reaction mixture

was kept in a hot water bath (60 °C) for 2 h to complete the precipitation. Then the precipitated complex compounds were filtered, washed with water and ethanol, and dried at room temperature in a vacuum oven. Structure of complexes are shown in (Figs. 2a–5).

4.4. Pharmacology

4.4.1. Cell culture

HL-60 promyeloic leukaemia cells were purchased from ATCC. Cells were grown in RPMI-1640 medium supplemented with 10% heat inactivated fetal calf serum, 1% L-glutamine and 1% penicillin/streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2. All media and supplements were obtained from Life Technologies.

4.4.2. Proliferation inhibition analysis

HL-60 cells were seeded in T-25 tissue culture flasks at a concentration of 1×10^5 cells per ml and incubated with increasing concentrations of agents (corresponding to 5, 10 and 20 μM of the drug). Cell counts and IC_{50} values were determined at 24 and 72 h using a Thoma slide. Experiments were done in triplicate. The percent of cell divisions compared to the untreated control were calculated as follows:

$$[(\text{C72 h} + \text{drug} - \text{C24 h} + \text{drug})/(\text{C72 h} - \text{drug} - \text{C24 h} - \text{drug})] \times 100 = \% \text{ cell division}$$

where C72 h + drug is the cell number after 72 h of drug treatment, C24 h + drug, is the cell number after 24 h of drug treatment, C72 h − drug is the cell number after 72 h without drug treatment, and C24 h − drug, is the cell number after 24 h without drug treatment.

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