



Synthesis, Characterization and Biological Evaluation of Some Novel Hydrazone Schiff's Bases and Their Metal Complexes

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N'-(4-Hydroxy-3-methoxyphenyl)methylidene]formic hydrazide (L-1), N'-[(4-hydroxy-3-methoxyphenyl)methylidene]-3'-hydroxynaphthalene-2'-carbohydrazide (L-2) N'-[(5-methylfuran-2-yl)methylidene]-3'-hydroxynaphthalene-2'-carbohydrazide (L-3), N'-[1''-(4-fluorophenyl)ethylidene]-3'-hydroxynaphthalene-2'-carbohydrazide (L-4) and N'-[(-1'-(4-chlorophenyl)ethylidene]formic hydrazide (L-5) were synthesized and tested for their antioxidant activity. All these newly synthesized Schiff's bases were treated with Cu(II), Co(II) and Zn(II) chlorides to form their respective complexes. Antioxidant activity of the synthesized ligands (L-1, L-2, L-3, L-4 and L-5), their corresponding metal(II) complexes, salts of the metal used in the complex formation and Trolox (as standard antioxidant) was measured in terms of their ability to scavenge the DPPH free radical. Ligand (L-4) was found to be the best antioxidant among all the ligands. As regards the activity of metal(II) complexes, Zn-L-4 exhibited the best antioxidant activity.

Key Words: Hydrazones, Hydrazides, Schiff's bases, Antioxidant activity.

INTRODUCTION

Oxidative stress is a well-known mechanism that is responsible for the development of vascular damage. Different pathogenic stimuli involved in cardiovascular diseases, such as activated macrophages, hyperglycaemia, oxidized low-density lipoprotein (LDL), exert their harmful effects, at least partially, through an increased local generation of reactive oxygen species. Reactive oxygen species (ROS) are normally produced throughout oxygen metabolism and play a major role in physiological and pathological cell redox signaling¹. Besides the direct free radical attack of cellular components, oxidative stress induces a lipid peroxidation of cellular membranes resulting in the generation of reactive carbonyl compounds (RCC) that react rapidly with free amino groups and thiol residues on proteins thereby forming adducts involved in the carbonyl stress^{2,3}. Both oxidative and carbonyl stress alter protein function and trigger the accumulation of modified proteins, which results in inflammation and apoptosis^{3,4}. Antioxidants inhibit the generation of ROS and the subsequent formation of lipid peroxidation products, thereby preventing both oxidative and carbonyl stress. Most antioxidants prevent LDL oxidation in cell-dependent and cell-free systems, and delay the formation of atherosclerotic lesions in animal models for atherosclerosis such as apoE^{-/-}mice⁵. The best compounds

in term of antioxidant and cytoprotective effects was found to be the symmetrical compound bearing two hydrazine scaffolds while all other ones were much less potent.

Hydrazones have been found with wide applications in synthetic chemistry⁶, to be used as indicators. Hydrazones are now being used extensively in detection and quantitative determination of several metals, for the preparation of compounds having diverse structures, analytical chemistry for the identification and isolation of carbonyl compounds^{7,8}. However, the most valuable property of hydrazones is, perhaps, their great physiological activity. The hydrazones have also been used for different purposes such as herbicides, insecticides, nematocides, redenticides and plant growth regulators⁹. The hydrazones are also important for their use as plasticizers and stabilizers for polymers^{10,11}, polymerization initiators and antioxidants¹². A polyphenol antioxidant is a type of antioxidant containing a polyphenolic substructure. Numbering over 4,000 distinct species, these compounds have antioxidant activity *in vitro* but are unlikely to have antioxidant roles *in vivo*¹³. Rather, they may affect cell-to-cell signalling, receptor sensitivity, inflammatory enzyme activity or gene regulation¹⁴.

In order to get insight on the efficiency of the hydrazone functionality, we report here the synthesis of some hydrazones. Keeping in view the promising use of potentially metal-based antimicrobial, antioxidant and antiinflammatory properties,

some metal-based [(Cu(II), Co(II) and Zn(II)] compounds incorporated with some novel formichydrazides and naphthalene-2-carbohydrazides (L-1-L-5) Schiff's bases are reported.

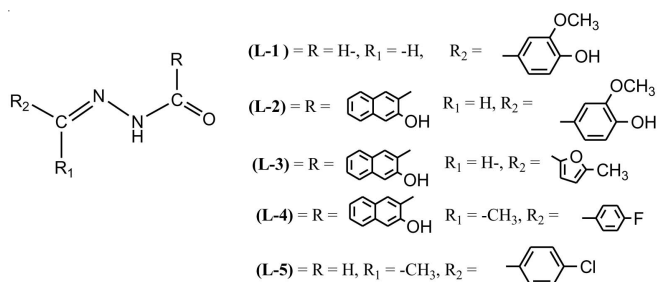


Fig. 1. Structure of ligands

EXPERIMENTAL

Solvents used were of analytical grade and all metals(II) were used as their chloride salts. IR spectra were recorded on 8400-FTIR Spectrophotometer using KBr disc method. NMR spectra were recorded on a Bruker-300 MHz NMR spectrophotometer. UV-Visible spectra were obtained in DMSO on IMRECO UV-VIS spectrophotometer, model U-2020. Conductance of the metal complexes was determined in DMSO on conductometer, Hitachi (Japan), model YSI-32. Melting points were recorded on a Gallenkamp melting point apparatus and are uncorrected. The complexes were analyzed for their metal contents by EDTA titrations¹⁵. Proton magnetic resonance spectra were recorded in CDCl₃ and DMSO-*d*₆ on Bruker AM 300 and AM 400 spectrometers (Rheinstetten-Forchheim, Germany) operating at 300 and 400 MHz, respectively. Tetramethyl-silance was used as an internal standard.

Preparation of Schiff's base

Synthesis of N'-(E)-(4-hydroxy-3-methoxyphenyl)methylidene] formic hydrazide (L-1): To a hot stirred solution of formic acid hydrazide (1 g, 0.017 mol) in ethanol (15 ml), vanillin (2.53 g, 0.017 mol) was added. The reaction mixture was heated under reflux for 1.5 h. During reflux precipitates were formed. The reaction was monitored through TLC. After completion of reaction, the mixture was cooled at room temperature. The solid material was collected by suction filtration. The precipitates were washed with hot ethanol, filtered and dried. Yield 55 %, white powder; m.p. 170 °C; IR (KBr, ν_{\max} , cm⁻¹): 3320 (OH), 3055 (NH), 1680 (>C=O), 1650 (>C=N), UV (DMSO) λ_{\max} (nm) 218; non-electrolyte; ¹H NMR (DMSO) δ : 6.82 (d, *J* = 8 Hz, 1H, C₅-H), 6.95 (m, 2H, C_{2,6}-H), 7.24 (s, 1H, CHN), 3.32 (s, 1H, C₄-OH), 3.87 (s, 3H, -OCH₃), 7.73 (s, 1H, NH), 8.65 (s, 1H, CHO) ppm. EIMS (*m/z*) = 194 (M⁺), 165, 148, 134, 123, 106.

Synthesis of 3'-hydroxy-N'-(Z)-(4-hydroxy-3-methoxyphenyl)methylidene]naphthalene-2'-carbohydrazide (L-2): To a hot stirred solution of 3-hydroxy-2-naphthoic hydrazide (1.5 g, 0.0074 mol) in a mixture of ethanol (50 mL) and 1,2-dioxane (2 mL), vanillin (1.13 g, 0.0074 mol) was added. The reaction mixture was heated under reflux for 10 h. The product was purified according to the previous procedure. Yield 95 %, yellow powder; m.p. 152 °C; IR (KBr, ν_{\max} , cm⁻¹): 3307, 3307 (-OH), 3145 (NH), 1660

(>C=O), 1595 (-HC=N-), 1520 (-OCH₃), UV (DMSO) λ_{\max} (nm) 227; non-electrolyte; ¹H NMR (DMSO) δ : 3.91 (s, 3H, -OCH₃), 7.00 (d, *J* = 8 Hz, 1H, C₂-H), 6.83 (d, *J* = 8 Hz, 1H, C₅-H), 7.26 (m, 4H, C_{6,1',4',7'}-H), 7.42 (m, 1H, C₆-H), 7.61 (d, *J* = 7.6 Hz, 2H, C_{5',8'}-H), 7.75 (s, 1H, CHN), 8.14 (s, 1H, C₃-OH), 8.39 (s, 1H, NH) ppm. EIMS (*m/z*) = 336 (M⁺), 171, 165, 143, 117.

Synthesis of 3'-hydroxy-N'-(E)-(5-methylfuran-2-yl)methylidene]naphthalene-2'-carbohydrazide (L-3): To a hot stirred solution of 3-hydroxy-2-naphthoic hydrazide (1.5 g, 0.0074 mol) in mixture of ethanol (35 mL) and 1,2-dioxane (2 mL), 5-methyl furfural (0.736 mL, 0.0074 mol) was added. The reaction mixture was heated under reflux for 2 h. The product was purified according to the previous procedure. Yield 87 %, yellow powder; m.p. 206 °C; IR (KBr, ν_{\max} , cm⁻¹): 3230 (OH), 3055 (NH), 1647 (>C=O), 1575 (>C=N-), UV (DMSO) λ_{\max} (nm) 348; non*electrolyte; ¹H NMR (DMSO) δ : 7.74 (d, *J* = 7.6 Hz, 1H, C₄-H), 7.67 (d, *J* = 8 Hz, 1H, C₃-H), 2.36 (s, 3H, CH₃), 7.29 (m, 3H, C_{1',4',7'}-H), 7.47 (m, 1H, C₆-H), 7.61 (d, *J* = 7.6 Hz, 2H, C_{5',8'}-H), 8.22 (s, 1H, C₃-OH), 8.39 (s, 1H, NH), 7.75 (s, 1H, CHN) ppm. EIMS (*m/z*) = 294 (M⁺), 171, 123, 143, 115, 79.

Synthesis of N'-(1Z)-1'-(4-fluorophenyl)ethylidene]-3'-hydroxy naphthalene-2'-carbohydrazide (L-4): To a hot stirred solution of 3-hydroxy-2-naphthoic hydrazide (1.5 g, 0.0074 mol) in ethanol (40 mL), 4-fluoroacetophenone (0.9 mL, 0.0074 mol) was added. The reaction mixture was heated under reflux for 12 h. The product was purified according to the previous procedure. Yield 87 %, cream coloured powder; m.p. 270 °C; IR (KBr, ν_{\max} , cm⁻¹): 3593 (OH), 3143 (-NH-), 1639 (>C=O), 1623, (-C=N-), UV (DMSO) λ_{\max} (nm) 336; non-electrolyte; ¹H NMR (DMSO) δ : 2.37 (s, 3H, CH₃), 7.29 (m, 3H, C_{1',4',7'}-H), 7.47 (m, 1H, C₆-H), 7.61 (d, *J* = 7.6 Hz, 2H, C_{5',8'}-H), 8.23 (s, 1H, C₃-OH), 8.43 (s, 1H, NH), 8.71 (d, *J* = 8.7 Hz, 2H, C_{3,5}-H), 7.90 (d, *J* = 8.7 Hz, 2H, C_{2,6}-H) ppm. EIMS (*m/z*) = 322 (M⁺), 171, 151, 115, 83.

Synthesis of N'-(1E)-1'-(4-chlorophenyl)ethylidene]-formic hydrazide (L-5): To a hot stirred solution of formic hydrazide (1 g, 0.017 mol) in ethanol (15 mL), 4-chloroacetophenone (1.37 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 5 h. The product was purified according to the previous procedure. Yield 88 %, white powder; m.p. 195 °C; IR (KBr, ν_{\max} , cm⁻¹): 3201 (-NH-), 1695 (>C=O), 1615 (>C=N-), 700 (>C-Cl), UV (DMSO) λ_{\max} (nm) 272; non-electrolyte; ¹H NMR (DMSO) δ : 2.22 (s, 3H, CH₃), 7.46 (d, *J* = 8.7 Hz, 2H, C_{3,5}-H), 7.77 (d, *J* = 8.7 Hz, 2H, C_{2,6}-H), 8.75 (s, 1H, NH), 11.12 (s, 1H, CHO) ppm. EIMS (*m/z*) = 196 (M⁺), 181, 167, 153, 137, 102.

Synthesis of metal(II) complexes

Synthesis of copper(II) complex (Cu-L-1): Copper(II) chloride dihydrate (1.3 mmol 0.218 g) was added to a magnetically stirred solution of L-1 (2.6 mmol, 0.5 g) in 1,4-dioxane (20 mL). The mixture was refluxed for 1 h. Precipitates were formed during reflux. The product was filtered, washed with ethanol and dried. The dried precipitates were kept in desiccator. All other Cu(II), Co(II) and Zn(II) complexes of ligands (L-1 to L-5) were synthesized according to the same method.

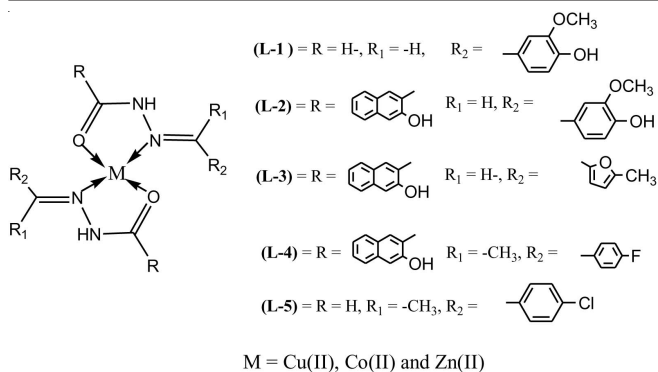


Fig. 2. Structures of metal(II) complexes

Antioxidant analysis

Method for antioxidant activity: The antioxidant activity of the ligands (L-1, L-2, L-3, L-4 and L-5) and their metal(II) complexes, salts of corresponding metals and Trolox (as standard antioxidant) was determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method of Brand-Williams *et al.*¹⁶. The methanolic solutions (0.1 mL) of the ligands, metal complexes, salts and Trolox were mixed with 40 μ M methanolic DPPH^{*} solution (2.9 mL) and absorbance was recorded after 0.5 h at 517 nm using a UV-Visible spectrophotometer. The antioxidant ability was calculated in terms of DPPH^{*} inhibition by a linear regression equation obtained from a calibration curve ($R^2 = 0.9902$) of DPPH^{*}:

$$\text{Abs}_{517\text{ nm}} = 0.2533 \times [\text{DPPH}^*](\mu\text{g})$$

The % DPPH^{*} remaining was calculated as:

$$[\text{DPPH}^*]_{\text{R}} (\%) = 100 \times [\text{DPPH}^*]_{\text{T}} \div [\text{DPPH}^*]_{\text{T}=0}$$

where, $[\text{DPPH}^*]_{\text{T}}$ is the remaining concentration of DPPH^{*} at 0.5 h time while $[\text{DPPH}^*]_{\text{T}=0}$ is the initial concentration of DPPH^{*}.

A kinetic study was also conducted to evaluate the free radical scavenging properties of the ligands, their complexes with metals(II), salts of corresponding metals and Trolox at varying time intervals. The percentage of remaining DPPH^{*} at every 5 min interval up to 30 min was plotted against time to obtain the initial time at which the samples inhibit the maximum concentration of DPPH^{*}.

RESULTS AND DISCUSSION

Chemistry of Schiff's base ligands (L-1-L-5) and their metal complexes: Ethanolic solutions of formic acid hydrazide

with vanillin and 4-chloroacetophenone were refluxed to synthesize Schiff bases (L-1) and (L-5) respectively. Similarly to get Schiff's bases (L-2), (L-3) and (L-4), 3-hydroxy-2-naphthoic acid hydrazide separately was refluxed with vanillin, 5-methyl-2-furfural and 4-fluoroacetophenone. The structures of these Schiff's bases formed were elucidated by IR, NMR, mass spectrometry and micro analytical data. These Schiff's bases were further used for the complexation reaction with Cu(II), Co(II) and Zn(II) metal ions. All of the newly synthesized metal complexes were found to be stable in air and moisture. They were prepared by the stoichiometric reaction with the corresponding metal(II) chlorides and the Schiff's bases (L-1 to L-5) in the molar ratios (M:L) 1:2. The complexes are amorphous solids, which are decomposed above 100 °C. These are insoluble in common organic solvents (chloroform, acetone, ethanol and methanol), but soluble in DMSO and DMF. All the complexes exhibited high values of molar conductance (31-116 $\Omega^{-1} \text{ cm}^2 \text{ mol}^{-1}$) determined in DMSO which show their ionic and electrolyte nature.

In the IR spectra of ligands the disappearance of absorption band at 1735 and 3420 cm^{-1} due to carbonyl $\nu(\text{C}=\text{O})$ and $\nu(\text{NH}_2)$ stretching vibrations and a new band appeared at *ca.* 1607 cm^{-1} assigned¹⁷ to the azomethine $\nu(\text{HC}=\text{N})$ linkage indicate that amino and aldehyde moieties of the starting reagents have been converted into their corresponding Schiff's bases. The $\nu(\text{NH})$ -amide, $\nu(\text{C}=\text{O})$ -amide and $\nu(\text{NNH})$ -imino stretching frequencies were present at 3440, 1679 and 1607 cm^{-1} , respectively. A comparison¹⁸ of the IR spectra of the Schiff's bases to their metal(II) complexes (Table-1) indicated that the Schiff's bases are coordinated to the metal atom mainly in two ways, thus the ligands are acting in a bidentate manner. The band absorption at 1607 cm^{-1} corresponding to azomethine group was shifted to lower frequencies by *ca.* 20 cm^{-1} , indicating participation of the azomethine nitrogen is coordinated¹⁹. A new medium strong band appearing at 540-533 cm^{-1} is assigned to $\nu(\text{M}-\text{O})$ ²⁰. This demonstrated that oxygen of the C=O-amide has formed a coordinate bond with the metal ions in an enolic form. A weak band at 420, 440 cm^{-1} is assigned to $\nu(\text{M}-\text{N})$. This further confirms that the nitrogen of the HN-imino group bonds to the metal atom. All of the data establishes that a conjugate chelate ring formed by ligand enolization exists in the complexes.

NMR spectrum of the ligand was determined in DMSO. ¹H NMR spectral data are reported along with possible

TABLE-1
MELTING/DECOMPOSITION POINTS AND UV-VISIBLE, IR SPECTRAL DATA OF THE SYNTHESIZED COMPLEXES

No.	Metal complexes / m.f.	m.p. / d.p. (°C)	λ_{max} (nm)	IR (cm^{-1})
1	[Cu(L-1) ₂ Cl ₂] / C ₁₆ H ₁₈ N ₄ O ₆ CuCl ₂ [522]	170	381	1650 (CO), 1585 (C=N), 510 (M-O), 435 (M-N)
2	[Co(L-1) ₂ Cl ₂] / C ₁₆ H ₁₈ N ₄ O ₆ CoCl ₂ [518]	300	415	1630 (CO), 1610 (C=N), 545 (M-O), 440 (M-N)
3	[Zn(L-1) ₂ Cl ₂] / C ₁₆ H ₁₈ N ₄ O ₆ ZnCl ₂ [524]	115	399	1650 (CO), 1600 (C=N), 532 (M-O), 430 (M-N)
4	[Cu(L-2) ₂ Cl ₂] / C ₃₈ H ₂₆ N ₄ O ₈ CuCl ₂ [806]	155	364	1637 (CO), 1593 (C=N), 535 (M-O), 415 (M-N)
5	[Co(L-2) ₂ Cl ₂] / C ₃₈ H ₂₆ N ₄ O ₈ CoCl ₂ [802]	220	331	1625 (CO), 1595 (C=N), 525 (M-O), 455 (M-N)
6	[Zn(L-2) ₂ Cl ₂] / C ₃₈ H ₂₆ N ₄ O ₈ ZnCl ₂ [808]	280	438	1632 (CO), 1601 (C=N), 533 (M-O), 435 (M-N)
7	[Cu(L-3) ₂ Cl ₂] / C ₃₄ H ₂₈ N ₄ O ₆ CuCl ₂ [722]	200	445	1635 (CO), 1602 (C=N), 530 (M-O), 425 (M-N)
8	[Co(L-3) ₂ Cl ₂] / C ₃₄ H ₂₈ N ₄ O ₆ CoCl ₂ [718]	230	437	1635 (CO), 1560 (C=N), 535 (M-O), 455 (M-N)
9	[Zn(L-3) ₂ Cl ₂] / C ₃₄ H ₂₈ N ₄ O ₆ ZnCl ₂ [724]	125	468	1632 (CO), 1595 (C=N), 505 (M-O), 435 (M-N)
10	[Cu(L-4) ₂ Cl ₂] / C ₃₈ H ₃₀ N ₄ O ₄ CuCl ₂ [778]	300	409	1638 (CO), 1550 (C=N), 520 (M-O), 442 (M-N)
11	[Co(L-4) ₂ Cl ₂] / C ₃₈ H ₃₀ N ₄ O ₄ CoCl ₂ [774]	242	458	1639 (CO), 1600 (C=N), 525 (M-O), 445 (M-N)
12	[Zn(L-4) ₂ Cl ₂] / C ₃₈ H ₃₀ N ₄ O ₄ ZnCl ₂ [780]	220	474	1630 (CO), 1610 (C=N), 520 (M-O), 433 (M-N)
13	[Cu(L-5) ₂ Cl ₂] / C ₁₈ H ₁₈ N ₄ O ₂ Cl ₂ CuCl ₂ [526]	170	431	1670 (CO), 1510 (C=N), 560 (M-O), 455 (M-N)

assignments. All the protons were found to be in their expected regions^{21,22}.

Mass spectra were determined by the instrument JEOL MS Route and MAT 312 that gives us different fragments of the Schiff bases. Molecular ion peaks were appeared at same value of the molecular weight of Schiff's bases.

The conclusions drawn from these studies further support to the mode of bonding discussed in their IR spectra. It was also observed that DMSO did not have any coordinating effect on the spectra of ligands or their metal complexes.

Antioxidant activity: The antioxidant activity of the synthesized ligands (L-1, L-2, L-3, L-4 and L-5), their corresponding metal(II) complexes, salts of the metal used in complex formation and Trolox (as standard antioxidant) was measured in terms of their ability to scavenge the DPPH free radical. Among the ligands, L-4 was found to be a good antioxidant followed by L-2 and L-1 while L-5 showed comparatively lowest antioxidant activity. Among the metal(II) complexes of these ligands, the salts of Zn(II) with each ligand showed good response regarding the inhibition of DPPH[•]. Zn-L-4 was found to be the best antioxidant followed by Co-L-2, Co-L-4 Zn-L-2 and Zn-L-5 which also showed more than 50 % inhibition of DPPH[•]. The Cu (II) complexes of each of the ligands were found to be poor antioxidants as compared to the respective ligand. The salts of all of the three metals also showed low antioxidant activities as compared to the ligands and their corresponding complexes. However, the antioxidant activity of all of the ligands, metal complexes and the salts were found to be low as compared to Trolox (Fig. 3).

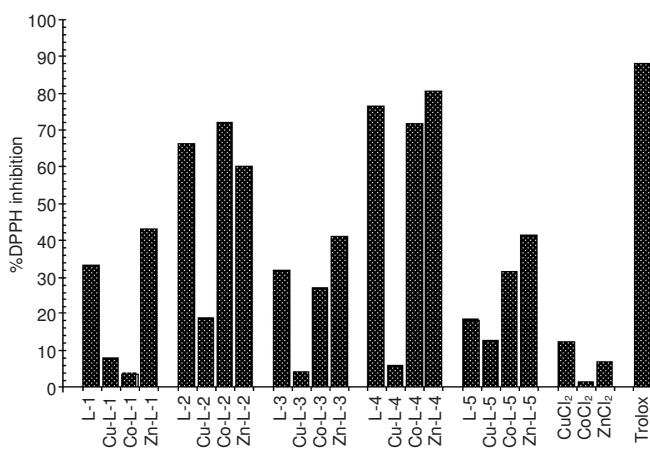
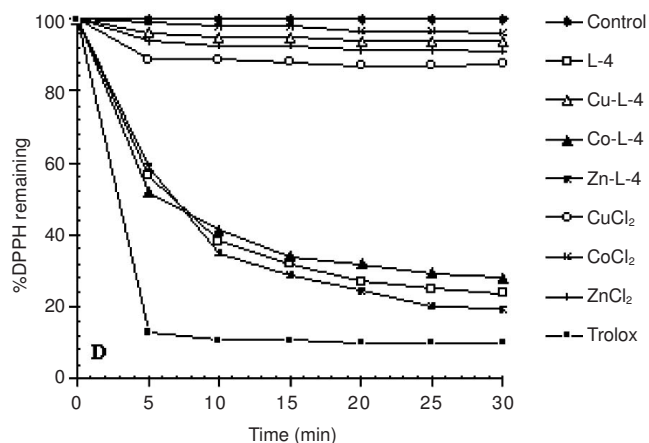
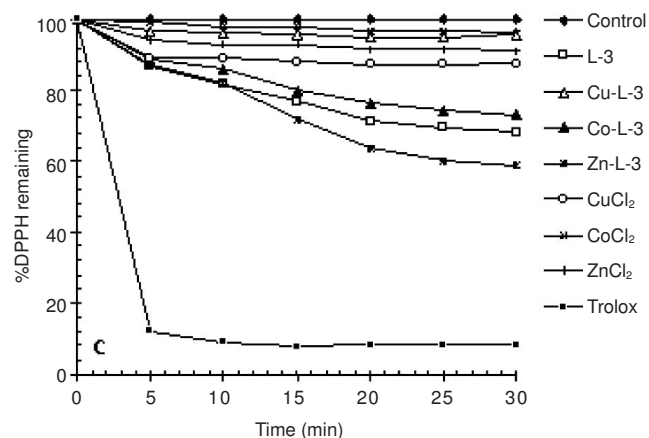
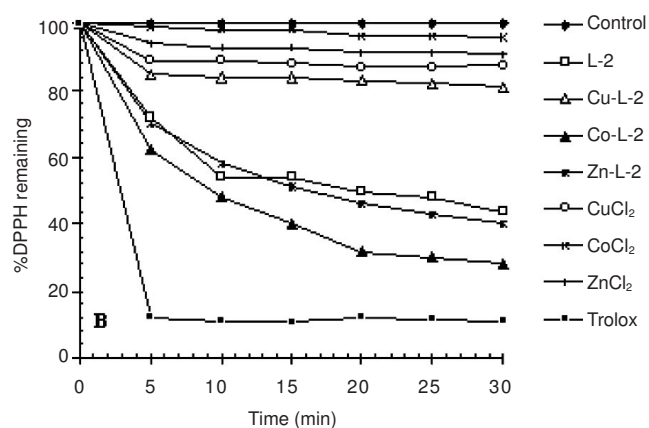
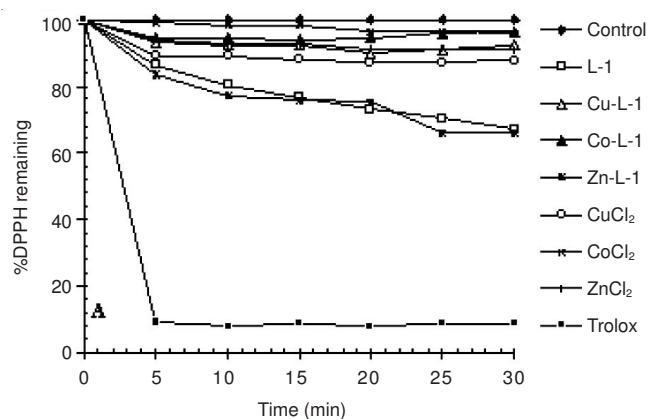


Fig. 3. Comparative antioxidant activity (% inhibition of DPPH[•]) of synthesized ligands, their metal complexes, salts of respective metals and a standard antioxidant (Trolox)

The kinetic behavior of DPPH[•] inhibition by the ligands, their metal complexes and salts of metals showed that these ligands and their complexes with cobalt(II) and Zn(II) cause a sharp decrease in DPPH[•] concentration within first 5 min (Fig. 4A,C). After 5 min a slow decrease in the remaining DPPH[•] concentration was observed to be continued for 0.5 h in each case. The salts of each metal and the complexes of the ligands with Cu(II) showed no further decrease in DPPH[•] concentration after 5 minutes. However, each of the ligands and complex showed low rate of DPPH[•] inhibition as compared to Trolox (Fig. 4).



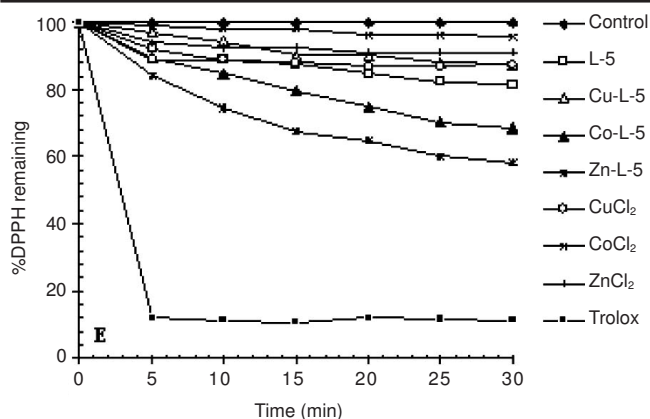


Fig. 4. Kinetic behavior of DPPH* inhibition by synthesized ligands, their metal complexes, salts of respective metals and a standard antioxidant (Trolox). A: L-1, B: L-2, C: L-3, D: L-4, E: L-5

Conclusion

All these newly synthesized Schiff's bases were characterized by UV-VIS, IR and ^1H NMR spectral data. The mass spectrometric data of these ligands was also in close resemblance with the structures supported by UV-VIS, IR and ^1H NMR spectroscopic tools. To determine antioxidant activity of the ligands (L-1, L-2, L-3, L-4 and L-5), their corresponding metal(II) complexes and metal chlorides, Trolox was used as standard antioxidant. Ligand (L-4) was found to be the best antioxidant among all the ligands while among the metal(II) complexes; Zn-L-4 exhibited the best antioxidant activity.

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