Med Chem Res (2014) 23:2476–2485
DOI 10.1007/s00044-013-0826-7

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Spectroscopic, antiproliferative and antiradical properties of Cu(II), Ni(II), and Zn(II) complexes with amino acid based Schiff bases

Zuhal Karagoz Genc · Sibel Selcuk · Suleyman Sandal · Naki Colak · Serhat Keser · Memet Sekerci · Mustafa Karatepe

Received: 24 January 2013 / Accepted: 3 October 2013 / Published online: 23 October 2013

Abstract Novel six Cu(II), Ni(II), and Zn(II) complexes with Schiff bases derived from 4-aminobenzoic acid with terephtaldehyde and amino acids (glycine, β-alanine). Structures have been proposed from elemental analysis, UV–Vis, IR, NMR, TGA, DTA, and magnetic measurements. Spectroscopic studies suggest that coordination occurs through azomethine nitrogen, hydroxyl group, and carbonyl oxygen of the ligands to the metal ions. The elemental analyses of the complexes where L is Schiff base ligands, are confined to the stoichiometry of the type $M_2L_2(CH_3COO)_2$ [M = Cu(II)]; and $M_2L(CH_3COO)_2$ [M = Ni(II) and Zn(II)]. The cytotoxicity activities of the compounds against human breast carcinoma MCF-7 cell line have been studied. Ligands and their Zn(II) compounds inhibited cell proliferation of MCF-7 cancer cell lines in a dose- and time-dependent manner. The free radical scavenging activity was measured by 1,1-diphenyl-2-picryl-hydrazil. Our results show that the synthesized compounds induced oxidative damage by increasing the lipid peroxidation in yeast since MDA formation was increased, and it could be concluded that the synthesized compounds caused oxidative stress. In addition, the antioxidant activities of the synthesized compounds were very much lower than those of standard antioxidants.

Keywords Spectroscopy · Schiff base · Antiproliferative activity · DPPH

Z. Karagoz Genc (✉) · S. Selcuk · S. Keser · M. Sekerci · M. Karatepe
Department of Chemistry, Faculty of Science, Firat University, Elazig, Turkey
e-mail: zuhalkaragoz23@gmail.com

S. Sandal
Department of Physiology, Faculty of Medicine, Inonu University, Malatya, Turkey

N. Colak
Department of Chemistry, Faculty of Science and Arts, Hitit University, Corum, Turkey

Introduction

Schiff bases form an interesting class of ligands that have enjoyed popular use in the coordination chemistry of transition, innertransition, and main group elements (Etaiw *et al*., 2011; Collinson and Fenton, 1996). Schiff bases have been reported to show a variety of biological actions by virtue of the azomethine linkage which is responsible for various antibacterial, antifungal, herbicidal, and clinical activities (Sharma and Chandra, 2011; Abd El-Halim *et al*., 2011; Ravoof *et al*., 2010). Transition metal ions play vital roles in a vast number of widely different biological processes (Priya *et al*., 2009). It is well known that some drugs exhibit increased activity when administered as metal complexes (Castillo-Blum and Barba-Behrens, 2000; El-Sherif and Eldebss, 2011; Campbell, 1975; Williams, 1972), and several metal chelates have been shown to inhibit tumor growth (Furst and Haro, 1969). Metal complexes of Schiff bases derived from heterocyclic compounds containing nitrogen, sulfur, and/or oxygen as ligand atoms are of interest as simple structural models of more complicated biological systems (Sakiyan *et al*., 2004).There have been several reports on metal complexes of the Schiff base ligands having a variety of applications including biological, clinical, analytic, and industrial fields in addition to their important roles in catalysis and organic synthesis (Dwyer *et al*., 1965; Ouyang *et al*., 2002; Raman *et al*., 2001; Jayabalakrishnan and Natarajan, 2002; Sharghi and Nasser, 2003; Gao and

Zheng, 2002). Moreover; the activity can be enhanced, when the biologically active ligand is coordinated to a transition metal ion.

In the present article, we report the synthesis and characterization of a series of copper(II), nickel(II), and zinc(II) complexes obtained from {4-[(4-{[4-(carboxymethyl-carbamoyl)-phenylimino]-methyl}-benzylidene)-amino]-benzoylamino}-acetic acid (L^1) and {4-[(4-{[4-(carboxymethyl-carbamoyl)-phenylimino]-methyl}-benzylidene)-amino]-benzoylamino}-propionic acid (L^2).

In addition, in vitro antitumoral activity and antioxidant effects of the ligands and their complexes have been studied, and the free radical scavenging activity of compounds was measured by 1,1-diphenyl-2-picryl-hydrazil ($DPPH^{\bullet}$). Malondialdehyde (MDA) levels were measured by high-performance liquid chromatography (HPLC) in *Saccharomyces cerevisiae* yeast cells (Fig. 1).

Results and discussion

All the Cu(II), Ni(II), and Zn(II) complexes were colored, stable at room temperature for extended periods, and decompose on heating. These complexes are insoluble in water and many common organic solvents but are readily soluble in strong coordinating solvents like DMF and DMSO. Therefore, the crystals were unsuitable for single crystal X-ray structure determination. The analytic data showed that the complexes had stoichiometry the type $M_2L_2(CH_3COO)_2$ [M = Cu(II)], $M_2L(CH_3COO)_2$ [M = Ni(II) and Zn(II)] respectively, where L is of base ligands (Table 1).

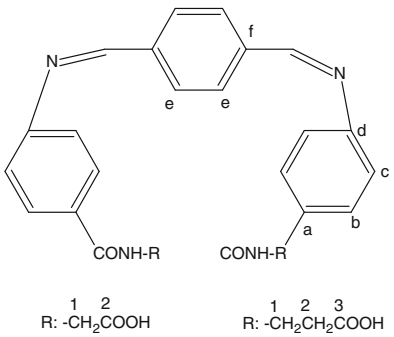


Fig. 1 Structures of the ligand (L^1 and L^2)

The ^{1}H-NMR spectra of ligands (L^1 and L^2) have been carried out in DMSO-d_6 at room temperature. Broad peaks at 13.00–13.06 were attributed to the –OH protons of carboxylic acid for the L^1 and L^2, respectively. The broad multiplets at 7.43–8.86 ppm were assigned to the aromatic ring protons. The singlet at 8.86 ppm was attributed to the proton of the azomethine group, and the multiplets at 3.66–4.15 ppm were assigned to the N–CH_2 group. The ^{1}H-NMR spectrum of the HL^2 showing a peak at 2.92 ppm was assigned to the protons of methyl group. In comparison with the ^{1}H-NMR spectrum of the L^1 and L^2 and their Zn(II) complexes, the mode of coordination between the ligands and metal ions was clarified. Through the discussion of ^{1}H-NMR spectrum of Zn(II) complexes, it was found that the peak (signals)-specialized OH proton of carboxylic acid disappeared. This behavior suggests participation of hydroxyl group in the ligand. For the CONH protons, a triplet was observed in the 8.63–8.91 ppm, which is shifted to 8.27–8.62 ppm in the Zn(II) complexes with a dramatic decrease in intensity, suggesting that the azomethine nitrogen was involved in coordination with Zn(II) in the complexes. The multisignals within the range of 2.5–2.7 are assigned to the protons of the acetate groups in the complex.

In order to clarify the mode of bonding and the effect of the metal ion on the ligand, the IR spectra of the free ligand and their Cu(II), Ni(II), and Zn(II) complexes were compared and assigned on the basis of the careful comparison (Table 2).

All complexes show similar IR spectral features, exhibiting a medium-to-strong bands at 1615 and 1631 cm^{-1} regions corresponding to υ(C=N) stretch of

Table 1 Elemental analyses of the ligands and their compounds

Compounds	FW (g/mol)	Color	M.P. (°C)	Yield (%)	μ_{eff} (B.M.)	Elemental analyses/(%) calculated (Found)		
						C	H	N
$C_{26}H_{20}N_4O_6$ (L^1)	484	Yellow	265–267	67	–	64.19 (63.68)	4.13 (4.25)	11.57 (10.52)
$C_{28}H_{26}N_4O_6$ (L^2)	514	Yellow	298–299	64	–	65.36 (65.22)	5.09 (4.98)	10.89 (11.01)
$[Cu_2L^1_2(CH_3COO)_2(H_2O)_2]\cdot 2H_20$	1356.7	Brown	335	56	1.40	49.53 (49.38)	4.56 (4.18)	8.25 (8.22)
$[Ni_2L^1(CH_3COO)_2]\cdot 3H_2O$	773.2	Green	325	64	2.46	46.66 (46.59)	4.14 (4.16)	7.25 (7.14)
$[Zn_2L^1(CH_3COO)_2]$	732.6	Yellow	330	66	–	49.14 (48.56)	3.54 (3.59)	7.64 (7.54)
$[Cu_2L^2_2(CH_3COO)_2(H_2O)_2]\ H_20$	1322.7	Brown	345	42	1.53	56.56 (56.89)	4.56 (4.30)	8.80 (8.92)
$[Ni_2L^2(CH_3COO)_2]\cdot 3.5H_2O$	830.2	Green	315	51	2.76	45.85 (46.71)	4.77 (4.67)	6.68 (6.65)
$[Zn_2L^2(CH_3COO)_2]$	762.6	Yellow	330	69	–	50.35 (50.24)	4.19 (3.93)	7.34 (7.02)

Table 2 Important IR spectral bands (cm^{-1}) of the ligands and their metal complexes

Compound	$\nu_{(OH)}$	$\nu_{(CON)}$	$\nu_{(COOH)}$	$\nu_{(C=N)}$	$\delta_{(N-H)}$	$\nu_{(M-N)}$	$\nu_{(M-O)}$	$\nu_{as}(OCO)$	$\nu_{s}(OCO)$
$C_{26}H_{20}N_4O_6$ (L^1)	3380–3100	1645	1708	1615	1567	–	–	–	–
$C_{28}H_{26}N_4O_6$ (L^2)	3550–3240	1660	1710	1631	1565	–	–	–	–
$[Cu_2L^1_2(CH_3COO)_2(H_2O)_2]\cdot 2H_20$	3300–3210	1610	1695	1617	1510	528	487–480	1552	1430
$[Ni_2L^1(CH_3COO)_2]\cdot 3H_2O$	3410–3210	1617	1690	1615	1520	521	497–490	1641	1463
$[Zn_2L^1(CH_3COO)_2]$	3450–3120	1610	1687	1621	1515	515	466–470	1635	1350
$[Cu_2L^2_2(CH_3COO)_2(H_2O)_2]\cdot H_20$	3340–3210	1635	1689	1635	1540	509	496–469	1555	1445
$[Ni_2L^2(CH_3COO)_2]\cdot 3.5H_2O$	3300–3100	1625	1691	1636	1545	506	490–470	1550	1310
$[Zn_2L^2(CH_3COO)_2]$	3430–3190	1620	1685	1639	1530	510	495–464	1610	1320

azomethine groups. These bands shifted slightly to higher wave number side relative to the free ligands, indicating noncoordination of "N" of C=N group in bonding. The observed strong bands at 1665, 1640 cm^{-1} and 1708, 1710 cm^{-1}, in the free ligands were attributed to υ(CO) stretches of the CONH and COOH groups, respectively. These bands shifted slightly to lower wave number side in all complexes indicating the participation of the carboxyl oxygen in bonding with metal ions (Zaki *et al*., 1998; Bellamy, 1964). Coordination of the Schiff base ligands to the metal ions through the nitrogen atom is expected to reduce electron density in the CONH link and lower the (NH) absorption frequency. These bands that shifted to a lower wave number side in all complexes indicate the coordination of the NH group to the metal ions. The IR spectra of the ligands reveal broad bands in the 3380–3100 cm^{-1} and 3550–3240 cm^{-1} regions, and are assigned to the υ(OH) groups. These bands must have disappeared in the spectra of the complexes, indicating deprotonation of hydroxyl group and coordinating through deprotonated OH groups, but the OH stretching frequency appearing in the spectra of complexes, are attributed to the presence of water of hydration and coordination. In addition, thermal analysis data have further confirmed the results of elemental analyses.

The new weak nonligand bands in the spectral regions 528–506 cm^{-1} and 464–497 cm^{-1} of the complexes are assigned to the stretching frequencies of υ(M–N) and υ(M–O) bonds, respectively. These indicate that azomethine nitrogen and carbonyl oxygen atoms are involved in coordination.

The electronic spectra of the two free ligands exhibit bands in the region of 350–250 nm, which could be attributed to $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions, respectively. The magnetic moments are reported at the room temperature and shown in Table 1. The electronic spectra of the ligands and their complexes were recorded in DMSO. The electronic spectra of metal complexes show different bands at different wavelengths, with each one corresponding to certain transition, which suggests the geometry of the complex compounds.

The electronic spectra of the L^1Ni and L^2Ni complexes showed two strong broad bands at 645–630 nm and 385–380 nm, respectively, attributed to $^3T_1 \rightarrow {}^3T_1$ (P) (ν_3) and charge transfer in a tetrahedral geometry. (Sonmez *et al*., 2004; Satyaranayana and Nagasundura, 2004). The observed magnetic moments (2.46 and 2.76 B.M.) confirm the tetrahedral geometry.

Electronic spectra of six-coordinate copper complexes have either D_{4h} or C_{4v} symmetry, and e_g and t_{2g} levels of ^2D free ion term will split into B_{1g}, A_{1g}, B_{2g}, and E_g levels. Thus, three spin-allowed transitions are expected in the visible and near IR region. However, only a few complexes are known, in which such bands are resolved by single crystal polarization studies (Mohan *et al*., 1990; Dubey and Sangwan, 1994). These bands may be assigned to following transitions: $^2B_{1g} \rightarrow {}^2A_{1g}$ ($d^2_{x-y} \rightarrow d^2_z$), $^2B_{1g} \rightarrow {}^2B_{2g}$($d^2_{x-y} \rightarrow d_{xy}$), and $^2B_{1g} \rightarrow {}^2Eg$($d^2_{x-y} \rightarrow d_{xz}\ d_{yz}$) in the order of increasing energy. The electronic spectra of complexes having molecular formulae L^1Cu and L^2Cu show broad bands in the ranges of 600–849 nm and 580–700 nm assigned to the $^2B_{1g} \rightarrow {}^2E_g$ transition, respectively. More intense bands at 438 and 450 nm are assigned to the LMCT transitions for L^2Cu complex (Dogan *et al*., 1998). These band positions are in agreement with those generally observed for octahedral copper (II) complexes. The magnetic moments of the copper complexes are 1.40 and 1.53 B.M. (Table 1) corresponding to one unpaired electron, which are lower than spin-only value, i.e., 1.73 BM for one unpaired electron for complexes. This reveals that these complexes are dimeric in nature and also show the presence of metal–metal interaction along the axial positions (Figs. 2, 3).

The thermogravimetric analysis (TGA) and differential thermal analysis (DTA) curves were obtained at a heating rate of 15 °C/min in nitrogen atmosphere over a temperature range of 25–1000 °C.

The $[Cu_2L^1_2(AcO)_2(H_2O)_2]\cdot 2H_2O$ complex was stable up to 35 °C, and their decomposition started at this

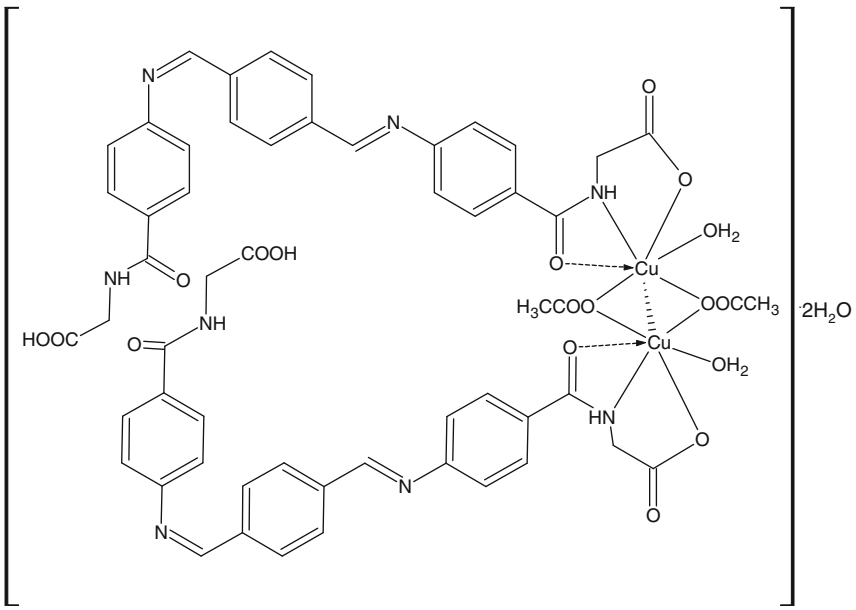


Fig. 2 Suggested structure of the Cu(II) complex of the ligand (L^1)

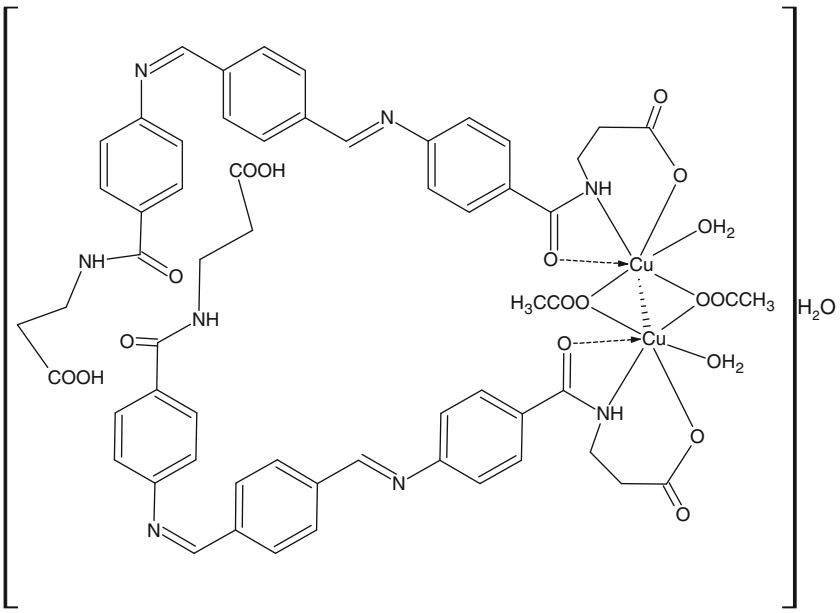


Fig. 3 Suggested structure of the Cu(II) complex of the ligand (L^2)

temperature. 2.73 % weight loss was observed at 195 °C corresponding to 2 mol of water of the crystallization, and 11.66 % weight loss was observed at 230 °C. These weight losses corresponded to the 2 mol of coordinated water molecule and two acetate groups. The $[Cu_2L^2_2(AcO)_2(H_2O)_2]$ H_2O complex was stable up to 55 °C, and its decomposition started at this temperature. 1.36 % weight loss was observed at 110 °C corresponding to a mole of water of the crystallization, and 11.64 % weight loss was observed at 270 °C; the weight losses corresponded to 2 mol of coordinated water molecule and two acetate groups. The decomposition processes of the Cu(II) complexes are irreversible.

The $[Ni_2L^1(AcO)_2]\cdot 3H_2O$ and $[Ni_2L^2(AcO)_2]\cdot 3.5H_2O$ complexes were stable up to 43 and 40 °C, and their decompositions started at these temperatures. In the decomposition process of the complexes, 6.38 and 7.69 % weight losses were observed at 170 and 150 °C, corresponding to 3 and 3.5 mol lattice water, respectively. In the second stage, 14.06 and 13.59 % weight losses were observed at 360 and 340 °C, respectively. These weight losses corresponded to two acetate groups. Also, coordinated water should exhibit frequency at 850, 575, and 500 cm^{-1} in the IR spectrum. The absence of spectral bands in these regions in the spectra of Ni(II) complexes indicates that the water molecules in these complexes are

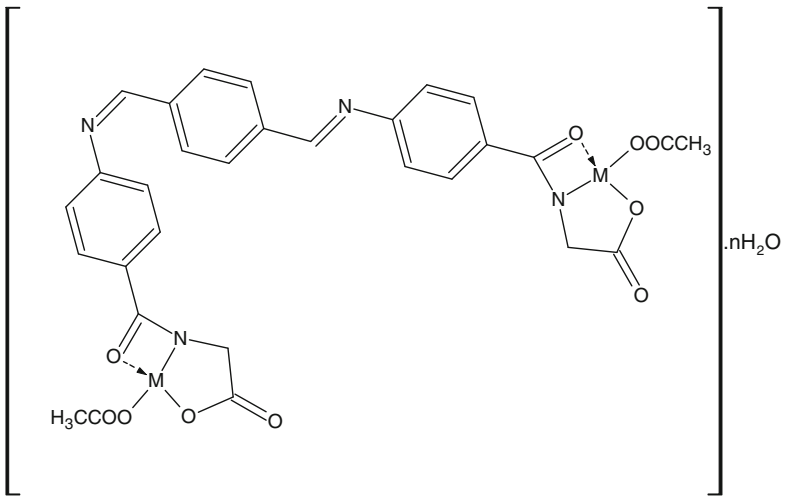


Fig. 4 Suggested structures of the Zn(II) and Ni(II) complexes of the ligand (L^1)

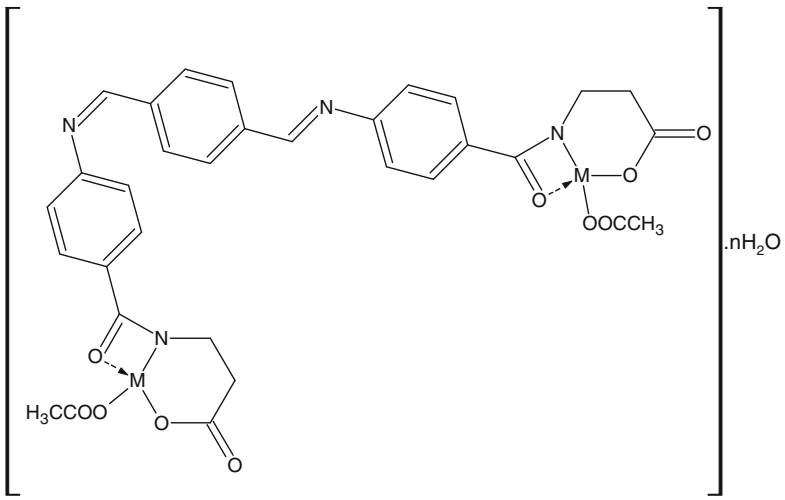


Fig. 5 Suggested structures of the Zn(II) and Ni(II) complexes of the ligand (L^2)

not coordinated but are present in the form of lattice water (Fig. 4).

The Zn(II) complexes were stable up to 315 and 330 °C, respectively. Their decomposition started at these temperatures. In the decomposition process of the Zn(II) complexes, the mass losses corresponded to two acetates in the first stage of the decomposition of the Zn(II) complexes. The higher dehydration temperatures of Zn(II) complexes suggest that the water molecule is not coordinated to the metal ions (Fig. 5).

Bioactivity

Cytotoxicity assays were carried out by means of trypan blue test. Cultures grown in each MCF-7 cell line were exposed to an higher concentration of test compounds (7.5–60 μM) for 24 and 48 h. A dose-dependent decrease in cell proliferation was clearly observed in the cell lines studied (Tables 3, 4). Treated samples were significantly different from the control in MCF-7 cells.

It is shown in MCF-7 cell lines that all compounds inhibit cell viability that may induce lipid peroxidation. Our study shows that L^1 and its compounds induced loss of cell proliferation in concentration- and time-dependent manner for cell lines. L^1Zn possess greater antitumoral activity against MCF-7 in comparison with L^1, Cu(II), and Ni(II) complexes.

L^2 and its metal complexes showed only time-dependent antitumoral activity in MCF-7 cell cultures. It was seen that cytotoxicity activities of the L^2 and L^2Zn complexes on MCF-7 cells were greater than those of the Cu(II) and Ni(II) complexes. The results show that the Zn(II) complexes have significant cytotoxic activity toward cell line (Turan *et al*., 2011).

Table 3 Dose- and time-dependent cell viability results in MCF-7 cells after exposure to L^1 ligand and its complexes

Groups ($N = 6$)	24 h 7.5 μM	24 h 15 μM	24 h 30 μM	24 h 60 μM	48 h 7.5 μM	48 h 15 μM	48 h 30 μM	48 h 60 μM
Control	93.00 ± 1.06	90.33 ± 0.56	82.83 ± 1.51	74.50 ± 0.76	83.83 ± 1.05	79.83 ± 1.37	83.16 ± 1.42	71.66 ± 0.76
L^1	68.00 ± 2.13[c]	49.00 ± 2.57[c]	50.66 ± 1.33[c]	18.17 ± 2.59[c]	49.33 ± 2.82[c]	36.50 ± 2.23[c]	34.66 ± 1.89[c]	11.00 ± 4.16[c]
L^1–Ni	56.33 ± 3.01[c]	50.83 ± 4.38[c]	34.50 ± 1.43[c]	15.33 ± 1.87[c]	34.17 ± 1.22[c]	27.66 ± 3.36[c]	13.83 ± 1.54[c]	0.83 ± 0.54[c]
L^1–Cu	81.33 ± 1.05[c]	77.00 ± 1.39[c]	60.00 ± 2.37[c]	29.66 ± 1.20[c]	69.83 ± 1.25[c]	37.17 ± 5.01[c]	45.66 ± 0.95[c]	5.00 ± 2.25[c]
L^1–Zn	39.16 ± 1.95[c]	30.50 ± 1.18[c]	30.00 ± 0.82[c]	9.17 ± 2.68[c]	16.83 ± 1.26[c]	6.66 ± 2.12[c]	3.00 ± 1.91[c]	2.83 ± 1.56[c]

[a] $p < 0.05$

[b] $p < 0.01$

[c] $p < 0.001$

Table 4 Dose- and time-dependent cell viability results in MCF-7 cells after exposure to L^2 ligand and its complexes

Groups ($N = 6$)	24 h 7.5 μM	24 h 15 μM	24 h 30 μM	24 h 60 μM	48 h 7.5 μM	48 h 15 μM	48 h 30 μM	48 h 60 μM
Control	88.83 ± 0.6	86.00 ± 0.97	81.00 ± 0.68	77.33 ± 0.67	87.17 ± 0.6	85.67 ± 0.88	79.83 ± 0.31	75.33 ± 0.42
L^2	63.33 ± 1.52[c]	46.33 ± 0.76[c]	50.83 ± 3.02[c]	42.00 ± 0.82[c]	34.00 ± 2.05[c]	24.50 ± 1.54[c]	28.17 ± 1.19[c]	8.50 ± 2.17[c]
L^2–Ni	74.50 ± 0.84[c]	42.83 ± 2.01[c]	58.00 ± 2.11[c]	34.17 ± 1.11[c]	31.17 ± 2.32[c]	24.17 ± 1.35[c]	24.83 ± 2.27[c]	3.00 ± 1.15[c]
L^2–Cu	77.00 ± 0.58[c]	63.17 ± 0.87[c]	51.50 ± 1.94[c]	34.67 ± 1.41[c]	41.67 ± 2.45[c]	36.50 ± 1.50[c]	21.50 ± 1.28[c]	0.50 ± 0.50[c]
L^2–Zn	57.50 ± 0.88[c]	46.67 ± 1.52[c]	54.50 ± 0.96[c]	33.33 ± 1.36[c]	29.33 ± 1.09[c]	6.67 ± 1.56[c]	22.50 ± 2.31[c]	2.00 ± 1.48[c]

[a] $p < 0.05$

[b] $p < 0.01$

[c] $p < 0.001$

Lipid peroxidation level

ROS are formed and degraded by all aerobic organisms, leading to either physiological concentrations required for normal cell function or excessive quantities, the state of which is called oxidative stress (Nordberg and Arner, 2001). ROS-mediated oxidation of membrane lipids results in the formation of lipid peroxidation products such as MDA (Maccarrone *et al*., 1997) and isoprostanes (Morrow, 2003). The cellular antioxidant systems can be divided into two major groups: enzymatic, and nonenzymatic. Some nonenzymatic low molecular weight antioxidant compounds such as ascorbic acid, α-tocopherol, and carotenoids are consumed and may fall below normal ranges. Determinations of enzymatic and/or nonenzymatic antioxidant levels and MDA levels in the samples are important in the evaluation of oxidative stress in biological systems.

To assess lipid peroxidation induced by the compounds tested, we determined MDA levels, an end product of lipid peroxidation considered as a late biomarker of oxidative stress, and cellular damage. Lipid degradation and consequently MDA production alter the structure and function of the cellular membrane and block cellular metabolism leading to cytotoxicity (Ennamany *et al*., 1995). MDA is generally considered to be an excellent indicator of lipid oxidation (Bird and Draper, 1984; Wendel and Reiter, 1984). Our results show that the compounds tested induced oxidative damage by increasing lipid peroxidation in yeast since MDA formation was increased (Table 5). In summary, it may be concluded that the compounds tested caused oxidative stress.

Table 5 *Saccharomyces cerevisiae* yeast cells' mean MDA levels (mg/2 × 10^6 cells) for 24 h, according to the doses, treated with L^1, L^2, and their complexes

MDA	(50 μM)	(100 μM)
Control	0.515 ± 0.02	0.515 ± 0.02
L^1	1.05 ± 0.08[b]	1.15 ± 0.00[b]
L^1–Ni	1.02 ± 0.06[a]	1.04 ± 0.00[b]
L^1–Cu	1.01 ± 0.03[a]	1.10 ± 0.03[b]
L^1–Zn	1.13 ± 0.00[a]	1.16 ± 0.02[b]
L^2	0.561 ± 0.05	0.587 ± 0.03
L^2–Ni	0.615 ± 0.01	0.803 ± 0.04[b]
L^2–Cu	0.63 ± 0.00	0.88 ± 0.02[a]
L^2–Zn	0.55 ± 0.15	0.63 ± 0.03

[a] $p < 0.05$

[b] $p < 0.01$

[c] $p < 0.001$

Table 6 Free radical scavenging activities of samples, trolox, and tocopherol by DPPH radicals (%)

Samples (1,000 μg/mL)	$DPPH^{\bullet}$ scavenging activity (%)
Control	0
L^1	29.53
L^1–Zn	33.43
L^1–Cu	31.76
L^1–Ni	31.33
L^2	32.99
L^2–Zn	54.72
L^2–Cu	34.94
L^2–Ni	34.29
Trolox	94.00
Tocopherol	89.45

DPPH radical scavenging activity

The model of scavenging the stable DPPH radical is a widely used method to evaluate antioxidant activities in a relatively short time in comparison with the other methods. The effect of antioxidants on DPPH radical scavenging was thought to be because of their hydrogen-donating ability. $DPPH^{\bullet}$ is a stable, free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Talaz *et al*., 2009). The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm induced by antioxidants. The decrease in the absorbance of DPPH radical was caused by antioxidants, because of the reaction between antioxidant molecules and radical progresses, which results in the scavenging of the radical by hydrogen donation. It is visually noticeable in the form of a discoloration from purple to yellow. Hence, $DPPH^{\bullet}$ is usually used as a substrate to evaluate antioxidative activity of antioxidants. DPPH scavenging activities of all complexes were lower than those of the standard antioxidants, tocopherol and trolox (Table 6).

According to lipid peroxidation and antiradical activity results, while the synthesized compounds showed increased MDA levels in yeast cells, these compounds showed lower antiradical activities in DPPH radical scavenging assay. In fact, these results are complementary to each other.

Experimental

All reagents were of commercial quality, and solvents were used without further purification. The human breast carcinoma MCF-7 cell line was obtained from the American type culture collection (ATCC). Dulbecco's modified eagle's medium (DMEM) and newborn calf serum were purchased from Hyclone (Waltham, MA, USA); and trypsin, penicillin, streptomycin. Infrared (IR) spectrum was determined on a Perkin-Elmer Spectrum One Fourier transform-infrared (FT-IR) spectrometer. Electronic spectral studies were conducted on a Shimadzu model UV-1700 Spectrophotometer in the wavelength range of 1000–200 nm. NMR spectra were recorded on Bruker 300 MHz spectrometer. Thermal analyses (TGA and DTA) of compounds were carried out in nitrogen atmosphere at a heating rate of 20 °C/min using Shimadzu TA 60WS thermal analyzer. Elemental analyses were performed on a LECO model 932 instrument. The cells were incubated under 5 % CO_2 air at 37 °C conditions in Nuaire humidified carbon dioxide incubator (Playmouth, MN, USA). Cells' conditions were checked by means of inverted microscope (Soif Optical Inc. China). Results are expressed as mean ± SE. Statistical analysis and comparison between mean values for cytotoxicity were performed by Tukey variance analysis (SPSS 10.0 for Windows; Chicago, IL, USA).

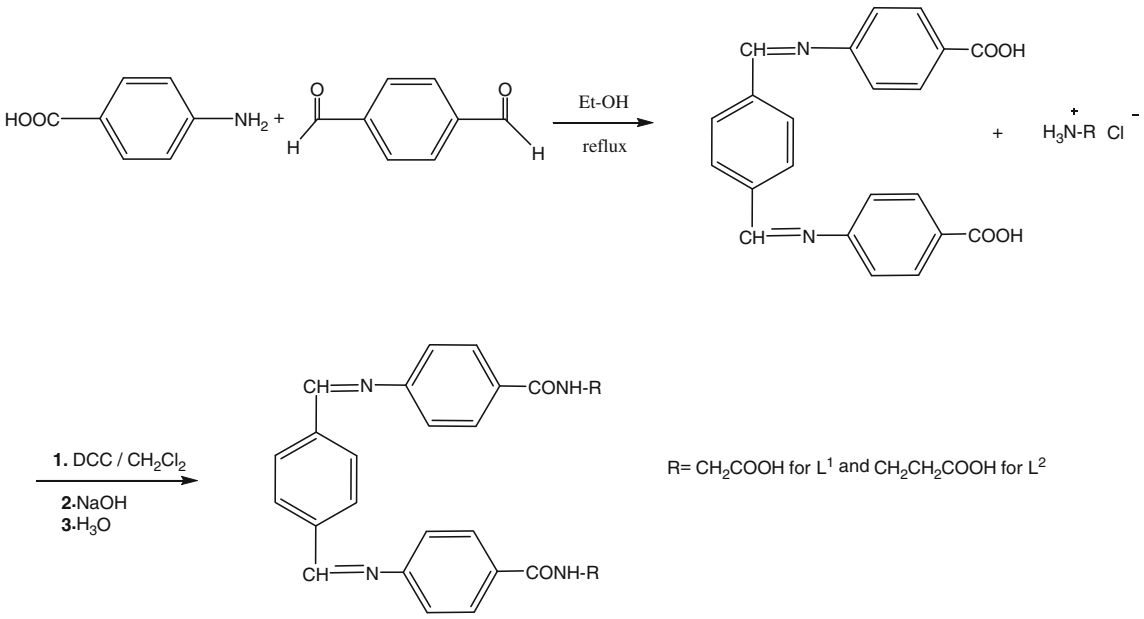
Synthesis of the ligands (L^1 and L^2)

Ligands (L^1 and L^2) were synthesized according to the literature (Colak and Yildirir, 2008). *Ligand (L^1)*: IR (KBr, cm^{-1}): *ν*(NH) 3,319, ν(Ar–H) 3,075, ν(CON) 1,708, *ν*(COOH) 1,645, *ν*(C=N) 1,615. ^{1}H-NMR (300 MHz, DMF-d_7/TMS): *δ* (ppm) 4.15 (d, 2H, H-1), 7.45 (d, 2H, H-b), 8.12 (d, 2H, H-c), 8.21 (s, 2H, H-e), 8.86 (s, 1H, CH=N), 8.91 (t, 1H, CONH), 12.95 (b, 1H, COOH). ^{13}C APT (75 MHz, DMF-d7/TMS): *δ* (ppm): Positive amplitude: 41.19, 129.95, 141.21, 154.42, 166.41 (CONH), 171.83 (COOH), Negative amplitude: 121.09, 126.76, 129.95, 161.20 (CH=N).

Ligand (L^2): IR (KBr, cm^{-1}): *ν*(NH) 3326, *ν*(Ar–H) 3,063, *ν*(C–H) 2,930, ν(CO) 1,708, ν(C=N) 1,631. ^{1}H-NMR (300 MHz, DMF-d_7/TMS): *δ* (ppm) 2.92 (t, 2H, CH_2), 3.66 (q, 2H, NCH_2), 7.43 (d, 2H, H-b), 8.12 (d, 2H, H-c) 8.24 (s, 2H, H-e), 8.63 (t, 1H, CONH), 8.86 (s, 1H, CH=N), 12.60 (b, 1H, COOH). ^{13}C APT (75 MHz, DMF-d_7/TMS): *δ* (ppm): Positive amplitude: 33.88, 36.05, 129.96, 141.22, 154.21, 166.11 (CONH), 173.06 (COOH), Negative amplitude: 121.02, 128.77, 129.52, 161.27 (CH=N) (Scheme 1).

Synthesis of the Ni(II), Cu(II), and Zn(II) Complexes of Ligand (L^1) and Ligand (L^2)

The proposed formula of the ligand is found to be in good agreement with the stoichiometry results as concluded from its analytic data (Table 1). Schiff base complexes under investigation were prepared by mixing 20 mL hot DMF of the Schiff base (1.02 g; 2.1 mmol for L^1 and 1.07 g; 2.1 mmol for L^2) with 25 mL ethanolic solution of the metal salts [$Cu(Ac)_2{\cdot}H_2O$ (0.419 g, 2.1 mmol), $Ni(Ac)_2{\cdot}4H_2O$ (1.045 g, 4.1 mmol), and $Zn(Ac)_2$ (0.770 g,



Scheme 1 General procedure of the synthesized ligands

4.2 mmol)]. The obtained mixture was refluxed for about 3 h, and then kept overnight to insure the complete reactions. Then, the formed complexes were filtered, collected, and washed several times with hot ethanol until the filtrate becomes colorless. The yield ranged from 42 to 69 %. The dried complexes were subjected to elemental and spectroscopic analyses.

Cell culture and treatment

MCF-7 cancer cell line was grown as monolayered culture in a high glucose (4.5 g/l). DMEM medium supplemented with 10 % fetal calf serum (FCS), 1 % L-glutamine (200 mM), and 1 % of penicillin/streptomycin incubated at 37 °C in an air-humidified incubator at 5 % CO2.

Cell viability

For the cytotoxicity analysis of test compounds, cells were seeded at 1 × 106 cell/mL of MCF-7 cells per eppendorf tubes in six replicates. The test compounds were dissolved in DMSO. Proliferative MCF-7 cells were seeded in flasks and incubated for 24 h. After preincubation, the cell-culture medium was replaced with fresh medium. Each test compound was added to the medium within the concentration range of 7.5, 15, 30, and 60 μM, and the cells were incubated under 5 % CO_2 air at 37 °C conditions. After 24 h and 48 h of incubation, cell viability was measured using Trypan blue exclusion method. Vehicle-treated tubes served as controls. The final concentration of DMSO in culture medium did not exceed 0.5 %.

Determination of MDA level

MDA levels in *S. cerevisiae* yeast cells in this study were measured by means of high-performance liquid chromatography (HPLC) using the previously described methods for MDA (Karatepe, 2004).

DPPH$^\bullet$ free radical scavenging activity

The free radical scavenging activities of complexes were measured by DPPH$^\bullet$ according to the method described by Shimada *et al*. (1992). In brief, 0.1 mM solution of DPPH$^\bullet$ in ethanol was prepared. 3 mL of the solution was added to 1000 μg/mL of complexes solution. The mixture was allowed to remain at room temperature for 30 min. Then, the absorbance was measured at 517 nm by means of a spectrophotometer (Shimadzu UV-1700 Spectrophotometer). Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The percent DPPH$^\bullet$ scavenging effect can be calculated using the following equation (Keser *et al*., 2012):

$$\% \text{ DPPH}^\bullet \text{ scavenging activity} = [A_C - A_S/A_C] \times 100$$

where A_C is the absorbance of the control reaction, and A_S is the absorbance in the presence of the synthesized compounds.

Statistical analysis

Results are expressed as mean ± SE Statistical analysis, and comparison between mean values for cytotoxicity were

performed by Tukey variance analysis (SPSS 10.0 for Windows). LSD test was used to analyze the antioxidant parameters. Level of statistical significance was set at $p < 0.05$.

Conclusions

The synthesized Schiff bases are tridentate ligands. The metal ions are coordinated through the oxygen and nitrogen groups of the CONH and OH groups. The bonding of ligands to the metal ion is confirmed by the analytic, spectral, magnetic, and thermal studies. Representative structures of Cu(II), Ni(II), and Zn(II) complexes are presented in Figs. 1, 2, 3, 4, 5. The Schiff bases and their metal complexes were found to be highly active against MCF-7 cell lines. In addition, it can be said that the synthesized compounds have low antioxidative as well as high oxidative properties.

References

Abd El-Halim HF, Omar MM, Mohamed GG (2011) Synthesis, structural, thermal studies and biological activity of a tridentate Schiff base ligand and their transition metal complexes. Spectrochim Acta A 78:36–44

Bellamy LJ (1964) The infrared spectra of complex molecules, 2nd edn. Wiley, New York

Bird RP, Draper HH (1984) Methods in enzymology. Academic, New York

Campbell MJM (1975) Transition metal complexes of thiosemicarbazide and thiosemicarbazones. Coord Chem Rev 15:279–319

Castillo-Blum SE, Barba-Behrens N (2000) Coordination chemistry of some biologically active ligands. Coord Chem Rev 196:3–30

Colak N, Yildirir Y (2008) Synthesis of {substituted-[(4-{[4-(carboxymethyl-carbamoyl)phenylimino]methyl}benzylidene)-amino] benzoylamino}carboxylic acids. Asian J Chem 20:2349–2357

Collinson SR, Fenton DE (1996) Metal complexes of bibracchial Schiff base macrocycles. Coord Chem Rev 148:19–40

Dogan HN, Rollas S, Erdeniz H (1998) Synthesis, structure elucidation and antimicrobial activity of some 3-hydroxy-2-naphthoic acid hydrazide derivatives. Farmaco 53:462–467

Dubey AK, Sangwan NK (1994) Synthesis and antifungal activity of 5-(3,5-diphenylpyrazol-4-yloxymethyl)-2-(4-oxo-2-substituted phenyl-3-thiazolidinyl)-1,3,4-oxadiazoles thiadiazoles and related-compounds. Indian J Chem Sect B 33:1043–1047

Dwyer FB, Mayhew E, Roe EMF, Shulman A (1965) Inhibition of Landschütz Ascites tumour growth by metal chelates derived from 3,4,7,8-tetramethyl-1,10-phenanthroline. Br J Cancer 19: 195–199

El-Sherif AA, Eldebss TMA (2011) Synthesis, spectral characterization, solution equilibria, in vitro antibacterial and cytotoxic activities of Cu(II), Ni(II), Mn(II), Co(II) and Zn(II) complexes with Schiff base derived from 5-bromosalicylaldehyde and 2-aminomethylthiophene. Spectrochim Acta A 79:1803–1814

Ennamany R, Marzetto S, Saboureau D, Creppy EE (1995) Lipid peroxidation induced by bolesatine, a toxin of *Boletus satanas*: implication in m(5)dC variation in vero cells related to inhibition of cell growth. Cell Biol Toxicol 11:347–354

Etaiw SEH, Abd El-Aziz DM, Abd El-Zaher EH, Ali EA (2011) Synthesis, spectral, antimicrobial and antitumor assessment of Schiff base derived from 2-aminobenzothiazole and its transition metal complexes. Spectrochim Acta A 79:1331–1337

Furst A, Haro RT (1969) A survey of metal carcinogenesis. Prog Exp Tumor Res 12:102–133

Gao WT, Zheng Z (2002) Synthetic studies on optically active Schiff-base ligands derived from condensation of 2-hydroxyacetophenone and chiral diamines. Molecules 7:511–516

Jayabalakrishnan C, Natarajan K (2002) Ruthenium(II) carbonyl complexes with tridentate Schiff bases and their antibacterial activity. Transition Met Chem 27:75–79

Karatepe M (2004) Simultaneous determination of ascorbic acid and free malondialdehyde in human serum by HPLC-UV. LC-GC North Am 22:362–365

Keser S, Turkoglu S, Celik S, Turkoglu I (2012) Determination of antioxidant capacities of *Phlomis pungens* Willd. var. *hispida* Hub.-Mor. Asian J Chem 24:2780–2784

Maccarrone M, Catani MV, Iraci S, Melino G, Agro AF (1997) A survey of reactive oxygen species and their role in dermatology. J Eur Acad Dermatol Venereol 8:185–202

Mohan J, Anjaneyulu GSR, Verma P, Yamini KVS (1990) Heterocyclic-systems containing bridge-head nitrogen atom-synthesis and anti-microbial activity of S-triazolo [3,4-B]-[1,3,4] thiadiazines, thiazolo [3,2-B]-S-triazoles and isomeric thiazolo-[2,3-C]-S-triazoles. Indian J Chem Sect B 29:88–90

Morrow JD (2003) The isoprostanes: their quantification as an index of oxidant stress status in vivo. Drug Metab Rev 32:377–385

Nordberg J, Arner ESJ (2001) Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. Free Radical Biol Med 31:1287–1312

Ouyang XM, Fei BL, Okamuro TA, Sun WY, Tang WX, Ueyama N (2002) Synthesis, crystal structure and superoxide dismutase (SOD) activity of novel seven-coordinated manganese(II) complex with multidentate di-Schiff base ligands. Chem Lett 31:362–363

Priya NP, Arunachalam S, Manimaran A, Muthupriya D, Jayabalakrishnan C (2009) Mononuclear Ru(III) Schiff base complexes: synthesis, spectral, redox, catalytic and biological activity studies. Spectrochim Acta A 72:670–676

Raman N, Raja YP, Kulandaismy A (2001) Synthesis and characterisation of Cu(II), Ni(II), Mn(II), Zn(II) and VO(II) Schiff base complexes derived from *o*-phenylenediamine and acetoacetanilide. Proc Indian Acad Sci (Chem Sci) 113:183–189

Ravoof TBSA, Crouse KA, Tahir MIM, How FNF, Rosli R, Watkins DJ (2010) Synthesis, characterization and biological activities of 3-methylbenzyl 2-(6-methyl pyridin-2-ylmethylene)hydrazine carbodithioate and its transition metal complexes. Transition Met Chem 35:871–876

Sakıyan I, Logoglu E, Arslan S, Sari N, Şakiyan N (2004) Antimicrobial activities of N-(2-hydroxy-1-naphthalidene)-amino acid(glycine, alanine, phenylalanine, histidine, tryptophane) Schiff bases and their manganese(III) complexes. Biometals 17:115–120

Satyaranayana S, Nagasundura KR (2004) Synthesis and Spectral Properties of the Complexes of Cobalt(II), Nickel(II), Copper(II), Zinc(II), and Cadmium(II) with 2-(Thiomethyl-2′-benzimidazolyl)-benzimidazole. Synth React Inorg Met–Org Chem 34:883–895

Sharghi H, Nasser MA (2003) Schiff-base metal(II) complexes as new catalysts in the efficient, mild and regioselective conversion of 1,2-epoxyethanes to 2-hydroxyethyl thiocyanates with ammonium thiocyanate. Bull Chem Soc Jpn 76:137–142

Sharma AK, Chandra S (2011) Complexation of nitrogen and sulphur donor Schiff's base ligand to Cr(III) and Ni(II) metal ions: synthesis, spectroscopic and antipathogenic studies. Spectrochim Acta A 78:337–342

Shimada K, Fujikawa K, Yahara K, Nakamura T (1992) Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J Agric Food Chem 40:945–948

Sonmez M, Levent A, Sekerci M (2004) Synthesis, characterization, and thermal investigation of some metal complexes containing polydentate ONO-donor heterocyclic Schiff base ligand. Russ J Coord Chem 30:655–659

Talaz O, Gulcin I, Goksu S, Saracoglu N (2009) Antioxidant activity of 5,10-dihydroindeno[1,2-b]indoles containing substituents on dihydroindeno part. Bioorg Med Chem 17:6583–6589

Turan N, Topcu MF, Ergin Z, Sandal S, Tuzcu M, Akpolat N, Yilmaz B, Sekerci M, Karatepe M (2011) Drug Chem Toxicol 34(4):369–378

Wendel A, Reiter R (1984) Oxygen radicals in chemistry and biology. Walter de Gruyter, Berlin

Williams DR (1972) Metals, ligands, and cancer. Chem Rev 72: 203–213

Zaki ZM, Haggag SS, Soayed AA (1998) Studies on some Schiff base complexes of Co(II), Ni(II) and Cu(II) Derived from salicylaldehyde and o-nitrobenzaldehyde. Spectrosc Lett 31:757–766