Spectral characterization and antimicrobial activity of Cu(II) and Fe(III) complexes of 2-(5-Cl/NO₂-1*H*-benzimidazol-2-yl)-4-Br/NO₂-phenols

Aydin Tavman[a]; Ismail Boz[b]; A. Seher Birteksöz[c]; Adem Cinarli[a]

[a] Faculty of Engineering, Department of Chemistry, Istanbul University, 34320 Avcilar, Istanbul, Turkey [b] Faculty of Engineering, Department of Chemical Engineering, Istanbul University, 34320 Avcilar, Istanbul, Turkey [c] Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Istanbul University, 34452 Beyazit, Istanbul, Turkey

First published on: 26 April 2010

PLEASE SCROLL DOWN FOR ARTICLE

Spectral characterization and antimicrobial activity of Cu(II) and Fe(III) complexes of 2-(5-Cl/NO$_2$-1*H*-benzimidazol-2-yl)-4-Br/NO$_2$-phenols

AYDIN TAVMAN*†, ISMAIL BOZ‡, A. SEHER BIRTEKSÖZ§
and ADEM CINARLI†

†Faculty of Engineering, Department of Chemistry, Istanbul University,
34320 Avcilar, Istanbul, Turkey
‡Faculty of Engineering, Department of Chemical Engineering,
Istanbul University, 34320 Avcilar, Istanbul, Turkey
§Faculty of Pharmacy, Department of Pharmaceutical Microbiology,
Istanbul University, 34452 Beyazit, Istanbul, Turkey

The compounds of 2-(5-chloro/nitro-1*H*-benzimidazol-2-yl)-4-bromo/nitrophenols (HL$_X$: X = 1–4) and their copper(II) nitrate and iron(III) nitrate complexes have been synthesized and characterized. The structures of the complexes were confirmed on the basis of elemental analysis, thermal gravimetric analysis, molar conductivity and magnetic moment measurements, FT-IR, mass, and UV-Vis spectroscopy techniques. The complexes show high-thermal stability with $> 350°$C m.p. In all complexes, the ligands are bidentate *via* one imine nitrogen and a phenolate oxygen. Cu(II) complexes having 1 : 2 M : L ratio are classified as non-electrolytes, whereas 1 : 1 M : L ratio is observed in Fe(III) complexes except [Fe(L$_3$)$_2$(H$_2$O)$_2$](NO$_3$)·3H$_2$O. The antimicrobial activities of the ligands and the complexes were evaluated using the disc diffusion method in DMSO as well as minimum inhibitory concentration dilution method against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*. Antifungal activities were reported for *Candida albicans*. The complexes [Fe(L$_3$)$_2$(H$_2$O)$_2$](NO$_3$)·3H$_2$O and [Cu(L$_3$)$_2$]·2H$_2$O are more effective against *S. epidermidis* than ciprofloxacin.

Keywords: Benzimidazole; Bromo/nitrophenols; Iron(III) and copper(II) complexes; Antimicrobial activity

1. Introduction

The compound 2-(1*H*-benzimidazol-2-yl)-phenol and its derivatives are used as chelating agents with various transition metal complexes reported in [1–4]. For example, a mixed-ligand Cr(III) complex [5], the tris-chelate Co(III) and the dinuclear Cr(III) coordination compounds [6], and a Cu(II) complex [7] with 2-(2-hydroxyphenyl)benzimidazole were reported. Complexes of iron(III) derived

---

*Corresponding author. Email: atavman@istanbul.edu.tr

from 2-(2′-hydroxyphenyl)benzimidazole (HPB) and 2-(2′-hydroxybenzyl)-N-methyl-benzimidazole (HMPB) were reported: Oxo-bridged and hydroxo complexes were obtained as $[Fe(PB)_2]_2O$ and $[Fe(MPB)_2(OH)_2] \cdot 1/2H_2O$ [3]. A series of binuclear square-planar complexes of Cu(II) with hydroxy bridges and Fe(II) and Fe(III) binuclear octahedral complexes with 1-(2′-hydroxybenzyl)-2-(2′-hydroxyphenyl)benz-imidazole were synthesized [8]. In some U(VI) and Ce(IV) complexes of 1-(2′-hydroxybenzyl)-2-(2′-hydroxyphenyl)-benzimidazole, the IR spectral data sug-gested that the ligand was monodentate through the tertiary nitrogen and that the phenolic oxygen was free from coordination [9]. On the other hand, X-ray crystal structure studies show coordination through C=N nitrogen and phenolate oxygen in the bis[2-(1H-benzimidazol-2-yl-κN^3)-phenolato-κO]metal(II) type complexes (metal : zinc [10], nickel [11], and cobalt [12]).

Antimicrobial activity of some 2-(1*H*-benzimidazol-2-yl)-phenol derivatives and their metal complexes were investigated against selected microorganisms [1, 2, 13]. The ligands were especially effective on *Staphylococcus aureus*; Ag(I) and Hg(II) complexes showed high antimicrobial activity toward all bacteria; Fe(III) complexes exhibited considerable activity against *S. aureus* and *Staphylococcus epidermidis* [1]. Some benzimidazolyl phenols and their Fe(III) complexes showed considerable activity on some bacteria and fungi [2, 13]. Co(II) and Ni(II) chelates of 2-(2′-hydroxyphenyl)-benzimidazole show antifungal activity against *Alternaria alternata* and *Aspergillus niger* [14, 15].

Iron and copper are the essential elements that play vital roles in biological systems. Thus, investigations of the coordination chemistry of Cu(II) continue to be stimulated by interest in developing models for copper proteins and understanding the way they react [16–18]. The extraordinary properties of these proteins, such as intense blue, are related to the specific coordination geometries as well as the nature of the donors, namely, histidine imidazole, cysteine sulfur, and methionine sulfur [19, 20]. One of the most abundant transition elements is iron and, as an essential element, it has more roles in biological systems than any other metal. One of the most important aspects is its oxygen-binding function to hemoglobin, myoglobin, and cytochromes [21].

Studies on characterization and antimicrobial activity of benzimidazolyl phenol type compounds and their complexes are rather scarce. In this study, we synthesize and characterize new benzimidazole derivatives (scheme 1) and their Fe(III) and Cu(II) complexes, and show their antimicrobial activities. The antibacterial and antifungal activity of the compounds is evaluated by the disc diffusion method against six types of bacteria and *Candida albicans* as yeast. Herein, we discuss the differences of metal ion complexes with the free ligands in their structural–biological activity relationship.

2. Experimental

2.1. *Chemistry and apparatus*

All the chemicals and solvents were of reagent grade and purchased from Merck and used without purification.

Analytical data were obtained with a Thermo Finnigan Flash EA 1112 analyzer. Melting points were determined using an electrothermal melting point apparatus.
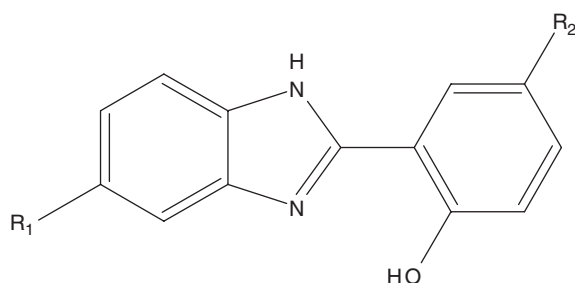
Scheme 1. The structure of the ligands: HL_1: $R_1 = Cl$, $R_2 = Br$; HL_2: $R_1 = Cl$, $R_2 = NO_2$; HL_3: $R_1 = NO_2$, $R_2 = Br$; HL_4: $R_1 = NO_2$, $R_2 = NO_2$.

Molar conductivity of the complexes was measured on a WTW Cond315i conductivity meter in DMSO at 25°C. FT-IR spectra were recorded as KBr discs on a Nicolet 380 FT-IR spectrometer. The electron spray ionization–mass spectroscopy (ESI-MS) analyses were carried out in positive ion modes using a Thermo Finnigan LCQ Advantage MAX LC/MS/MS. UV-Vis spectra were obtained on a Perkin Elmer Lambda 25 UV-Vis spectrometer. Thermogravimetric studies were made on a TG-60WS Shimadzu, with a heating rate of $10°C\,min^{-1}$ and air flow at the rate of $50\,mL\,min^{-1}$. Magnetic measurements were carried out using MK1 Sherwood Scientific apparatus at room temperature by Gouy's method. Diamagnetic corrections were made using Pascal's constants.
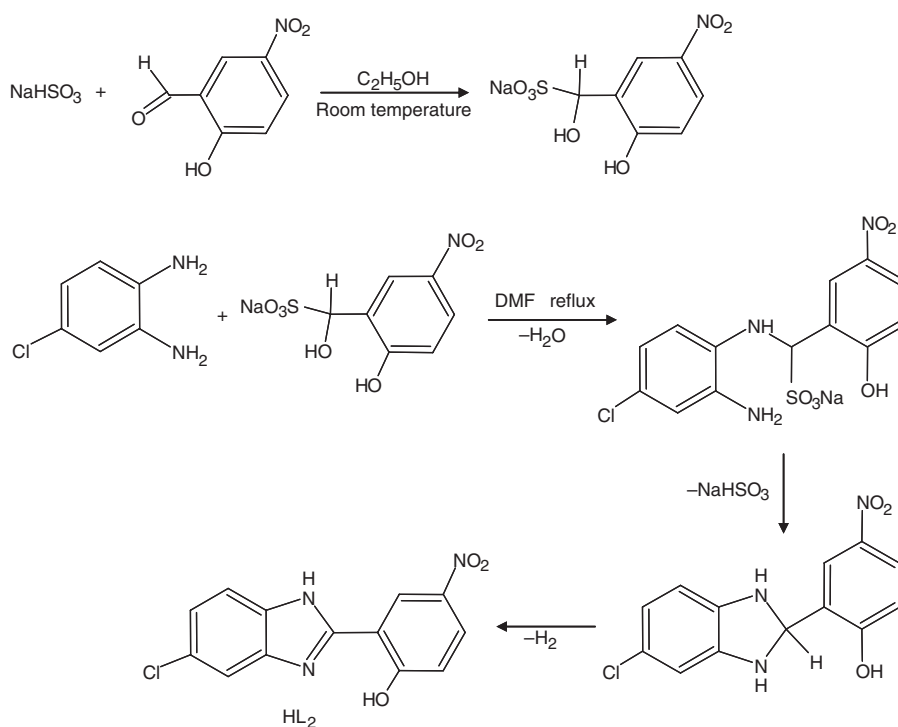
2.2. *Synthesis of the ligands: general procedure*

The ligands were prepared according to the procedures in literature [4, 22]. For instance, 2-(5-chloro-1*H*-benzimidazol-2-yl)-4-nitrophenol (HL_2) has been obtained by the reaction of 2-hydroxy-5-nitrobenzaldehyde (1.67 g, 10 mmol) with an equivalent of $NaHSO_3$ (1.04 g, 10 mmol) at room temperature in 25 mL ethanol for ~5 h. The mixture was treated with 4-chloro-1,2-phenylenediamine (1.42 g, 10 mmol) in dimethylformamide (15 mL) and gently refluxed for 2 h (scheme 2). The reaction mixture was then poured into ice water (500 mL), filtered, and crystallized from ethanol.

2.3. *Synthesis of the complexes*

2.3.1. $[Cu(L_1)_2] \cdot 2H_2O$. 162 mg HL_1 (~0.5 mmol) suspended in methanol (10 mL) and 121 mg $Cu(NO_3)_2 \cdot 3H_2O$ (0.5 mmol) in methanol (5 mL) were added to the ligand solution. After 2 h of reflux, the precipitate was filtered, washed with methanol (5 mL), and dried at 70–80°C. The other Cu(II) complexes were prepared in a manner similar to $[Cu(L_1)_2] \cdot 2H_2O$.

2.3.2. $[Fe(L_1)(OH)(H_2O)_3](NO_3) \cdot 3H_2O$. 162 mg HL_1 (~0.5 mmol) suspended in isopropanol (10 mL) and 210 mg $Fe(NO_3)_3 \cdot 9H_2O$ (>0.5 mmol) in isopropanol (5 mL) were added to the ligand solution. After 2 h of reflux, the precipitate was filtered, washed with isopropanol (5 mL), and dried at 70–80°C. The other Fe(III)

NaHSO$_3$ +   (2-hydroxy-5-nitrobenzaldehyde) $\xrightarrow[\text{Room temperature}]{C_2H_5OH}$ NaO$_3$S (bisulfite adduct)

(4-chlorobenzene-1,2-diamine) + NaO$_3$S (adduct) $\xrightarrow[-H_2O]{\text{DMF} \quad \text{reflux}}$ (amino intermediate with SO$_3$Na)

$\xrightarrow{-NaHSO_3}$ (dihydrobenzimidazole)

(dihydrobenzimidazole) $\xrightarrow{-H_2}$ HL$_2$ (benzimidazole phenol)

Scheme 2.   Synthesis of HL$_2$.

complexes were prepared in a manner similar to $[Fe(L_1)(OH)(H_2O)_3](NO_3) \cdot 3H_2O$; the results are presented in table 1.

2.4. *Determination of antimicrobial activity*

Antimicrobial activities against *S. aureus* ATCC 6538, *S. epidermidis* ATCC 12228, *Escherichia coli* ATCC 8739, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 14153, and *C. albicans* ATCC 10231 were determined by the microbroth dilutions technique using the National Committee for Clinical Laboratory Standards (NCCLS) recommendations [23, 24]. Mueller-Hinton broth for bacteria, RPMI-1640 medium buffered to pH 7.0 with morpholinepropanesulfonic acid (MOPS) for yeast strain was used as the test medium. Serial twofold dilutions ranging from 5000 to $4.9 \, \mu g \, mL^{-1}$ were prepared in the medium. As control, antimicrobial effects of the solution were investigated against test microorganisms. The inoculum was prepared using a 4–6 h broth culture of each bacteria and 24 culture of yeast strains adjusted to a turbidity equivalent to a 0.5 McFarland standard, diluted in broth media to give a final concentration of $5 \times 10^5 \, cfu \, mL^{-1}$ for bacteria and from 0.5×10^3 to $2.5 \times 10^3 \, cfu \, mL^{-1}$ for yeast in the test tray. The trays were covered and placed in plastic bags to prevent evaporation. Trays containing Mueller-Hinton broth were incubated at 35°C for 18–20 h and the trays containing RPMI-1640 medium were incubated at 35°C for 46–50 h.

Table 1. Analytical data and physical properties of the ligands and complexes.

Compound	Elemental analysis: Found (Calcd) (%)			Y (%)	m.p. (°C)	M (B.M.)	Λ (Ω^{-1} cm^2 mol^{-1})	Color
	C	H	N					
HL$_1$ C$_{13}$H$_8$BrClN$_2$O	48.4 (48.2)	2.8 (2.5)	8.6 (8.7)	64	309	0	–	Dirty white
HL$_2$ C$_{13}$H$_8$ClN$_3$O$_3$	54.2 (53.9)	3.1 (2.8)	14.4 (14.5)	63	328	0	–	Yellow
HL$_3$ C$_{13}$H$_8$BrN$_3$O$_3$	46.4 (46.7)	2.6 (2.4)	12.8 (12.6)	58	277	0	–	Dark yellow
HL$_4$ C$_{13}$H$_8$N$_4$O$_5$	52.3 (52.0)	3.1 (2.7)	18.4 (18.7)	70	318	0	–	Dark yellow
[Cu(L$_1$)$_2$] · 2H$_2$O C$_{26}$H$_{18}$Br$_2$Cl$_2$CuN$_4$O$_4$	42.7 (41.9)	2.8 (2.4)	7.8 (7.5)	83	>350	1.59	12	Tile red
[Fe(L$_1$)(OH)(H$_2$O)$_3$](NO$_3$) · 3H$_2$O C$_{13}$H$_{20}$BrClFeN$_3$O$_{11}$	27.1 (27.6)	3.3 (3.6)	6.8 (7.4)	89	>350	5.06	71	Black
[Cu(L$_2$)$_2$] · H$_2$O C$_{26}$H$_{16}$Cl$_2$CuN$_6$O$_7$	47.5 (47.4)	2.8 (2.4)	12.3 (12.7)	85	>350	1.58	16	Dark brown
[Fe(L$_2$)(OH)$_2$(H$_2$O)$_2$] · 3H$_2$O C$_{13}$H$_{15}$ClFeN$_4$O$_{12}$	32.7 (33.3)	3.6 (4.1)	9.1 (9.0)	78	>350	3.96	24	Dark brown
[Cu(L$_3$)$_2$] · 2H$_2$O C$_{26}$H$_{18}$Br$_2$CuN$_6$O$_8$	41.1 (40.8)	2.9 (2.4)	11.0 (11.0)	80	>350	1.65	9.5	Dark brown
[Fe(L$_3$)$_2$(H$_2$O)$_2$](NO$_3$) · 3H$_2$O C$_{26}$H$_{24}$Br$_2$FeN$_7$O$_{14}$	35.8 (35.7)	3.3 (2.8)	11.4 (11.2)	85	>350	4.61	94	Black
[Cu(L$_4$)$_2$] · 2H$_2$O C$_{26}$H$_{18}$CuN$_8$O$_{12}$	44.2 (44.7)	3.0 (2.6)	15.6 (16.0)	85	>350	1.66	10	Dark brown
[Fe(L$_4$)(OH)$_2$H$_2$O] · H$_2$O C$_{13}$H$_{11}$FeN$_4$O$_8$	37.2 (36.7)	3.6 (3.1)	13.6 (13.2)	80	>350	4.18	22	Black

Y denotes yield; M, magnetic moment; and Λ, molar conductivity (25 ± 1°C).

The experiments were performed three times to minimize the error and the average values were presented. The minimum inhibitory concentrations (MIC) were defined as the lowest concentrations of compound giving complete inhibition of visible growth. Ciprofloxacin and fluconazole were used as reference antibimicrobials for bacteria and yeast, respectively. As control, antimicrobial effects of DMSO were investigated against test microorganisms. According to the values of the controls, the results were evaluated.

3. Results and discussions

The analytical data and physical properties of the ligands and the complexes are summarized in table 1.

3.1. *General properties*

All the Cu(II) complexes are in the ratio of 1:2 according to the analytical data. The Fe(III) complexes have 1:1 M:L ratio except the HL_3 complex. Molar conductivities of the complexes support the suggested structures. $[Fe(L_3)_2(H_2O)_2](NO_3) \cdot 3H_2O$ and $[Fe(L_1)(OH)(H_2O)_3](NO_3) \cdot 3H_2O$ are ionic; the other Fe(III) complexes and the Cu(II) complexes are non-ionic. High melting points of the complexes ($> 350°C$) show that the compounds have high-thermal stability.

In the Fe(III) complexes, number of hydroxyl ions present are anomalous. Fe(III) complexes of HL_1, HL_2, and HL_4 which have 1:1 M:L ratio contain hydroxide as ligand, whereas HL_3 complex with 1:2 M:L ratio does not. The presence or absence of hydroxide in the complexes is correlated with pH. Fe(III) salts hydrolyze easily above pH 5 and thus, this easy hydrolysis leads to the formation of hydroxo complexes [3, 13, 25]. Other factors which determined the number of hydroxides are acidity of the ligands, chemical properties of the solvents, and excess of Fe(III) salt. All these factors play a role in the hydrolysis of Fe(III) salts. The degree of hydrolysis is lower for Fe(III) complex of HL_1 than those of HL_2 and HL_4; there was no hydrolysis in Fe(III) complex of HL_3. Elemental analysis and ESI–MS spectral analysis results are in agreement with the suggested composition formulae of the complexes (tables 1 and S2).

The room temperature effective magnetic moment values of Fe(III) complexes are in the 3.96–5.06 BM range, indicating stabilization of the species having high-ferric spin ($S = 5/2$) state. These complexes may have six- or five-coordinate ferric complexes (scheme 3) [13, 26, 27]. Magnetic moment values of Cu(II) complexes (between 1.58 and 1.66 BM) are closer to the expected value (1.73 BM) for a simple mononuclear Cu(II) complex (scheme 4). These magnetic moment values can be assumed to square-planar structure [25].

3.2. *Thermal analysis*

Major features of the thermal analysis of the complexes are summarized in table 2. Thermal gravimetric analysis (TGA) curves of $[Fe(L_4)(OH)_2H_2O] \cdot H_2O$ and $[Cu(L_4)_2] \cdot 2H_2O$ are shown in figure S1. Samples were heated from room temperature to 400°C. Thermal analysis results exhibited valuable information about water
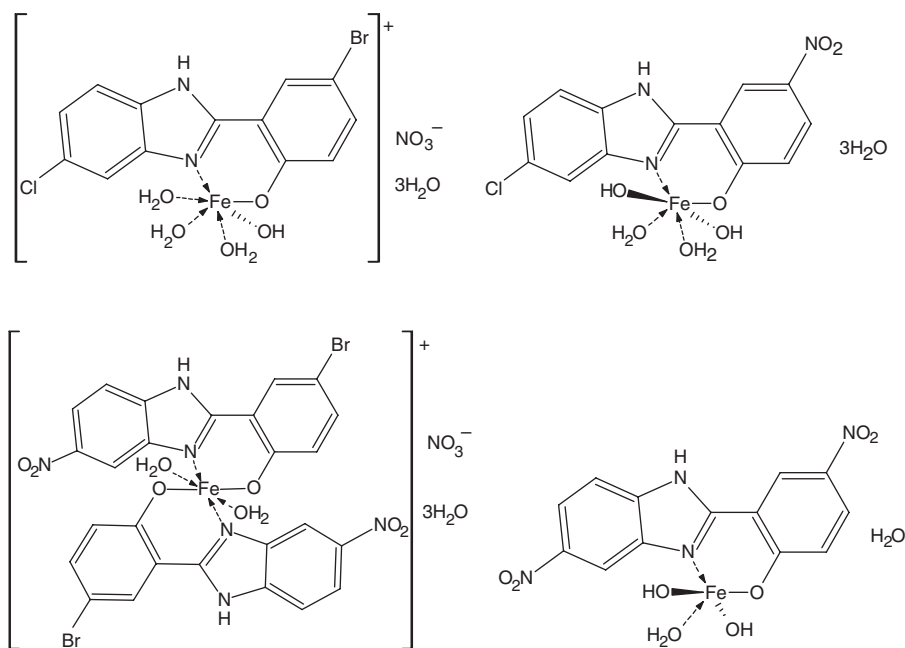
Scheme 3. The proposed structures for the Fe(III) complexes.
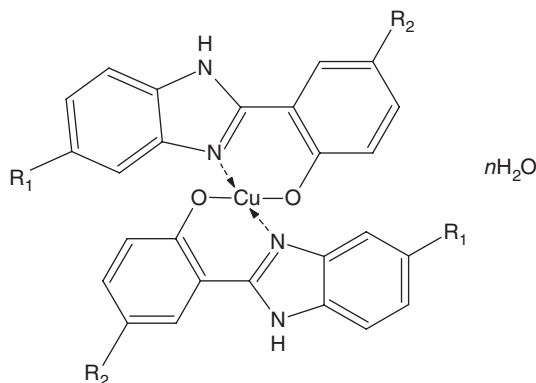
Scheme 4. The proposed structure for the Cu(II) complexes ($R_1 = Cl$, NO_2, $R_2 = Br$, NO_2; $n = 1$ or 2).

coordination of the complexes. Thermal degradation occurred in three stages. At the first stage, uncoordinated lattice water was lost through evaporation from 50°C to 100°C (table 2, first column). At the second stage, coordinated water was removed, especially from Fe(III) complexes, at temperatures between 100°C and 150°C (table 2, second column) [25, 28]. For the Cu(II) complexes, there are no weight losses corresponding to removal of coordinated water, consistent with four coordination (no water coordination). The Fe(III) complexes have coordinated and uncoordinated water molecules and are five- or six-coordinate according to the analytical and thermal analyses. For example, the TGA data are very informative for coordinated and

Table 2. TGA data of the complexes (thermal decomposition).

Complex	Weight loss (%)						
Temperature (°C)	100	150	200	250	300	350	400
$[Cu(L_1)_2] \cdot 2H_2O$	4.2	4.9	5.2	5.4	7.1	8.7	22.0
$[Fe(L_1)(OH)(H_2O)_3](NO_3) \cdot 3H_2O$	8.7	11.7	13.0	16.2	19.8	24.5	32.0
$[Cu(L_2)_2] \cdot H_2O$	2.9	3.3	3.6	4.4	5.6	10.7	37.8
$[Fe(L_2)(OH)_2(H_2O)_2] \cdot 3H_2O$	9.9	13.1	17.7	25.3	42.7	56.0	67.2
$[Cu(L_3)_2] \cdot 2H_2O$	4.1	4.3	4.4	6.8	8.3	11.5	63.2
$[Fe(L_3)_2(H_2O)_2](NO_3) \cdot 3H_2O$	5.9	7.4	13.3	15.1	20.0	67.3	74.5
$[Cu(L_4)_2] \cdot 2H_2O$	4.6	5.5	5.9	6.7	8.2	10.7	32.1
$[Fe(L_4)(OH)_2H_2O] \cdot H_2O$	4.7	6.4	8.7	16.1	19.2	24.5	72.3

uncoordinated water molecules of the five-coordinate $[Fe(L_4)(OH)_2H_2O] \cdot H_2O$: 4.7% and 4.2% found and calculated, respectively, for one uncoordinated water molecule of $[Fe(L_4)(OH)_2H_2O] \cdot H_2O$ (figure S1).

In all complexes, weight losses above 250°C can be explained in terms of cleavage of Cl, Br, and nitro groups, and also as decomposition of nitrate ions. Above 350°C, all other organic parts of complexes are oxidized to carbon dioxide and water.

3.3. *IR spectra*

FT-IR spectral data of the ligands and the complexes are given in table S1. The $v(O–H)$ and $v(N–H)$ vibration frequencies of the ligands exhibit only a single strong band at *ca* 3300 cm^{-1} caused by double intramolecular hydrogen bonding between the phenoxy hydrogen and one of the imine nitrogens [13, 29–31]. These bands change significantly upon metal complexation indicating deprotonation and subsequent coordination of the phenoxy [32]. Appearance of strong or medium, broad bands between 3200 and 3500 cm^{-1} in the complexes strongly support the presence of coordinated and uncoordinated water molecules (figures S2 and S3). N–H stretching vibrations of the ligands are observed between 3120 and 3275 cm^{-1} in the complexes, suggesting that the imine proton remains attached at N-1.

The $v(C–H)$ and $\delta(C–H)$ modes of ring residues in the ligands and the complexes are observed between 3145–3070 cm^{-1} and 845–730 cm^{-1} (table S1), respectively. The ring residue C=C and the imidazole C=N stretching frequencies are expected to appear between 1650 and 1600 cm^{-1} with their own characteristics for the ligands and the complexes. Also, these bands are combined with coupled vibrations, such as $\delta(C–H)$ and $\delta(N–H)$. Specific differences between spectra of the free ligands and the complexes in this region support the formation of new complexes. In the spectra of free ligands and their complexes, there is a band at around 1280 cm^{-1} which is assigned to $v(C–O)$ of the phenolic group [33]. Phenolic C–O bands of the uncoordinated ligand shift to higher frequencies during complex formation [34]; retention of the 1280 cm^{-1} band in spectra of the presented complexes suggests that the phenolic oxygens are coordinated to the metal [26].

In IR spectra of HL$_2$, HL$_3$, HL$_4$, and their complexes, the strong or medium bands near 1500 cm^{-1} (1532–1483) and in the 1377–1305 cm^{-1} range are assigned to symmetric
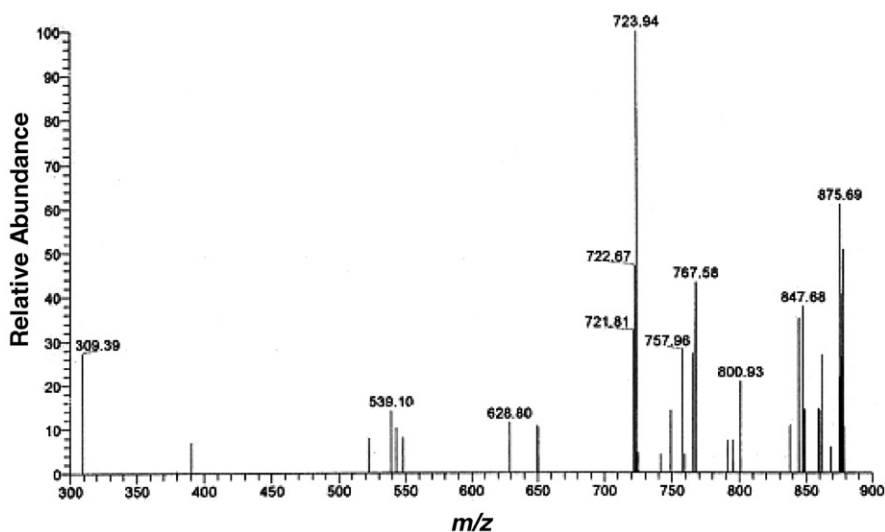
Figure 1.   ESI-MS spectra of $[Fe(L_3)_2(H_2O)_2](NO_3) \cdot 3H_2O$.

and asymmetric $\nu(NO_2)$, respectively. The C–Br stretching vibration is at 500–$550\,cm^{-1}$ as medium bands for HL_1, HL_3, and their complexes [35]. The C–Cl stretching vibration is at 600–$650\,cm^{-1}$ as medium or weak bands for HL_1, HL_2, and their complexes [36]. Fe(III) complexes of HL_1 and HL_3 show strong bands at $1386\,cm^{-1}$ in their IR spectra, supporting the presence of uncoordinated nitrate [37], also confirmed by conductivity data.

3.4. *Mass spectra*

The ESI-MS spectral data of the complexes are given in table S2 as molecular ions with relative abundance. The assignments of some intense ions are given in table S2 also. The ESI-MS spectra of $[Fe(L_3)_2(H_2O)_2](NO_3) \cdot 3H_2O$ and $[Cu(L_3)_2] \cdot 2H_2O$ are shown in figures 1 and S4. Molecular ions of the complexes have been determined. The molecular ions for $[Cu(L_1)_2] \cdot 2H_2O$ and $[Cu(L_2)_2] \cdot H_2O$ were observed with 100% relative abundance. The other complexes showed their ions peak with various ratios. Also, the peaks of the ligands (shown in table S2 as L) are easily determined in the mass spectra of the complexes. It is known that chlorine, bromine, and copper have two isotopes: $^{35}Cl(75.8\%) : ^{37}Cl(24.2\%)$ $(3:1)$; $^{79}Br(50.7\%) : ^{81}Br(49.3\%)$ $(1:1)$; and $^{63}Cu(69.2\%) : ^{65}Cu(30.8\%)$ $(2:1)$. Many isotopic patterns are identified for copper complexes that include chlorine and bromine. For example, in the mass spectra of $[Cu(L_3)_2] \cdot 2H_2O$, the isotopic patterns are observed clearly (table S2): 764.6 (21.7%, $[M-1]^+$, ^{65}Cu); 762.6 (36.8%, $[M-3]^+$, ^{63}Cu); 334.3 (100%, L, ^{79}Br); and 332.3 (97.6%, L, ^{81}Br). Also, the fragment of $[Cu(L_1)_2] \cdot 2H_2O$ complex produces many peaks. For example, $M+2$ peak is observed at 746.7 mass number with 100% relative abundance. There are many peaks between m/z 744 and m/z 748 because of various combinations of Cl, Br, and Cu isotopes. No isotopic patterns are observed in spectra of $[Fe(L_4)(OH)_2H_2O] \cdot H_2O$, as expected.

3.5. *UV-Vis spectra*

The UV-Vis spectral data are given in table 3. The sample visible electronic spectra for Fe(III) and Cu(II) complexes are shown in figures S5 and S6.

The lower wavelength bands (200–300 nm) in the electronic spectra of the ligand and the complexes correspond to $\pi \to \pi^*$ transitions of the aromatic rings. The medium or weak bands above 300 nm are due to $n \to \pi^*$ transitions. Fe(III) complexes have broad, strong bands in the 516–556 nm region caused by phenolic oxygen $\to$ metal charge transfer ($L \to Fe^{3+}$ charge transfer; figure S5) [2]. The electronic spectra of the Cu(II) complexes showed two or three bands at the visible region. Among them, the lowest wavenumber bands are attributed to ligand to metal charge transfer transitions ($O \to Cu^{2+}$). For example, in the spectra of $[Cu(L_1)_2] \cdot 2H_2O$, the 497 nm band is due to the $L \to Cu^{2+}$ charge transfer transition (figure S6). The other two weak bands, 584 and 628 nm, may be assigned to $^2B_{1g} \to {}^2A_{1g}$ and $^2B_{1g} \to {}^2E_g$ transitions, respectively. These assignments can be attributed to a square-planar geometry for Cu(II) complexes (scheme 4) [25, 38, 39].

3.6. *Antimicrobial activity*

The results concerning *in vitro* antimicrobial activity of the ligands and the complexes together with MIC values of compared antibiotic and antifungal agents are presented in table 4.

Table 3. UV-Vis spectral data of the compounds (in methanol).

Compound	Wavelength (λ_{max} (nm)) (log ε)	Solution color
HL_1	226(4.58), 292sh(4.27), 300(4.37), 327(4.44), 341(4.42)	Colorless
HL_2	212(4.70), 297(4.57), 325(4.62), 334sh(4.57), 356(4.21), 404br(3.83)	Yellow
HL_3	214(4.68), 230(4.30), 263sh(4.19), 269 (4.20), 349(4.11)	Yellow
HL_4	227(4.44), 262sh(4.48), 269(4.49), 342(4.40), 413br(3.85)	Yellow
[A]	227(4.75), 230(4.73), 252sh(4.65), 300(4.41), 342(4.35), 548br(3.28)	Red
$[Cu(L_1)_2] \cdot 2H_2O$	227(4.02), 261(3.65), 268sh(3.60), 303(3.41), 366(3.10), 497(2.04), 584(1.99), 628(1.96)	Light brown
[B]	214(4.59), 253(4.01), 268sh(3.60), 297(4.01), 337(4.02), 527br(2.89)	Red–violet
$[Cu(L_2)_2] \cdot H_2O$	213(4.52), 254(3.62), 264sh(3.62), 284(3.37), 372(3.11), 383(3.11), 476sh(2.37), 632(2.14)	Dark yellow
[C]	217(4.67), 242(4.67), 252sh(4.60), 288(4.39), 348br(4.34), 388(4.07), 429(2.54), 556br(3.21)	Purple–violet
$[Cu(L_3)_2] \cdot 2H_2O$	223(4.63), 268(4.08), 276sh(4.00), 299(3.87), 375(3.19), 425(3.51), 436(3.52), 488 (2.82)	Orange
$[Fe(L_4)(OH)_2H_2O] \cdot H_2O$	214(4.57), 223(4.63), 256(4.33), 267sh(4.31), 355br(4.28), 516br(3.00)	Dark red
$[Cu(L_4)_2] \cdot 2H_2O$	214(4.66), 236(4.68), 245sh(4.65), 252(4.64), 261sh(4.62), 274(4.61), 381(3.56), 459(3.07), 640(2.36)	Light brown

[A]: $[Fe(L_1)(OH)(H_2O)_3](NO_3) \cdot 3H_2O$, [B]: $[Fe(L_2)(OH)_2(H_2O)_2] \cdot 3H_2O$, and [C]: $[Fe(L_3)_2(H_2O)_2](NO_3) \cdot 3H_2O$.

Six bacteria and only one fungus have been studied in the antimicrobial activity tests. Most of the compounds (HL$_1$, HL$_2$, HL$_4$, and their complexes) and also Fe(NO$_3$)$_3 \cdot$ 9H$_2$O and Cu(NO$_3$)$_2 \cdot$ 3H$_2$O have no significant antimicrobial activity in this study. The antibacterial data indicate that both the bromo- and the nitro-substituted ligand (HL$_3$) have very strong and penetrating activity against *S. aureus* (2.4 μg mL^{-1}) and *S. epidermidis* (19.5 μg mL^{-1}) which are Gram-positive bacteria. Fe(III) and Cu(II) complexes of HL$_3$ have a similarly very strong activity against the above-mentioned bacteria. For instance, [Fe(L$_3$)$_2$(H$_2$O)$_2$](NO$_3$) $\cdot$ 3H$_2$O and [Cu(L$_3$)$_2$] $\cdot$ 2H$_2$O show superior activity against *S. epidermidis* organism (MIC value is 39 μg mL^{-1} for both compounds) compared to the reference ciprofloxacin (MIC = 156 μg mL^{-1}). Superior activity of HL$_3$, [Fe(L$_3$)$_2$(H$_2$O)$_2$](NO$_3$) $\cdot$ 3H$_2$O and [Cu(L$_3$)$_2$] $\cdot$ 2H$_2$O against *S. aureus* and *S. epidermidis* is a valuable contribution to research on metal-based drugs, indicating selective activity toward Gram-positive bacteria.

In the literature, there are numerous studies dealing with the antimicrobial activity of coordination compounds [40–48]. Some complexes showed higher antimicrobial activity than the ligands alone and the reference compounds [49–51]. In line with the results published, there are several promising complexes which show some prospects in antimicrobial activity. Therefore, there is a room for further studies on the metal complexes. The results of our study indicate that HL$_3$ ligand, [Fe(L$_3$)$_2$(H$_2$O)$_2$](NO$_3$) $\cdot$ 3H$_2$O, and [Cu(L$_3$)$_2$] $\cdot$ 2H$_2$O complexes have potential to generate novel metabolites, by displaying high affinities toward most of the receptors. The strong antimicrobial activities of these complexes against *S. aureus* and *S. epidermidis* warrant further investigation.

HL$_3$ and its Fe(III) and Cu(II) complexes are effective against *Staphylococcus*-type bacteria (Gram positive) only. However, at this stage it is impossible to find a simple explanation for the antibacterial effect of HL$_3$ and its complexes, while the other complexes and HL$_1$ ligand have no activity on these bacteria and further studies will be needed to elucidate this phenomenon.

Table 4. *In vitro* antimicrobial activity of the compounds (MIC, μg mL^{-1}).

Compound	Microorganisms						
	Sa[a]	*Se*[a]	*Ec*[b]	*Kp*[b]	*Pa*[b]	*Pm*[b]	*Ca*
HL$_1$	–	–	–	–	–	–	–
HL$_2$	312	–	–	–	–	–	–
HL$_3$	2.4	19.5	–	–	–	–	–
HL$_4$	78	–	–	–	–	–	–
[A]	9.8	39	–	–	–	–	–
[B]	19.5	39	–	–	–	–	–
Fe(NO$_3$)$_3 \cdot$ 9H$_2$O	–	–	–	–	–	–	–
Cu(NO$_3$)$_2 \cdot$ 3H$_2$O	–	–	–	–	–	–	–
Ciprofloxacin	0.125	156	0.0625	0.0625	2.00	0.0312	–
Fluconazole	–	–	–	–	–	–	1.00

[A], [Fe(L$_3$)$_2$(H$_2$O)$_2$](NO$_3$) $\cdot$ 3H$_2$O; [B], [Cu(L$_3$)$_2$] $\cdot$ 2H$_2$O.
Sa, *Staphylococcus aureus* ATCC 6538; *Se*, *Staphylococcus epidermidis* ATCC 12228; *Ec*, *Escherichia coli* ATCC 8739; *Kp*, *Klebsiella pneumoniae* ATCC 4352; *Pa*, *Pseudomonas aeruginosa* ATCC 1539; *Pm*, *Proteus mirabilis* ATCC 14153; and *Ca*, *Candida albicans* ATCC 10231.
[a]Gram-positive bacteria; [b]Gram-negative bacteria.
– Denotes antimicrobial activity was not detected.

4. Conclusion

In this study, 2-(5-chloro/nitro-1*H*-benzimidazol-2-yl)-4-bromo/nitro-phenols ligands (scheme 1) and their copper(II) nitrate and iron(III) nitrate complexes were synthesized and characterized. Their antibacterial and antifungal activities were evaluated by the disc diffusion method against six bacteria and *C. albicans* as yeast.

All of the Cu(II) complexes are of the bis-type (1 : 2) according to the analytical data. The Fe(III) complexes have 1 : 1 M : L ratio except the HL_3 complex. Fe(III) complexes of HL_1, HL_2, and HL_4 contain hydroxide as ligand because of hydrolysis, whereas the HL_3 complex does not. According to the magnetic moment measurements, Fe(III) complexes are high spin ($S = 5/2$) and are five- or six-coordinate. From the TGA data, the presence of coordinated and uncoordinated water molecules was confirmed; Fe(III) complexes have both coordinated and uncoordinated water molecules. Cu(II) complexes do not contain coordinated water molecules; however, they contain water molecules in the crystal. Complexation occurred *via* the nitrogen of C=N and the oxygen of phenol. Cu(II) complexes are square-planar structure according to the UV-Vis spectra. Molar conductivity measurements also confirm the structures. Molecular ions of the complexes and also the isotopic patterns have been determined in the ESI–MS spectra.

The antibacterial data indicate that HL_3 ligand having both the bromo and the nitro substituents and its complexes show a very strong activity against *S. aureus* and *S. epidermidis* (Gram-positive bacteria). $[Fe(L_3)_2(H_2O)_2](NO_3) \cdot 3H_2O$ and $[Cu(L_3)_2] \cdot 2H_2O$ complexes are more effective against *S. epidermidis* than ciprofloxacin. The antimicrobial activity results of this study extend the literature. Various Cu(II) complexes have considerable antimicrobial activities [44, 45, 50, 52], and Fe(III) complexes are effective on various microorganisms [1, 2, 13, 41, 51, 53] with some Fe(III) and Cu(II) complexes more effective than the free ligand [51, 54]. The presented compounds warrant further investigation.

The proposed structures for the complexes are shown in schemes 3 and 4, in accordance with the experimental data obtained from analytical data, magnetic moments, molar conductivities, TGA, MS, FT-IR, and UV-Vis spectroscopic techniques.

References

[1] B. Ülküseven, A. Tavman, G. Ötük, S. Birteksöz. *Folia Microbiol.*, **47**, 481 (2002).
[2] A. Tavman, N.M. Agh-Atabay, A. Neshat, F. Gücin, B. Dülger, D. Haciu. *Transition Met. Chem.*, **31**, 194 (2006).
[3] C.G. Wahlgren, A.W. Addison, S. Burman, L.K. Thompson, E. Sinn, T.M. Rowe. *Inorg. Chim. Acta*, **166**, 59 (1989).
[4] A. Tavman, B. Ülküseven. *Main Group Met. Chem.*, **24**, 205 (2001).
[5] Y.-P. Tong, Y.-W. Lin. *Inorg. Chim. Acta*, **362**, 2167 (2009).

[6] X. Quezada-Buendía, A. Esparza-Ruiz, A. Pena-Hueso, N. Barba-Behrens, R. Contreras, A. Flores-Parra, S. Bernes, S.E. Castillo-Blum. *Inorg. Chim. Acta*, **361**, 2759 (2008).
[7] Y.-P. Tong, S.-L. Zheng. *J. Mol. Struct.*, **841**, 34 (2007).
[8] L.D. Prabhakar, K.M.M.S. Prakash, M.C. Chowdary. *Inorg. Chim. Acta*, **133**, 233 (1987).
[9] K.M.M.S. Prakash, L.D. Prabhakar, M.C. Chowdary. *Polyhedron*, **6**, 285 (1987).
[10] Y.-P. Tong, Y.-W. Lin, S.W. Ng. *Acta Crystallogr.*, **E61**, m1915 (2005).
[11] Y. Wu, B. Xie, L. Zou, J.-S. Feng, Z. Hu. *Acta Crystallogr.*, **E63**, m2298 (2007).
[12] H.-Y. Bu, Y.-J. Liu, Q.-F. Liu, J.-F. Jia. *Acta Crystallogrt.*, **E61**, m1986 (2005).
[13] A. Tavman, N.M. Agh-Atabay, S. Güner, F. Gücin, B. Dülger. *Transition Met. Chem.*, **32**, 172 (2007).
[14] G. Seth, Y. Garg, N.K. Mouraya. *Asian J. Chem.*, **14**, 919 (2002).
[15] G. Seth, Y. Garg, N.K. Mouraya. *Asian J. Chem.*, **15**, 546 (2003).
[16] J.J. da Silva, R.J.P. Williams. *The Biological Chemistry of the Elements – The Inorganic Chemistry of Life*, Clarendon, Oxford, England (1991).
[17] H. Sigel (Ed.). *Metal Ions in Biological Systems*, Dekker, New York (1981).
[18] D.E. Fenton. In *Perspectives in Coordination Chemistry*, A.F. Williams, C. Floriani, A.E. Merbach (Eds), P. 203, Verlag Helvetica Chimica Acta, Basel (1992).
[19] J.M. Guss, H.D. Bartunik, H.C. Freeman. *Acta Crystallogr.*, **B48**, 790 (1992).
[20] R. Balamurugan, M. Palaniandavar, R.S. Gopalan, G.U. Kulkarni. *Inorg. Chim. Acta*, **357**, 919 (2004).
[21] D.E. Fenton. *Biocoordination Chemistry*, Oxford University Press, Oxford (1997).
[22] H.F. Ridley, R.G.W. Spickett, G.M. Timmis. *J. Heterocyl. Chem.*, **2**, 453 (1965).
[23] Clinical and Laboratory Standards Institute (CLSI). *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically: Approved Standard*, M7-A5, Clinical and Laboratory Standards, Wayne, PA, USA (2006).
[24] Clinical and Laboratory Standards Institute (CLSI). *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts: Approved Standard*, M27-A2, 2nd Edn, Clinical Laboratory Standards, Wayne, PA, USA (2002).
[25] K.K.M. Yusuff, R. Sreekala. *Thermochim. Acta*, **179**, 313 (1991).
[26] A.K. El-Sawaf, D.X. West, R.M. El-Bahnasawy, F.A. El-Saied. *Transition Met. Chem.*, **23**, 227 (1998).
[27] S. Padhye, G.B. Kauffman. *Coord. Chem. Rev.*, **63**, 127 (1985).
[28] A.A. Soliman, W. Linert. *Thermochim. Acta*, **338**, 67 (1999).
[29] A. Tavman. *Spectrochim. Acta*, **A63**, 343 (2006).
[30] D. Kanamori, Y. Yamada, A. Onoda, T.A. Okamura, S. Adachi, H. Yamamoto, N. Ueyama. *Inorg. Chim. Acta*, **358**, 331 (2005).
[31] N. Ueyama, N. Nishikawa, Y. Yamada, T. Okamura, A. Nakamura. *Inorg. Chim. Acta*, **283**, 91 (1998).
[32] V.M. Leovac, L.S. Jovanovic, V.S. Cesljevic, L.J. Bjwlica, V.B. Arion, N.V. Gerbelu. *Polyhedron*, **13**, 3005 (1994).
[33] K. Nakanishi, P.H. Solomon. *Infrared Absorption Spectroscopy*, 2nd Edn, Holden-Day, San Francisco (1977).
[34] T.M. Aminabhavi, N.S. Biradar, V.L. Roddabasanagoudar, W.E. Rudzinski, D.E. Hoffman. *Inorg. Chim. Acta*, **121**, L45 (1986).
[35] N. Sundaraganesan, C. Meganathan, B. Anand, C. Lapouge. *Spectrochim. Acta*, **A66**, 773 (2007).
[36] N. Sundaraganesan, B. Anand, F.-F. Jian, P. Zhao. *Spectrochim. Acta*, **A65**, 826 (2006).
[37] K. Nakamoto. *Infrared and Raman Spectra of Inorganic and Coordination Compounds, Part B*, 5th Edn, John Wiley & Sons, New York (1997).
[38] M.M. Abd-Elzaher. *Appl. Organomet. Chem.*, **18**, 149 (2004).
[39] A.B.P. Lever. *Inorganic Electronic Spectroscopy*, Elsevier, Amsterdam (1984).
[40] R. Moya-Hernandez, R. Gomez-Balderas, A. Mederos, S. Dominguez, M.T. Ramirez-Silva, A. Rojas-Hernandez. *J. Coord. Chem.*, **62**, 40 (2009).
[41] L.-C. Yu, Z.-L. Tang, P.-G. Yi, S.-L. Liu. *J. Coord. Chem.*, **62**, 894 (2009).
[42] A. Kulkarni, P.G. Avaji, G.B. Bagihalli, S.A. Patil, P.S. Badami. *J. Coord. Chem.*, **62**, 481 (2009).
[43] K.V. Sharma, V. Sharma, R.K. Dubey, U.N. Tripathi. *J. Coord. Chem.*, **62**, 493 (2009).
[44] L.-C. Yu, Z.-L. Tang, P.-G. Yi, S.-L. Liu, X. Li. *J. Coord. Chem.*, **62**, 903 (2009).
[45] A.A.A. Abu-Hussen, W. Linert. *J. Coord. Chem.*, **62**, 1388 (2009).
[46] J.R. Anacona, H. Rodriguez. *J. Coord. Chem.*, **62**, 2212 (2009).
[47] P. Tharmaraj, D. Kodimunthiri, P. Prakash, C.D. Sheela. *J. Coord. Chem.*, **62**, 2883 (2009).
[48] A.D. Kulkarni, G.B. Bagihalli, S.A. Patil, P.S. Badami. *J. Coord. Chem.*, **62**, 3060 (2009).
[49] N. Raman, J.D. Raja, A. Sakthivel. *J. Chem. Sci.*, **119**, 303 (2005).
[50] P. Tharmaraj, D. Kodimunthiri, C.D. Sheela, C.S.S. Priya. *J. Coord. Chem.*, **62**, 2220 (2009).
[51] A.A.A. Emara, H.S. Selem, A.M. Madyan. *J. Coord. Chem.*, **62**, 2569 (2009).
[52] M.A. Phaniband, S.D. Dhumwad. *J. Coord. Chem.*, **62**, 2399 (2009).
[53] P.A. Ajibade, G.A. Kolawe. *J. Coord. Chem.*, **61**, 3367 (2008).
[54] C.J. Dhanaraj, M.S. Nair. *J. Coord. Chem.*, **62**, 4018 (2009).