SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF SOME TRANSITION METAL COMPLEXES OF *N*-(5-CHLORO-2-HYDROXYPHENYL)-3-METHOXY-SALICYLALDIMINE

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ABSTRACT. Cr(III), Fe(II), Fe(III), Co(II), Ni(II), Cu(II), Zn(II) complexes of *N*-(5-chloro-2-hydroxyphenyl)-3-methoxy-salicylaldimine (H$_2$L), an ONO type tridentate ligand, were synthesized and characterized by elemental analysis, magnetic moment, molar conductivity, TGA, NMR, IR, UV-vis. and ESI-MS spectral techniques. All of the complexes except [Zn(L)(H$_2$O)$_2$] are paramagnetic. The CoCl$_2$ gives a complex with 1:2 M:L ratio while the others form 1:1 non-ionic complexes with H$_2$L. The ligand coordinated to the metal ions through the both OH oxygen and the azomethine nitrogen atoms acting tridentate behaviour. Octahedral geometry has been proposed for the Fe(II), Cr(III), Co(II), Ni(II) complexes considering the magnetic moment values and the electronic spectral data. The Zn(II) and Fe(III) complexes exhibit five coordination geometries and the Cu(II) complex is four-coordinated having acetato bridged dimeric structure. Antimicrobial activities of the compounds were determined against six bacteria and *C. albicans* as fungi. The Ni(II) and Co(II) complexes exhibit considerable selective activity on *S. epidermidis* and *C. albicans* whereas the ligand has no activity on the microorganisms. In addition, the Cr(III) complex shows antibacterial activity toward *S. epidermidis* and the Zn(II) complex has antifungal activity on *C. albicans*.

KEY WORDS: Schiff base, Salicylaldimine, 5-Chloro-2-hydroxyphenyl, Transition metal complexes, Antimicrobial

INTRODUCTION

Salicylaldimines are known the *o*-hydroxy derivatives of Schiff bases and they are of particular interest due to an asymmetric intramolecular hydrogen bond formed between the oxygen and nitrogen atoms [1, 2]. Transition metal complexes with these types Schiff bases are of particular interest because of their ability to possess unusual configurations, be structurally labile and their sensitivity to molecular environments [3-5]. They have wide applications in fields such as antibacterial, antiviral, antifungal agents [6], homogeneous or heterogeneous catalysis [7]. Schiff bases are potential anticancer drugs [8] and when administered as their metal complexes, the anticancer activity of these complexes is enhanced in comparison to the free ligand [9]. It has been shown that Schiff base complexes derived from 4-hydroxysalicylaldehyde and amines have strong anticancer activity, e.g., against Ehrlich ascites carcinoma (EAC) [10, 11]. A large number of Schiff bases and their complexes have been investigated for their interesting and important properties, such as their ability to reversibly bind oxygen, catalytic activity in the hydrogenation of olefins, photochromic properties and complexing ability towards some toxic metals, furthermore complexes of Schiff bases showed promising applications in biological activity and biological modeling applications [12]. It was reported that complexes of Schiff bases have been used as antibacterial, antifungal, anticancer, antitubercular, hypertensive, and hypothermic reagents [13].

In this study Cr(III), Fe(II), Fe(III), Co(II), Ni(II), Cu(II), Zn(II) complexes of *N*-(5-chloro-2-hydroxyphenyl)-3-methoxy-salicylaldimine (H$_2$L) (Figure 1) were synthesized and characterized. Antimicrobial activity of the compounds was evaluated against six bacteria and *Candida albicans* as fungus.

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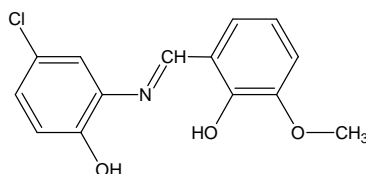*Corresponding author. E-mail: atavman@istanbul.edu.tr

Figure 1. The chemical structure of *N*-(5-chloro-2-hydroxyphenyl)-3-methoxy-salicylaldimine.

EXPERIMENTAL

All chemicals and solvents were of reagent grade and were used without further purification. Elemental analysis data were obtained with a Thermo Finnigan Flash EA 1112 analyzer. Melting points were determined using an Electro thermal melting-point apparatus. ^{1}H- and ^{13}C-NMR spectra were run on a Varian Unity Inova 500 NMR spectrometer. The residual DMSO-d_6 signal was also used as an internal reference. FT-IR spectra were recorded in KBr disks on a Mattson 1000 FT-IR spectrometer. UV-Visible spectra was performed on a Perkin Elmer Lambda 25 UV/Visible Spectrometer in MeOH. 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. Magnetic moments of the paramagnetic complexes were carried out on a Sherwood Scientific apparatus (MK1) at room temperature by Gouy's method. Molar conductivity of the complexes was measured on a WTW Cond315i conductivity meter in dimethyl formamide (DMF) at 25 ºC. Thermogravimetric studies were made on a TG-60WS Shimadzu, with a heating rate of 10 ºC/min and air flowing at the rate of 50 mL/min.

Synthesis of the ligand (H₂L)

H_2L was prepared by mixing an ethanolic solution (15 mL) of 4-chloro-2-aminophenol (1.44 g, 10 mmol) with 3-methoxy-salicylaldehyde (1.52 g, 10 mmol) in same volume of ethanol. This mixture was then refluxed with stirring for 3 h and the solution was allowed at the room temperature to crystallize H_2L [1].

Syntheses of the complexes: general procedure

100 mg ligand (0.36 mmol) and equivalent amounts of metal salts (e.g. 96 mg $CrCl_3 \cdot 6H_2O$, 0.36 mmol) were dissolved in ethanol (20 mL) and the mixture was refluxed for 4 h. The mixture was allowed to stand at room temperature for a few days. Then the precipitate formed was filtered and washed with ethanol (2 mL) and diethylether (5 mL) and dried at room temperature. The other complexes were obtained similarly. The salts used for synthesis of the complexes are: $CrCl_3 \cdot 6H_2O$, $FeSO_4 \cdot 7H_2O$, $FeCl_3 \cdot 6H_2O$, $CoCl_2 \cdot 6H_2O$, $NiCl_2 \cdot 6H_2O$, $Cu(CH_3COO)_2 \cdot H_2O$ and $Zn(CH_3COO)_2 \cdot 2H_2O$.

Determination of antimicrobial activity

Antimicrobial activity against *Staphylococcus aureus ATCC 6538*, *Staphylococcus epidermidis ATCC 12228*, *Escherichia coli ATCC 8739*, *Klebsiella pneumoniae ATCC 4352*, *Pseudomonas aeruginosa ATCC 27853*, *Proteus mirabilis ATCC 14153* and *Candida albicans ATCC 10231* were determined by the microbroth dilutions technique following the Clinical and Laboratory Standards Institute (CLSI) recommendations [14, 15]. Mueller-Hinton broth for bacteria, RPMI-1640 medium buffered to pH 7.0 with MOPS for yeast strain was used as the test medium. Serial two-fold dilutions ranging from 5000 µg/mL to 4.9 µg/mL were prepared in medium. 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 5×10^5 cfu/mL for bacteria and 0.5×10^3 to 2.5×10^3 cfu/mL for yeast in the test tray. The trays were covered and placed in plastic bags to prevent evaporation. The trays containing Mueller-Hinton broth were incubated at 35 ºC for 18–20 h and the trays containing RPMI-1640 medium were incubated at 35 ºC for 46–50 h. The minimum inhibitory concentrations (MIC) were defined as the lowest concentration of compound giving complete inhibition of visible growth. Antimicrobial effects of the solvents were investigated against test microorganisms. The medium with DMSO as solvent was used as a negative control whereas media with Ciprofloxacin (standard antibiotic) and Fluconazole (standard antifungal drug) were used as the positive controls. The experiments were performed in triplicate.

RESULTS AND DISCUSSION

General properties

In this study, Cr(III), Fe(II), Fe(III), Co(II), Ni(II), Cu(II) and Zn(II) complexes of *N*-(5-chloro-2-hydroxyphenyl)-3-methoxy-salicylaldimine (H_2L), an ONO type tridentate ligand, were synthesized and characterized by elemental and thermal analyses, magnetic moment, molar conductivity measurements and NMR, IR, UV-Vis, ESI-MS spectral techniques. The data of the ligand were reported in our previous study [1a]. The analytical data and physical properties of the ligand and the complexes are summarized in Table 1. The complexes are paramagnetic except $[Zn(L)(H_2O)_2]$ and they have 1:1 M:L ratio except that of Co(II). Molar conductivity of the complexes showed that all complexes are non-electrolytes in DMF (Table 1).

Table 1. The analytical data and physical properties of the ligand and the complexes.

Compound	Elemental analysis found (calcd) %			Y %	m.p. ºC	μ_{eff} BM	Λ	Color
	C	H	N					
H_2L $C_{14}H_{12}ClNO_3$	60.19 (60.55)	4.64 (4.36)	5.03 (5.04)	87	210	0	--	Brick red
$[Cr(L)Cl(H_2O)_2] \cdot 4H_2O$ $C_{14}H_{22}Cl_2CrNO_9$	35.73 (35.68)	4.38 (4.71)	3.21 (2.97)	75	207	3.65	36	Dark brown
$[Fe(L)(H_2O)_3]$ $C_{14}H_{16}ClFeNO_6$	43.52 (43.61)	3.80 (4.18)	3.60 (3.63)	86	>400	5.18	9.0	Black
$[Fe(L)(H_2O)(OH)]$ $C_{14}H_{13}ClFeNO_5$	45.42 (45.87)	3.88 (3.57)	3.67 (3.82)	77	>400	4.70	16	Black
$[Co(HL)_2] \cdot 2H_2O$ $C_{28}H_{26}Cl_2CoN_2O_8$	52.10 (51.87)	3.94 (4.04)	4.67 (4.32)	70	204	5.13	27	Reddish brown
$[Ni(L)(H_2O)_3]$ $C_{14}H_{16}ClNiNO_6$	43.73 (43.29)	3.87 (4.15)	3.24 (3.61)	84	>400	3.37	15	Light brown
$[Cu_2(HL)(L)(Ac)]$ $C_{30}H_{24}Cl_2Cu_2N_2O_8$	48.79 (48.79)	3.69 (3.28)	4.06 (3.79)	61	387	1.38	2.3	Army green
$[Zn(L)(H_2O)_2]$ $C_{14}H_{14}ClNO_5Zn$	44.88 (44.59)	3.96 (3.74)	3.67 (3.71)	92	>400	0	0.3	Dark yellow

Y, Yield; μ_{eff}, effective magnetic moment; Λ, Molar conductivity, $\Omega^{-1}cm^2mol^{-1}$ (25±1 ºC).

Melting points of the Cr(III) and Co(II) complexes are lower than that of the others. Relatively lower melting point of the Cr(III) complex, $[Cr(L)Cl(H_2O)_2] \cdot 4H_2O$, may be resulted from its' high water content coordinated and uncoordinated. The Co(II) complex, $[Co(HL)_2] \cdot 2H_2O$, has 1:2 M:L ratio as a difference from the others. Also, this complex compound has lower melting point degree according to the others except the Cr(III) complex. This situation can be explained as follows: Probably only one of the OH protons is removed on

complexation and a complex compound is formed in [Co(HL)$_2$] form. This type complex may be has lower thermal stability according to the others; and the lower melting point of Co(II) complex may be related to its structure. Similarly, higher molar conductivity of Co(II) complex than expected may be related with OH coordination, also. The following weak dissociation should be occured when the Co(II) complex is dissolved in DMF: [Co(HL)$_2$] ⇆ [Co(L)$_2$]$^{2-}$ + 2H$^+$ [16]. As a result of this dissociation, the molar conductivity of the Co(II) complex is higher than the expected. According to the molar conductivity measurements all of the complexes have non-ionic character.

The Fe(II) and Ni(II) complexes are very similar to each other structurally. The coordination number of these complexes is 6. Probably, geometry of the Fe(II) and Ni(II) complexes is distorted octahedral. The FeCl$_3$ gave a hydroxo complex having ampirical formula [Fe(L)(H$_2$O)(OH)]. Presence of a hydroxyl ion is due to the hydrolysing of Fe(III) salt. It is known that Fe(III) salts hydrolyses easily above pH 5 and thus, this easy hydrolysis leads to the formation of hydroxo complexes [17]. Elemental analysis and ESI-MS spectral analysis results are in agreement with the suggested composition formulas of the complexes (Tables 1 and 5).

Magnetic moments

Room temperature magnetic moment values of the solid complexes are presented at Table 1. Magnetic moment value of the Fe(III) complex, 4.70 BM, indicates stabilization of the species having intermediate ferric spin (S = 3/2) state. The occurrence of such an intermediate spin state is typical for the six and five coordinate ferric complexes [18]. The magnetic moment of the Fe(II) complex, μ = 5.18 BM, is in the range for the high spin Fe(II) state [19].

The Cr(III) complex shows magnetic moment (3.65 BM) corresponding to three unpaired electrons, i.e. 3.72 BM, expected for high-spin octahedral Cr(III) complexes [20].

Observed magnetic moment value for the Co(II) complex at room temperature is 5.13 BM. This value can be considered as characteristic of complexes with an high-spin octahedral environment around Co(II) in the range 4.7–5.2 BM [21]. It is known that both tetrahedral and high-spin octahedral Co(II) complexes possess three unpaired electrons but may be distinguished by the magnitude of the deviation of μ$_{eff}$ from the spin-only value. The magnetic properties of high spin octahedral Co(II) complexes are governed by the orbitally degenerate ground term ^{4}T$_{1g}$; this provides an orbital contribution to the magnetic moment so that room temperature moments are experimentally found to be in the range 4.7–5.2 BM [18, 21, 22].

Magnetic moment value for the Ni(II) complex is 3.37 BM, which is near to the expected range for octahedral geometry around the central metal ion [23]. Magnetic moment of the Cu(II) complex, 1.38 BM, is lower than the expected value for the S = 1/2 system. This may be explained by a Cu-Cu interaction in acetate bridging dinuclear complex [24].

FT-IR spectra

FT-IR spectral data of the ligand and the complexes are given in Table 2. The IR spectrum of the ligand showed medium or strong intensity absorption bands at 1644 and 1495 cm^{-1} assigned to C=N and C=C stretching modes. The presence of aromatic rings has been identified by their characteristic ring vibrations at 1500–1400, 1100–1050 and 900–700 cm^{-1} regions. The broad bands between 2900 and 2600 cm^{-1} in the spectrum of the ligand demonstrate the formation of the OH⋯N intramolecular hydrogen bond between OH and N atom of the ligand [1, 25].

The broad bands between 3400 and 3500 cm^{-1} are due to stretching vibrations of OH. Besides, the broad bands between 3000 and 2600 cm^{-1} are due to the intra- and intermolecular hydrogen bonding because of water molecules in the spectra of the complexes [26]. These bands are especially observed at the spectra of Fe(II), Fe(III), Cr(III), Co(II) and Zn(II) complexes. High water content of Cr(III) complex appears in the IR spectra prominently (very broad bands between 3450 and 2800 cm^{-1}). TGA curve of Cr(III) complex confirms this evaluation. The

strong band at 1644 cm^{-1}, ν(C=N), in the ligand spectrum is considerably changed in the complexes: This band shifted to the lower wavenumber in the complexes. This indicates that the azomethine nitrogen is coordinated to the metal ions [27]. The ν(C–O) bands of C–OH and C–O–CH$_3$ groups of the ligand appear between 1200 and 1290 cm^{-1} as strong or medium bands. In the complexes, the considerable changes at the 1209 and 1286 cm^{-1} bands showed the phenolic oxygen coordination to the metal ions (C–O→M coordination) [28]. In the Cu(II) complex, the strong bands at 1482 and 1439 cm^{-1} are may be due to the coordination of the acetate group oxygen atoms as bidentate [29].

Table 2. The FT-IR spectral data of the ligand and the complexes.

Compound	Frequency (cm^{-1})
H$_2$L	3433 w,br, 3050 m, 2955 m, 2829 m, 2663 m, 1644 s, 1595 m, 1510 s, 1428 m, 1345 m, 1286 m, 1252 m, 1209 s, 1170 m, 1066 m, 901 m, 811 m, 750 m, 731 m, 573 m, 435 m
[Cr(L)Cl(H$_2$O)$_2$]·4H$_2$O	3168 br (3450–2800), 1640 m, 1606 s, 1548 m, 1498 m, 1440 m, 1274 m, 1253 m, 1216 m, 1176 m, 1084 w, 967 w, 915 w, 863 w, 740 m, 657 m, 574 m, 540 m, 512 m, 453 w
[Fe(L)(H$_2$O)$_3$]	3597 m, 3055 m,br, 2936 m,br, 2763 m,br, 1606 s, 1582 m, 1545 m, 1480 s, 1431 s, 1382 m, 1311 m, 1275 s, 1257 s, 1226 m, 1118 m, 1051 s, 1017 s, 974 m, 838 m, 740 m, 688 m, 581 w, 424 w
[Fe(L)(H$_2$O)(OH)]	3456 m,br, 3289 m, 2965 w, 1596 m, 1577 m, 1544 m, 1479 s, 1426 m, 1377 m, 1309 w, 1275 m, 1260 s, 1222 s, 1084 w, 982 w, 840 m, 738 m, 689 m, 587 w, 535 m, 430 w
[Co(HL)$_2$]·2H$_2$O	3388 m,br, 3197 m,br, 3055 m, 2963 m, 1640 m, 1616 m, 1548 w, 1478 s, 1441 m, 1379 w, 1290 m, 1247 m, 1219 s, 1177 m, 1084 w, 971 w, 912 w, 863 w, 740 m, 663 w, 540 w, 461 w
[Ni(L)(H$_2$O)$_3$]	3452 s, 3317 m, 2926 m,br, 1614 m, 1590 m, 1548 m, 1502 m, 1445 s, 1392 m, 1282 s, 1254 m, 1224 s, 1184 m, 1098 m, 976 m, 924 m, 825 m, 746 m, 676 m, 606 w, 531 w, 462 w
[Cu$_2$(HL)(L)(Ac)]	3432 m,br, 3007 w, 2930 w, 1608 s, 1577 w, 1540 m, 1482 s, 1439 s, 1386 w, 1281 m, 1253 m, 1232 s, 1080 w, 985 m, 923 w, 834 m, 750 m, 689 m, 538 m, 427 w.
[Zn(L)(H$_2$O)$_2$]	3420 m, 3061 m,br, 2936 m,br, 1634 s, 1584 m, 1542 m, 1482 s, 1445 s, 1392 m, 1315 m, 1278 m, 1245 m, 1225 s, 1087 w, 982 m, 917 w, 828 w, 742 m, 677 m, 522 w, 403 w

m = medium, s = strong, sh = shoulder, w = weak, br = broad.

UV-Visible spectra

The UV-Visible spectral data are given in Table 3. The UV-Visible absorption spectra were obtained in methanol at room temperature. The electronic spectra of the compounds exhibited intense bands in the 200–400 nm region, which may be assignable to the *n→σ**, *n→π** and *π→π** transitions. The bands below 250 nm are due to the *n→σ** transitions. Schiff base ligands exhibit intense intra-ligand absorption bands in the 250–350 nm region. The *π→π** transitions of the aromatic rings are observed in the 250–300 nm region whereas the *π→π** transitions of the C=N bond are in between 300 and 350 nm [30].

The Cr(III) complex showed three bands at 725 (m,br), 668 (m,br) and 489 nm (sh) in the UV-visible spectra. These bands are consistent with the octahedral geometry of Cr(III) complexes. The medium band at 391 nm is due to LMCT transition [31].

Three bands are observed in the Fe(III) complex at 624 (m), 554 (sh) and 513 nm (w) which do not suggest the octahedral or tetrahedral geometry around the metal atom. These bands may be considered as a support for five coordinate square pyramidal Fe(III) complex [32]. And, the strong absorption at 404 nm is due to LMCT transition.

Table 3. The UV-Visible spectral data of the compounds.

Compound	Wavelength (λ_{max}, nm) in MeOH (10^{-4} M)
H_2L	462 m,br, 353 m,br, 278 s,br, 220 s
$[Cr(L)Cl(H_2O)_2]\cdot 4H_2O$	738 w,br, 668 w,br, 487sh, 462 w,br, 392 m,br, 316 m,br, 286 m,br, 227 sh, 211 s
$[Fe(L)(H_2O)_3]$	605 w, 505 m,br, 403 m,br, 297 m, 231 s
$[Fe(L)(H_2O)(OH)]$	754 sh, 624 m, 554 sh, 513 w, 404 s, 299 s, 231 s
$[Co(HL)_2]\cdot 2H_2O$	462 sh, 433 m,br, 305 sh, 262 sh, 254 m, 212 s
$[Ni(L)(H_2O)_3]$	459 sh, 438 m, 359 sh, 342 m, 311 sh, 300 m, 248 sh, 216 s
$[Cu_2(HL)(L)(Ac)]$	752 w, 457 sh, 432 s, 397 m,br, 326 sh, 312 m, 301 m, 253 sh
$[Zn(L)(H_2O)_2]$	457 sh, 434 w, 390 sh, 299 m,br, 268 m, 254 sh, 213 s

The electronic spectra of the other complexes are of little help in the present case at the visible region, since the d→d transitions are masked by the strong charge-transfer transition bands [33]. 505, 438 and 433 nm absorptions for the Fe(II), Ni(II) and Co(II) complexes, respectively, can be accepted CT transitions. On the other hand, the shoulders at 459 nm for the Ni(II) complex and 462 nm for the Co(II) complex, and the weak absorption at 605 nm in the Fe(II) complex may be related with their octahedral structures [23, 34].

There is no sufficient information from electronic spectra of the Cu(II) and Zn(II) complexes. The absorptions of the both complexes at the 430–460 nm range are due to the CT transitions. The weak band at 752 nm in the Cu(II) complex is due to the d-d transitions and can be considered as indicative of a tetragonal environment for the metal ion and in agreement with the values obtained for dimeric Cu(II) complexes [35].

NMR spectra

The ^{1}H-NMR spectral data of the ligand and its' Zn(II) complex are given in Table 4. The ligand has two hydroxyl protons: OH-2 and OH-2' (Figure 2). The OH-2' proton of the salicylaldimine moiety (phenolic proton) appeared at 13.64 ppm as a broad singlet and OH-2 proton of chlorohydroxyphenyl at 10.00 ppm as singlet. According to the above observation, it may be estimated that OH-2' (phenolic) proton will be removed firstly on complexation. Removing of the second proton is unlikely in 1:2 M:L complex, namely Co(II) complex, with regard to electroneutrality. The both OH protons disappear on complexation with the Zn(II) ion.

The azomethine proton of the ligand and Zn(II) complex appeared at 8.96 and 8.87 ppm, respectively. The upfield shift (shielding) in the Zn(II) complex shows that the azomethine nitrogen coordinated to Zn(II) ion [36]. The very broad signal at 6.67 ppm is remarkable in Zn(II) complex: It may be belongs to H4' proton. This proton appears as a broad signal at the ligand (7.19 d-d, br). This proton gains high acidic character as a result of complexation because of the perturbing effect of Zn(II) ion. Thus, it appears as a very broad signal and showed upfield shift (lower ppm value). The suggested structure of the Zn(II) complex is shown in Figure 2.

Table 4. The ^{1}H-NMR spectral data of the ligand and its Zn(II) complex (δ_H, as ppm, in DMSO-d_6).

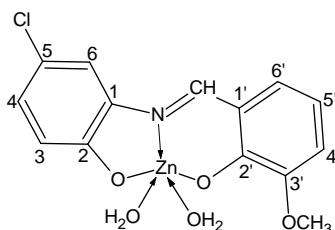| Compound	Chloro-hydroxyphenyl protons				C$\underline{H}$=N	Phenolic (salicylaldimine) protons				
	H3	H4	H6	OH(2)		OH(2')	OCH$_3$	H4'	H5'	H6'
H_2L	7.10 dd,br J = 7.8;1.5	7.15 dd J = 2.6;8.2	7.48 d J = 2.6	10.0 s	8.96 s	13.64 s,br	3.80 s	7.19 dd,br J = 1.3; 8.0	6.87 t J = 8.3; 7.8	6.95 d J = 8.8
$[Zn(L)(H_2O)_2]$	6.91 d J = 7.32	6.97 d J = 7.35	7.57 s	--	8.87 s	--	3.73 s	6.67 s,br	6.39 t J = 7.81; 7.80	6.81 d J = 7.32

Figure 2. The suggested structure of the Zn(II) complex.

Mass spectra

The ESI-MS spectral data of the complexes are given in Table 5 as molecular ions with the relative abundance. It is known that chlorine, bromine and copper have two isotopes: These isotopes, their percent abundance and approximately relative proportions are as the following: $^{35}Cl(75.8\%)$:$^{37}Cl(24.2\%)$ (3:1); $^{79}Br(50.7\%)$:$^{81}Br(49.3\%)$ (1:1); $^{63}Cu(69.2\%)$:$^{65}Cu(30.8\%)$ (2:1). On the other hand, Cr, Fe, Zn and Ni have various isotopes whereas Co has only one isotope. Because of all these reasons, there are many mole peaks at the mass spectra of the complexes. Besides, the peaks of the ligand are easily determined in the mass spectra of the Fe(III), Ni(II) and Zn(II) complexes. The ligand is shown as L in Table 5.

Table 5. The ESI-MS spectral data of the compounds.

Compound and MW (g/mol)	Molecular ions (m/z) with relative abundance (%) and isotopic patterns
H_2L 277.5	276.77 (100, $[M-1]^+$ {^{35}Cl}), 277.78 (39.2, $[M]^+$ {^{35}Cl}), 278.71 (86.3, $[M+1]^+$ {$^{35}Cl+^{37}Cl$}), 279.98 (16.1, $[M+2]^+$ {^{37}Cl})
$[Cr(L)Cl(H_2O)_2]\cdot4H_2O$ 471.1	467.7 (14.2, $[M-3]^+$), 468.8 (16.4, $[M-2]^+$), 470.9 (11.9, M^+), 417.9 (21.7, [M-3H$_2$O]), 422.9 (100, [M-3H$_2$O+5]),
$[Fe(L)(H_2O)_3]\cdot H_2O$ 403.6	401.2 (100, $[M-2]^+$), 403.2 (82.5, $[M]^+$), 405.2 (27.6, $[M+2]^+$), 365.8 (17.5, [L+Fe+2H$_2$O])
$[Fe(L)(H_2O)(OH)]$ 366.6	365.0 (100, $[M-2]^+$), 367.0 (88.6, M^+), 369.0 (29.5, $[M+2]^+$), 278.3 (42.6, L)
$[Co(HL)_2]\cdot2H_2O$ 648.35	609.2 (100, [M-2H$_2$O-3]), 611.2 (65.7, [M-2H$_2$O-1]), 610.2 (28.9, [M-2H$_2$O-2]), 612.2 (18.0, [M-2H$_2$O])
$[Ni(L)(H_2O)_3]$ 388.14	384.5 (100, $[M-4]^+$), 386.6 (26.4, $[M-2]^+$), 387.5 (10.1, $[M-1]^+$), 388.3 (3.6, $[M]^+$), 276.2 (44.1, L-2), 278.2 (11.4, L)
$[Cu_2(HL)(L)(Ac)]$ 738.6	737.4 (25.1, $[M-2]^+$), 736.5 (22.3, $[M-1]^+$), 739.4 (19.4, $[M]^+$), 675.8 (100, [M-Ac-H$_2$O])
$[Zn(L)(H_2O)_2]$ 377.1	376.1 (12.1, $[M-1]^+$), 358.0 (15.3, $[M-H_2O]^+$), 339.4 (20.2, [M-2H$_2$O]), 278.4 (42.2, $[L+1]^+$), 606.2 (100, [2L+Zn+H$_2$O])

Thermal analysis

The major features of the thermal analysis of the complexes are summarized in Table 6. The thermal analysis curves are shown in Figures 3 and 4. The samples of the complexes were heated from room temperature up to 800ºC. Thermal degradation of complexes occurred in three stages. At the first stage, uncoordinated lattice water was lost through evaporation from 50 to 100 ºC. At the second stage, the coordinated water molecules are removed from Cr(III), Fe(II), Fe(III) and Zn(II) complexes at temperatures between 100 and 200 ºC. Cr(III) complex has coordinated and uncoordinated water molecules according to the analytical and thermal analysis data. TGA values are compatible with the suggested structures of the complexes. TGA curve of Co(II) complex draws attention different from the others because of its' 1:2 M:L ratio and

different coordination. Molecular weight ratio of the amount of metal oxide show very good agreement for the proposed structures according to the TGA data. In the all complexes, weight losses observed above 300 ºC can be explained in terms of cleavage of Cl, OH and OCH_3 groups. Above 350 ºC, all other organic parts of complexes are oxidized to carbon dioxide and water.

Table 6. The TGA data of the complexes (thermal decomposition).

| Temperature (ºC) →	100	150	200	250	300	350	400	450	500	550
Complex	Weight loss (%)									
[Cr(L)Cl(H$_2$O)$_2$]·4H$_2$O	9.3	15.2	23.6	29.3	32.6	67.0	84.0	85.2	85.1	85.1
[Fe(L)(H$_2$O)$_3$]	2.6	3.3	4.4	9.4	9.6	11.3	80.3	81.2	81.9	81.7
[Fe(L)(H$_2$O)(OH)]	1.2	2.2	6.6	7.0	7.5	78.7	80.1	80.3	82.6	82.5
[Co(HL)$_2$]·2H$_2$O	5.9	6.7	8.2	27.5	31.8	35.3	38.4	42.9	62.0	89.9
[Ni(L)(H$_2$O)$_3$]	2.1	3.6	11.3	27.1	39.5	45.1	54.7	61.5	78.9	78.6
[Cu$_2$(HL)(L)(Ac)]	0.4	0.5	1.0	2.1	18.9	69.4	71.5	75.9	78.4	83.4
[Zn(L)(H$_2$O)$_2$]	0.8	6.8	9.2	9.9	10.8	17.1	53.3	61.2	72.0	76.5
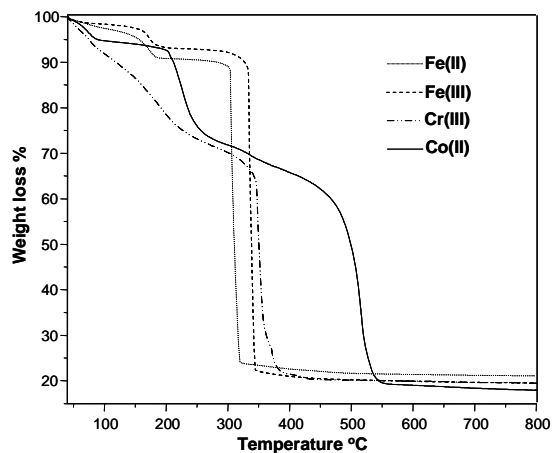
Figure 3. The TGA curves of the Fe(II), Fe(III), Cr(III) and Co(II) complexes.
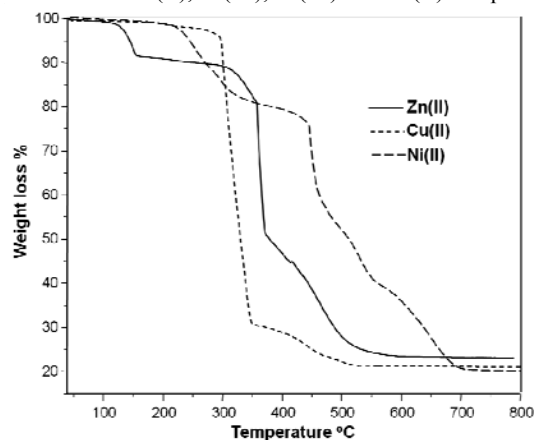

Figure 4. The TGA curves of the Zn(II), Cu(II) and Ni(II) complexes.

As a conclusion, according to the experimental data, the structures in Figure 5 can be proposed for the complexes as a conclusion.
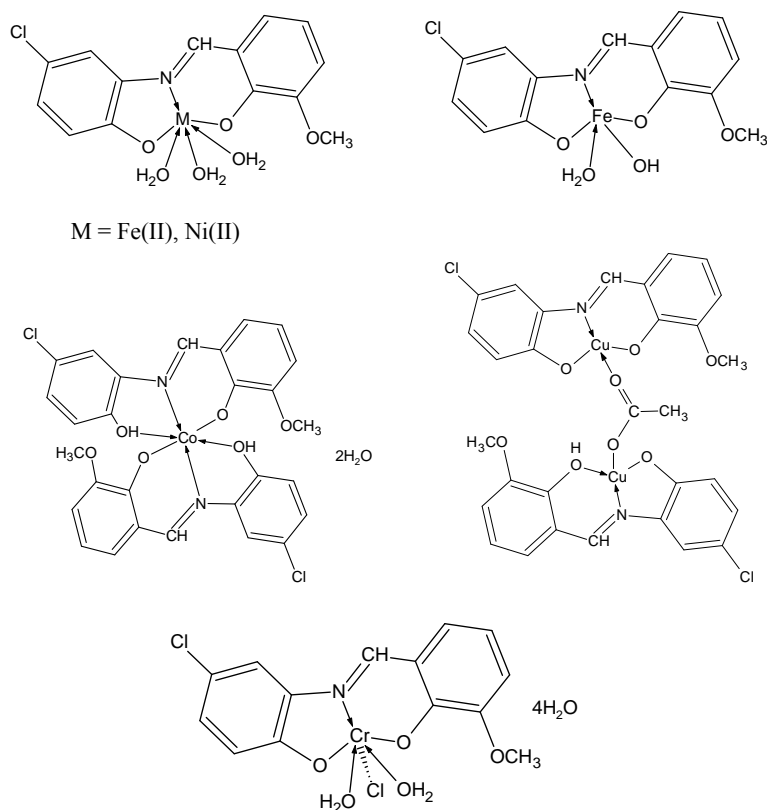

Figure 5. The proposal structures for the Cr(III), Fe(II), Fe(III), Co(II), Ni(II) and Cu(II) complexes.

Antimicrobial activity

The antibacterial activities of the free ligand and the complexes were evaluated using the microbroth dilution method toward six bacteria, and the results are compared with those of Ciprofloxacin. Antifungal activities were referenced against Fluconazole. The antimicrobial activity results were presented in Table 7. Table 7 contains *S. epidermidis* and *C. albicans* as microorganisms because of some of the compounds showed antimicrobial activity toward these two microorganisms only.

From antimicrobial activity tests the significant results were obtained. The Ni(II), Co(II), Cr(III) and Zn(II) complexes are effective on some microorganisms whereas the ligand itself and its Fe(II), Fe(III) and Cu(II) complexes has no activity on the microorganisms. The Co(II) and Ni(II) complexes exhibit considerable antimicrobial activity on *S. epidermidis* and *C. albicans* (MIC value of the Co(II) complex on *S. epidermidis* 12.5 µg/mL; MIC value of the Ni(II) complex on *C. albicans* 31.2 µg/mL). These results can be considered as selective activity. In addition, the Zn(II) complex is effective on *C. albicans* and the Cr(III) complex

shows antibacterial activity on *S. epidermidis* selectively. The activity of the Co(II) complex can be explained with its dissociation in DMSO; the dissociation of the Co(II) complex can be interpreted as a factor increasing the antimicrobial activity. The antifungal activity of the Ni(II) complex may be related with its coordinated water content. In the Ni(II) complex three moles of water were coordinated to the central atom. Water is a weak ligand and it cannot compete the DNA of the microorganisms, and consequently they leave the Ni(II) ion and the microorganisms may coordinated to the central atom instead of the water molecules. This evaluation may also be suggested for the Cr(III) and Zn(II) complexes that contain three and two coordinated water molecules, respectively. As a result, the moderate antimicrobial activities of the Ni(II), Co(II), Zn(II) and Cr(III) complexes against *S. epidermidis* and *C. albicans* warranted further investigation on similar compounds.

Table 7. The *in vitro* antimicrobial activity of the complexes (MIC, µg/mL)[*].

Compound	Microorganisms	
	Se	*Ca*
H_2L	-	-
$[Cr(L)Cl(H_2O)_2] \cdot 4H_2O$	156	-
$[Fe(L)(H_2O)_3]$	-	-
$[Fe(L)(H_2O)(OH)]$	-	-
$[Co(HL)_2] \cdot 2H_2O$	12.5	39
$[Ni(L)(H_2O)_3]$	156	31.2
$[Cu_2(HL)(L)(Ac)]$	-	-
$[Zn(L)(H_2O)_2]$	-	312
Ciprofloxacin	0.0625	-
Fluconazole	-	1.00

Se: Staphylococcus epidermidis ATCC 12228. *Ca: Candida albicans* ATCC 10231. -: Antimicrobial activity was not detected. [*] Only two microorganisms that affected by the compounds under study were showed.

CONCLUSIONS

Salicylaldehyde derivatives, with one or more halo-atoms in the aromatic ring, showed variety of biological activities such as antibacterial and antifungal activities. In this study, Cr(III), Fe(II), Fe(III), Co(II), Ni(II), Cu(II), Zn(II) complexes of *N*-(5-chloro-2-hydroxyphenyl)-3-methoxy-salicylaldimine (H_2L) were prepared and characterized by elemental analysis, magnetic moment, molar conductivity, TGA and NMR, IR, UV-Vis and ESI-MS spectral techniques. It is observed that all of the complexes except $[Zn(L)(H_2O)_2]$ are paramagnetic. The $CoCl_2$ gave a complex with 1:2 M:L ratio while the others form 1:1 non-ionic complexes with H_2L. H_2L, an ONO type ligand, acts tridentate behaviour coordinating to the metal ions through the both OH oxygen and the azomethine nitrogen atoms. Considering the magnetic moment values and the electronic spectral data, octahedral geometry is proposed for the Fe(II), Cr(III), Co(II), Ni(II) complexes. The Zn(II) and Fe(III) complexes have five coordination geometries whereas the Cu(II) complex is four-coordinated having acetato bridged dimeric structure. Antibacterial activities of the compounds were determined by the microbroth dilution technique against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis*. Antifungal activities were reported for *Candida albicans*. It is observed that Ni(II) and Co(II) complexes exhibit considerable activity on *S. epidermidis* and *C. albicans* whereas the ligand has no activity on the microorganisms. In addition, the Cr(III) complex shows antibacterial activity toward *S. epidermidis* and the Zn(II) complex has antifungal activity on *C. albicans*.

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REFERENCES

1. a) Çinarli, A.; Gürbüz, D.; Tavman, A.; Birteksöz, A.S. *Bull. Chem. Soc. Ethiop.* **2011**, 25, 407; b) Çinarli, A.; Gürbüz, D.; Tavman, A; Birteksöz, A.S. *Chinese J. Chem.* **2012**, 30, 449; c) Gürbüz, D.; Çinarli, A.; Tavman, A.; Birteksöz, A.S. *Chinese J. Chem.* **2012**, 30, 970; d) Tavman, A. *Anal. Sci.* **2008**, 24, x145.
2. a) Popovic, Z.; Pavlovic, G.; Matkovic-Calogovis, D.; Roje, V.; Leban, I. *J. Mol. Struct.* **2002**, 615, 23; b) Elmali, A.; Elerman, Y.; Svoboda, I. *Acta Cryst.* **2001**, C54, 485; c) Ünver, H.; Karakaş, A.; Elmali, A. *J. Mol.Struct.* **2008**, 877, 152.
3. You, Z.-L.; Zhu, H.-L.; Liu, W.-S. Z. *Anorg. Allg. Chem.* **2004**, 630, 1617.
4. You, Z.-L.; Zhu, H.-L. Z. *Anorg. Allg. Chem.* **2004**, 630, 2754.
5. Gölcü, A.; Tümer, M.; Demirelli, H.; Wheatley, R.A. *Inorg. Chim. Acta* **2005**, 358, 1785.
6. Chandra, S.; Sangeetika, X. *Spectrochim. Acta* **2004**, A60, 147.
7. a) Fujita, E.; Brunschwig, B.S.; Ogata, T.; Yanagida, S. *Coord. Chem. Rev.* **1994**, 132, 195; b) Kimura, E.; Wada, S.; Shiyonoya, M.; Okazaki, Y. *Inorg. Chem.* **1994**, 33, 770; c) De Clercq, B.; Verpoort, F. *Macromolecules* **2002**, 35, 8943; d) Opstal, T.; Verpoort, F. *Angew. Chem. Int. Ed.* **2003**, 42, 2876; e) De Clercq, B.; Lefebvre, F.; Verpoort, F. *Appl. Catal.* **2003**, A247, 345.
8. a) Ren, S.; Wang, R.; Komatsu, K.; Bonaz-Krause, P.; Zyrianov, Y.; McKenna, C.E.; Csipke, C.; Tokes, Z.A.; Lien, E.J. *J. Med. Chem.* **2002**, 45, 410; b) Hodnett, E.M.; Dunn, W.J. *J. Med. Chem.* **1970**, 13, 768.
9. a) Yang, Z.-Y.; Yang, R.-D.; Li, F.-S.; Yu, K.-B. *Polyhedron* **2000**, 19, 2599; b) Chakraborty, J.; Patel, R.N. *J. Ind. Chem. Soc.* **1996**, 73, 191; c) Zhong, X.; Yi, J.; Sun, J.; Wei, H.-L.; Liu, W.-S.; Yu, K.-B. *Eur. J. Med. Chem.* **2006**, 41, 1090; d) Adsule, S.; Barve, V.; Chen, D.; Ahmed, F.; Dou, Q.P.; Padhye, S.; Sarkar, F.H. *J. Med. Chem.* **2006**, 49, 7242.
10. Majumder, A.; Rosair, G.M.; Mallick, A.; Chattopadhyay, N. *Polyhedron* **2006**, 25, 1753.
11. Zishen, W.; Zhiping, L.; Zhenhuan, Y. *Transit. Met. Chem.* **1993**, 18, 291.
12. a) Khalil, M.M.H.; Aboaly, M.M.; Ramadan, R.M. *Spectrochim. Acta* **2005**, A61, 157; b) Chantarasiri, N.; Ruangpornvisuti, V.; Muangsin, N.; Detsen, H.; Mananunsap, T.; Batiya, C.; Chaichit, N. *J. Mol. Struct.* **2004**, 701, 93; c) Abdallah, S.M.; Mohamed, G.G.; Zayed, M.A.; El-Ela, M.S.A. *Spectrochim. Acta* **2009**, A73, 833.
13. a) Radhakrishanan, P.K.; Indrasen, P.; Nair, C.G.R. *Polyhedron* **1998**, 18, 67; b) Ispir, E.; Kurtoglu, M.; Purtas, F.; Serin, S. *Transit. Met. Chem.* **2005**, 30, 1042; c) Sreedaran, S.; Bharathi, K.S.; Kalilur-Rahiman, A.; Rajesh, K.; Nirmala, G.; Jagadish, L.; Kaviyarasan, V.; Narayana, V. *Polyhedron* **2008**, 27, 1867.
14. Clinical and Laboratory Standards Institute (CLSI) *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, Approved Standard M7-A5*, Wayne: PA, USA; **2006**.
15. Clinical and Laboratory Standards Institute (CLSI) *Reference method for broth dilution antifungal susceptibility testing of yeasts, Approved Standart M27-A2*, 2nd ed., Wayne: PA, USA; **2002**.
16. a) Sanchiz, J.; Esparza, P.; Dominguez, S.; Brito, F.; Mederos, A. *Inorg. Chim. Acta* **1999**, 291, 158; b) Türkel, N.; Aydin, R.; Özer, U. Turk. *J. Chem.* **1999**, 23, 249; c) Kopecka, B.; Springer, V.; Majer, J. *Chem. Zvesti.* **1981**, 35, 641.
17. a) Wahlgren, C.G.; Addison, A.W.; Burman, S.; Thompson, L.K.; Sinn, E.; Rowe, T.M. *Inorg. Chim. Acta* **1989**, 166, 59; b) Tavman, A.; Agh-Atabay, N.M.; Güner, S.; Gücin, F.;

Dülger, B. *Transit. Met. Chem.* **2007**, 32, 172; c) Tavman, A.; Boz, I.; Birteksöz, A.S.; Cinarli, A. *J. Coord. Chem.* **2010**, 63, 1398.

18. a) El-Sawaf, A.K.; West, D.X.; El-Saied, F.A.; El-Bahnasawy, R.M. *Transit. Met. Chem.* **1998**, 23, 565; b) Padhye, S.; Kauffman, G.B. *Coord. Chem. Rev.* **1985**, 63, 127; c) Tavman, A.; Agh-Atabay, N.M.; Neshat, A.; Gücin, F.; Dülger, B.; Haciu, D. *Transit. Met. Chem.* **2006**, 31, 194.

19. Ruminski, R.R.; Nelson, J.; Culver, W. *Inorg. Chim. Acta* **2004**, 357, 839.

20. Shukla, D.; Gupta, L.K.; Chandra, S. *Spectrochim. Acta* **2008**, A71, 746.

21. a) Vinuelas-Zahinos, E.; Maldonado-Rogado, M.A.; Luna-Giles, F.; Barros-Garcia, F.J. *Polyhedron* **2008**, 27, 879; b) Avaji, P.G.; Patil, S.A.; Badami, P.S. *Transit. Met. Chem.* **2008**, 33, 275.

22. a) Sonmez, M. *Turk. J. Chem.* **2001**, 25, 181; b) Anacona, J. R.; Gomez, J. *J. Chil. Chem. Soc.* **2008**, 53, 1694.

23. Jacob, W.; Mukherjee, R. *Inorg. Chim. Acta* **2006**, 359, 4565.

24. a) Nawar, N.A.; Shallaby, A.-H. M.; Hosny, N. M.; Mostafa, M.M. *Transit. Met. Chem.* **2001**, 26, 180; b) Drew, M.G.B.; Yates, P.C. *Inorg. Chim. Acta* **1986**, 118, 37.

25. a) Yildiz, M.; Kiliç, Z.; Hökelek, T. *J. Mol. Struct.* **1998**, 441, 1; b) Schilf, W.; Szady-Chelmieniecka, A.; Grech, E.; Przybylski, P.; Brzezinski, B. *J. Mol. Struct.* **200**2, 643, 115.

26. Redshaw, C, Wilkinson, G, Bates, B.H., Hursthouse, M.B. *J. Chem. Soc. Dalton Trans.* **1992**, 1803.

27. a) Koley, M.K.; Sivasubramanian, S.C.; Varghese, B.; Manoharan, P.T.; Koley, A.P. *Inorg. Chim. Acta* **2008**, 361, 1485; b) Purohit, S.; Koley, A.P.; Prasad, L.S.; Manoharan, P.T.; Ghosh, S. *Inorg. Chem.* **1989**, 28, 3735.

28. a) Kaya, İ.; Moral, F.; Özdemir, E. *Bull. Chem. Soc. Ethiop.* **2009**, 23, 55; b) Anitha, C.; Sheela, C.D.; Tharmaraj, P.; Hema, V.V. *J. Chem.* **2013**, in press, http://dx.doi.org/10.1155/2013/724163.

29. a) Yilmaz, V.T.; Hamamci, S.; Andaç, O.; Güven, K. Z. *Anorg. Allg. Chem.* **2003**, 629, 172; b) Dianu, M.L.; Kriza, A.; Stanica, N.; Musuc, A. M. *J. Serb. Chem. Soc.* **2010**, 75, 1515.

30. a) Akkus, Ö.; Uzman, S. *Spect. Lett.* **2000**, 33, 445; b) Ambroziak, K.; Rozwadowski, Z.; Dziembowska, T.; Bieg, B. *J. Mol. Struct.* **2002**, 615, 109; c) Issa, R.M.; Khedr, A.M.; Rizk, H.F. *Spectrochim. Acta* **2005**, A62, 621; d) Habibi, M.H.; Montazerozohori, M.; Laleganı, A.; Harrington, R.W.; Clegg, W. *J. Fluorine Chem.* **2006**, 127, 769.

31. a) Maples, D.L.; Maples, R.D.; Hoffert, W.A.; Parsell, T.H.; van Asselt, A.; Silversides, J.D.; Archibald, S.J.; Hubin, T.J. *Inorg. Chim. Acta* **2009**, 362, 2084; b) Wright-Garcia, K.; Basinger, J.; Williams, S.; Hu, C.J.; Wagenknecht, P.S. *Inorg. Chem.* **2003**, 42, 4885.

32. a) Singh, D.P.; Kumar, R.; Singh, J. Eur. *J. Med. Chem.* **2009**, 44, 1731; b) Lever, A.B.P. *Inorganic Electronic Spectroscopy*, Elsevier: Amsterdam; **1984**.

33. Salem, N.M.H.; El-Sayed, L.; Iskander, M.F. *Polyhedron* **2008**, 27, 3215.

34. Cotton, F.A.; Wilkinson, G.; Murillo, C.A.; Bochmann, M. *Advanced Inorganic Chemistry*, 6th ed., Wiley: New York; **1999**.

35. a) Rodriguez-Infante, C.; Esteban, D.; Avecilla, F.; de Blas, A.; Rodriguez-Blas, T.; Mahia, J.; Macedo, A.L.; Geraldes, C.F.G.C. *Inorg. Chim. Acta* **2001**, 317, 190; b) Piu, P.; Leoni, V.; Zoroddu, M.A.; Manca, G.; Deiana, S.; Gessa, C. *Transit. Met. Chem.* **1992**, 17, 283; c) Alves, W.A.; de Almeida-Filho, S.A.; de Almeida Santos, R.H.; Paduan-Filho, A.; da Costa Ferreira, A.M. *J. Braz. Chem. Soc.* **2004**, 15, 872.

36. a) Montazerozohori, M.; Yadegari, S.; Naghiha, A.; Veyseh, S. *J. Ind. Eng. Chem.* 2014, 20, 118; b) Montazerozohori, M.; Zahedi, S.; Naghiha, A.; Zohour, M.M. *Mater. Sci. Eng. C Mater. Biol. Appl.* **2014**, 35, 195.