



## SYNTHESIS, CHARACTERIZATION, ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF 1,5-BIS(6-CHLORO-1H-BENZIMIDAZOL-2-YL)PENTAN-3-ONE AND ITS Fe(III), Co(II), Cu(II), Zn(II) AND Ru(II) COMPLEXES

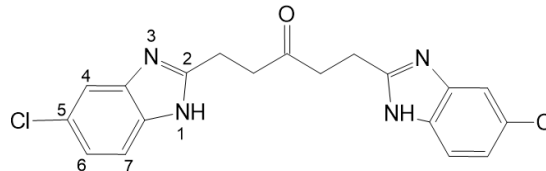
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A new bis-benzimidazole ligand, 1,5-bis(6-chloro-1H-benzimidazol-2-yl)pentan-3-one (**L**, C<sub>19</sub>H<sub>16</sub>Cl<sub>2</sub>N<sub>4</sub>O), and its complexes with Fe(III), Co(II), Cu(II), Zn(II) and Ru(II) chlorides are synthesized and characterized by elemental analysis, molar conductivity, magnetic moment, TGA, FT-IR, <sup>1</sup>H-NMR, fluorescence and UV-visible spectroscopy. The spectral data suggest that the chelating ligand acted as tridentate towards to Cu(II) ion however bidentate in the other complexes. According to the molar conductance data, [Fe(**L**)<sub>2</sub>Cl<sub>2</sub>]Cl·2H<sub>2</sub>O and [Cu(**L**)Cl]Cl·2H<sub>2</sub>O complexes are 1:1 electrolytes whereas [Co(**L**)Cl<sub>2</sub>]·2H<sub>2</sub>O, [Zn(**L**)(Cl)<sub>2</sub>(H<sub>2</sub>O)]·H<sub>2</sub>O and [Ru(**L**)(DMSO)(H<sub>2</sub>O)Cl<sub>2</sub>] complexes are non-ionic. In addition, antibacterial and antioxidant activity of the compounds were tested. The Ru(II) and Co(II) complexes showed moderate activity against to all tested bacteria whereas the ligand itself had no activity. According to the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH•) scavenging results, the Ru(II) complex of **L** had quite significant radical scavenging activity almost the same as that of 3,5-di-tert-butylhydroxytoluene (BHT), a commercial standard.



### INTRODUCTION

Benzimidazole is an attractive heterocyclic ring because it is present in cyanocobalamin (vitamin B12) and various drugs such as omeprazole, albendazole, mebendazole etc. It is also plays a role in clinically confirmed antivirals, antihistamines, anthelmintics and antiulcers.<sup>1</sup> There are published studies of benzimidazole derivatives that exhibit antitumor and antimicrobial properties and act as thrombopoietin receptor agonists.<sup>2,3</sup>

Bis-benzimidazoles have attracted attention because of the coordination of the double bonded heterocyclic ring nitrogen atom (–C=N–), which acts as a ligand in transition metal complexes.<sup>4</sup> They give chelate complexes coordinating through both the C=N nitrogen atoms<sup>4,5</sup> and have metallic polymer forming characteristics as typical multidentate ligands.<sup>6–8</sup> In addition, there are studies showing that bis-benzimidazoles show significant antimicrobial activities such as

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antibacterial and antifungal<sup>9-11</sup> and anticancer effect.<sup>12</sup> Transition metal complexes of bis-benzimidazole ligands having various bridging groups were investigated as potential models for the structure of metal binding sites in metalloproteins such as hemocyanin, (a copper-containing protein chemically unlike hemoglobin, is found in some crustaceans), azurin (a bacterial blue copper protein), tyrosinase (a copper-containing enzyme) and hemerythrin (a monomeric O<sub>2</sub>-binding non-heme iron protein found in the muscles of marine invertebrates).<sup>13-21</sup>

There are very limited studies on bis-benzimidazoles including keto group and their metal complexes. In the one of them, we reported preparation and spectral characterization of 1,3-bis(1*H*-benzimidazol-2-yl)-propan-1-one that an asymmetric bis-benzimidazole and its various complexes with Zn(II) salts.<sup>22</sup> There are a few studies on the synthesis and some properties of bis(benzimidazol-1-yl)-methanone<sup>23,24</sup>, bis(1-

methyl-1*H*-benzimidazol-2-yl)methanone<sup>25</sup> and bis(4-(1*H*-benzimidazol-1-yl)phenyl)methanone.<sup>26</sup> The dicopper complex of bis(4-(1*H*-benzimidazol-1-yl)phenyl)methanone is also reported.<sup>26</sup> Ketonization of methylene of bis(benzimidazol-2-yl)methane by molecular oxygen under the catalysis of cobalt(II) ion was reported.<sup>27</sup> Bis(1-methylbenzimidazol-2-yl)ketone was synthesized by lithiation of 1-methylbenzimidazole and its Cu(I) and Cu(II) complexes were prepared by Gorun *et al.*<sup>28</sup>

In this study, a new bis-benzimidazole derivative, 1,5-bis(6-chloro-1*H*-benzimidazol-2-yl)pentan-3-one (**L**), and its complexes with Fe(III), Co(II), Cu(II), Zn(II) and Ru(II) chlorides are synthesized and characterized by elemental analysis, molar conductivity, magnetic moment, FT-IR, <sup>1</sup>H-NMR, fluorescence and UV-visible spectroscopy. In addition, antibacterial and antioxidant activities of the obtained compounds were investigated.

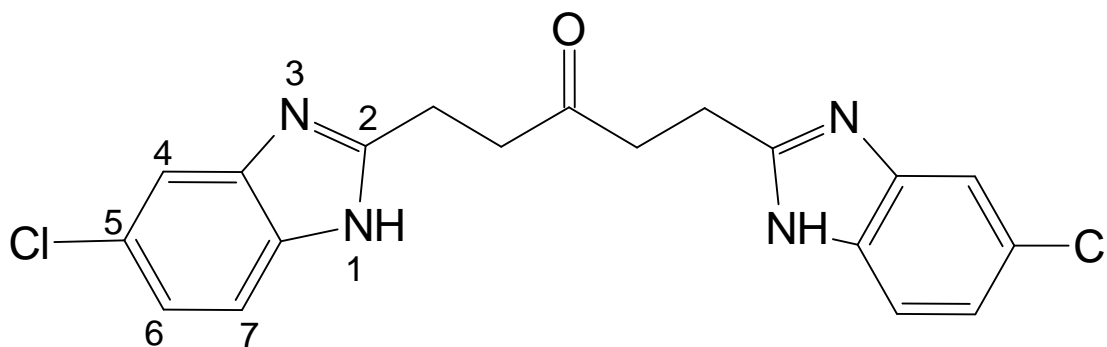


Fig. 1 – Chemical structure of 1,5-bis(6-chloro-1*H*-benzimidazol-2-yl)pentan-3-one (**L**).

## RESULTS AND DISCUSSION

Some physicochemical data of the ligand and its complexes were given in Experimental section. All the complexes were obtained by refluxing in ethanol at a ratio of 1:1 in the same conditions. The Fe(III) complex appears to form in a 1:2 M:L ratio whereas the others 1:1.

The ligand and complexes have well solubility in polar solvents such as ethanol, acetonitrile, dimethylformamide (DMF) etc whereas low solubility in apolar solvents such as chloroform, acetone etc.

### Molar Conductivity and Magnetic Moment

According to the molar conductivity data, Fe(III) and Cu(II) complexes are 1:1 ionic (108.3 and 68.0  $\Omega^{-1}\text{cm}^2\text{mol}^{-1}$ , respectively, in DMF), whereas Co(II), Zn(II) and Ru(II) complexes are

non-ionic (51.1, 39.4 and 29.0  $\Omega^{-1}\text{cm}^2\text{mol}^{-1}$ , respectively).<sup>29</sup>

Magnetic moment values of the Fe(III), Co(II) and Cu(II) complexes were measured as 6.12, 4.15 and 1.68 BM, respectively. The magnetic moment value of the Fe(III) complex, 6.12 BM, is very close to spin only magnetic moment value is 5.92 BM for five unpaired electrons ( $d^5: t_{2g}^3 e_g^2$ ) and indicates an octahedral structure. The magnetic moment value of 4.15 BM of the Co(II) complex shows that orbital contributions are high in the complex, considering that only the spin magnetic moment value is 3.88 BM for three unpaired electrons. The blue color of this complex suggests a tetrahedral coordination geometry of the Co(II) ions (and the value of the magnetic moment at room temperature might support this affirmation. The room temperature magnetic moment value of the Cu(II) complex (1.68 BM) are very closer to the theoretical value (1.73 BM) for a  $d^9$  metal ion.

### Infrared Spectroscopy

FT-IR spectral data of the ligand and its complexes are given in Experimental Section. The infrared spectra of all compounds are given in Fig. 2 comparatively.

The C=O is a very characteristic group for the ligand and is detected at the wavenumber  $1712\text{ cm}^{-1}$  as strong band. It is possible to detect whether the complexes are coordinated over the C=O oxygen

or not. The weakening of the C=O band (at  $1695\text{ cm}^{-1}$ , weak shoulder) in the Cu(II) complex can be considered as an evidence for the C=O oxygen coordination. In the other complexes, the C=O band is seen as strong or medium in the range of  $1704\text{--}1725\text{ cm}^{-1}$  similarly in that of the free ligand. According to this finding, it is possible to argue that the C=O oxygen is not coordinated to the metal ion in the complexes other than the Cu(II) complex (Fig. 3).

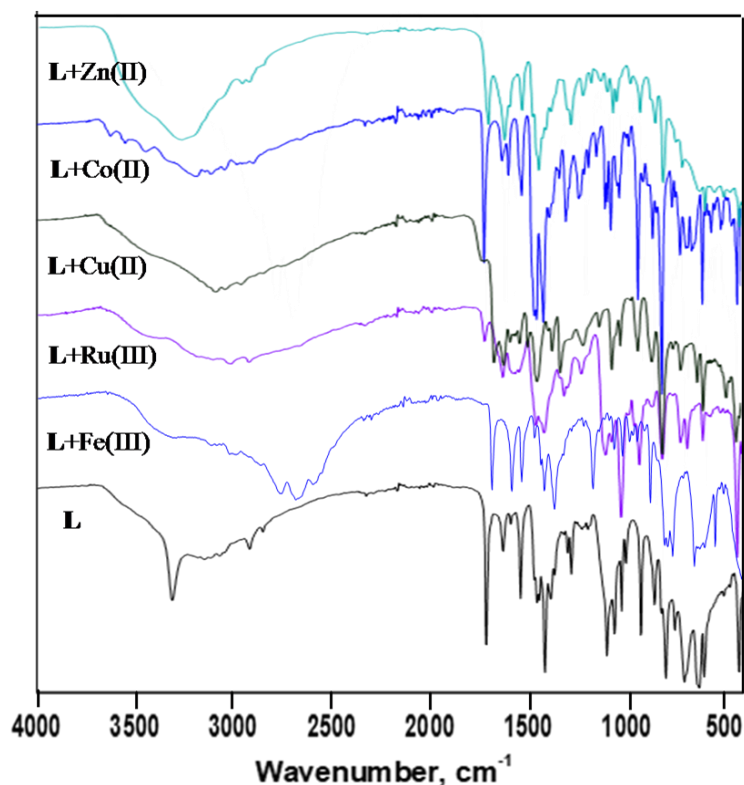


Fig. 2 – FT-IR spectra of all the compounds between  $4000$  and  $400\text{ cm}^{-1}$ .

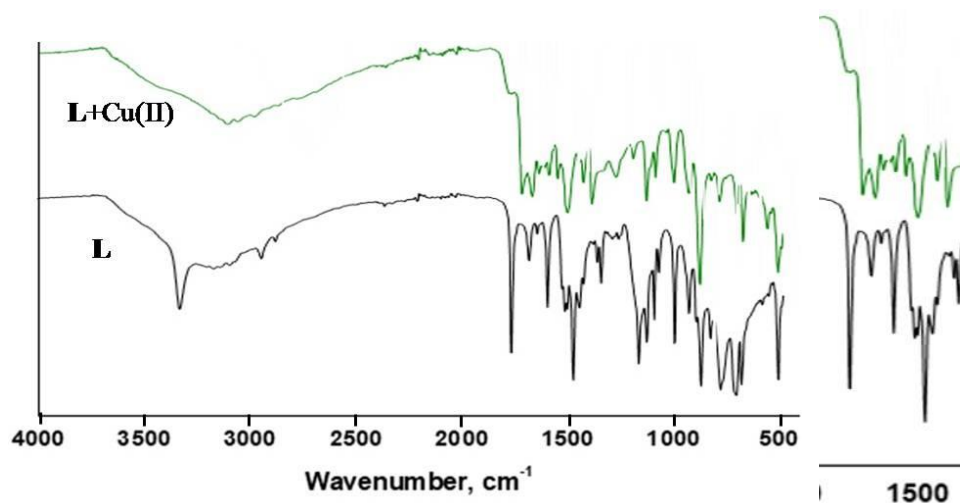


Fig. 3 – FT-IR spectra of the ligand (L) and its Cu(II) complex at  $4000\text{--}400$  and  $1500\text{--}1600\text{ cm}^{-1}$  regions.

The stretching vibration of the NH groups of **L** is detected as medium band at  $3311\text{ cm}^{-1}$ . The stretching vibration of the C=N groups is observed at  $1629\text{ cm}^{-1}$ . The C-Cl stretching vibrations could be detected at  $702\text{--}719\text{ cm}^{-1}$  range as medium bands.

The difference in the Fe(III) complex compared to other complexes (broad band between  $3000\text{--}2500\text{ cm}^{-1}$ ) indicates strong inter- and intramolecular hydrogen bonding.

In the complexes, the new bands with medium characteristics between  $664\text{ and }686\text{ cm}^{-1}$  with respect to the ligand can be assigned to the  $\nu(\text{N-M})$  band.<sup>30,31</sup>

### NMR Spectroscopy

NMR spectral data of **L** and its Zn(II) and Ru(II) complexes are presented at Experimental Section. At the  $^1\text{H-NMR}$  spectra, the aromatic ring protons (H4, H6 and H7) were detected at the  $7.10\text{--}7.69\text{ ppm}$  range. These protons showed downfield shift of  $0.08\text{--}0.19\text{ ppm}$  in the Zn(II) and Ru(II) complexes whereas their doublet characters changed to broad singlet. The methylene protons of the bridging section  $[(-\text{CH}_2\text{-CH}_2\text{-})_2\text{C=O}]$  appear at  $3.10\text{ and }3.02\text{ ppm}$  as multiplet and they shifted to the  $3.14\text{--}3.17\text{ and }3.07\text{--}3.12\text{ ppm}$  ranges, respectively, in the complexes. In the  $^{13}\text{C-NMR}$  spectra, the C=O carbon shows signal at  $208.15\text{ and }207.80\text{ ppm}$  in the ligand and the Zn(II) complex, respectively. According to this finding, it is possible to assert that the C=O oxygen is not coordinated to the Zn(II) ion. IR data also support

this interpretation. The methylene carbon atoms at the bridging section  $[(-\text{CH}_2\text{-CH}_2\text{-})_2\text{C=O}]$  appear at  $39.71\text{ and }23.20\text{ ppm}$  at the ligand, and  $39.87\text{ and }23.07\text{ ppm}$  in the Zn(II) complex.  $^{13}\text{C-NMR}$  signals of the Ru(II) complexes could not be obtained.

### UV-visible Spectroscopy

UV-visible spectral data of the compounds obtained in acetonitrile are presented in Experimental section.

The electronic spectra of the compounds are obtained in the  $200\text{ to }800\text{ nm}$ . They exhibit intense bands in the  $200\text{--}290\text{ nm}$  region, which can be assigned to the  $n \rightarrow \sigma^*$ ,  $n \rightarrow \pi^*$  and  $\pi \rightarrow \pi^*$  transitions. The bands below  $250\text{ nm}$  are due to the  $n \rightarrow \sigma^*$  transitions. The  $\pi \rightarrow \pi^*$  transitions of the aromatic rings are observed between  $250\text{ and }290\text{ nm}$ .<sup>32</sup> The electronic spectra of the complexes are of little assist in the present case in the visible region, as the  $d \rightarrow d$  transitions are masked by the strong charge-transfer transition bands.

The UV-vis spectrum of Co(II) complex shows a broad band with a maxima at approximately  $630\text{ nm}$  which can be assigned  $^4\text{A}_2(^4\text{F}) \rightarrow ^4\text{T}_1(^4\text{P})$ , with two shoulders. This absorption can be considered as an indication that the Co(II) complex has a tetrahedral geometry. The other two spin allowed d-d transitions band occur in NIR region above  $800\text{ nm}$ , falls outside of scope of our study. The magnetic moment value ( $4.15\text{ BM}$ ) and the blue color of the cobalt(II) complex, also suggest tetrahedral stereochemistry.<sup>33</sup>

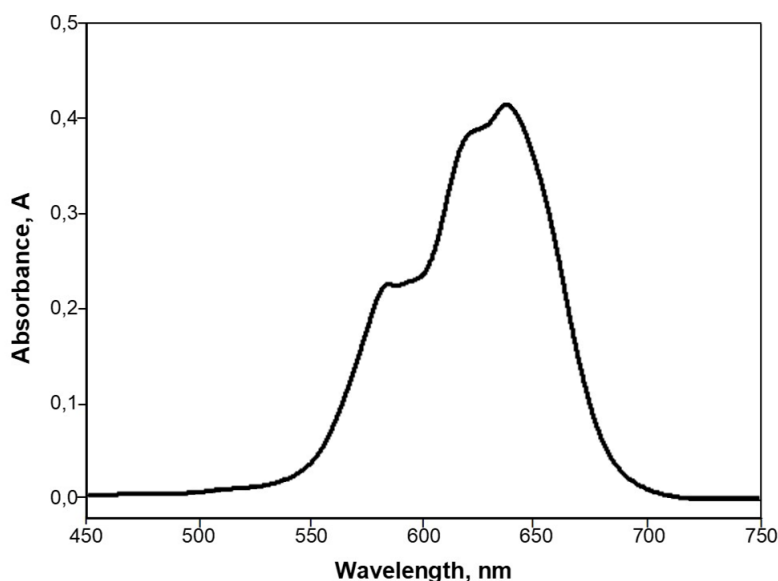


Fig. 4 – The  $450\text{--}750\text{ nm}$  region of the UV-visible spectrum of the Co(II) complex in MeCN.

Dark smoked colored Ru(II) complex showed a medium broad band at 575 nm is due to the charge transfer transition.

### Thermogravimetric Studies

The major features of the thermal analysis of the complexes are given in Experimental section. The thermal analysis curves of Fe(III), Co(II), Cu(II), Zn(II) and Ru(II) complexes are shown in Fig. 5. The samples of the complexes were heated from room temperature up to 500 °C in air atmosphere. It was observed that the thermal degradation of the complexes occurred at four stages in the most of the complexes. At the first stage, uncoordinated lattice water was lost through elimination from 40 to 100 °C. At the second stage, coordinated water molecules are removed above 100 °C. At the third stage, a small weight losses observed around 300 °C can be explained in terms of leaving of the carbonyl group. TGA curves of the Co(II) and

Cu(II) complexes are similar to each other up 100 °C. A mass loss of 6.5% was observed in all of them below 100 °C. According the TGA data, it is possible to suggest that two moles of H<sub>2</sub>O in Fe(III), Co(II) and Cu(II) complexes are lattice water. The mass of two moles of water corresponds to a mass of about 3.5% for the Fe(III) complex and about 6.3% for the Co(II) and Cu(II) complexes. In the Zn(II) complex, one of the H<sub>2</sub>O molecules is coordinated and the other one is lattice water, weight loss 3.1% up to 90 °C and 6.3% up 170 °C (theoretical value for two moles of water for the Zn(II) complex is 6.45%). It is possible to argue that one mole of water in the Ru(II) complex is coordinated considering about 3% weight loss at the 150–190 °C range (theoretical value: 2.75%). Based on the TGA data, it can be argued that the Zn(II) complex has a five-coordinate structure, such as square pyramid stereochemistry, [Zn(L)(Cl)<sub>2</sub>(H<sub>2</sub>O)]·H<sub>2</sub>O (Fig. 7).

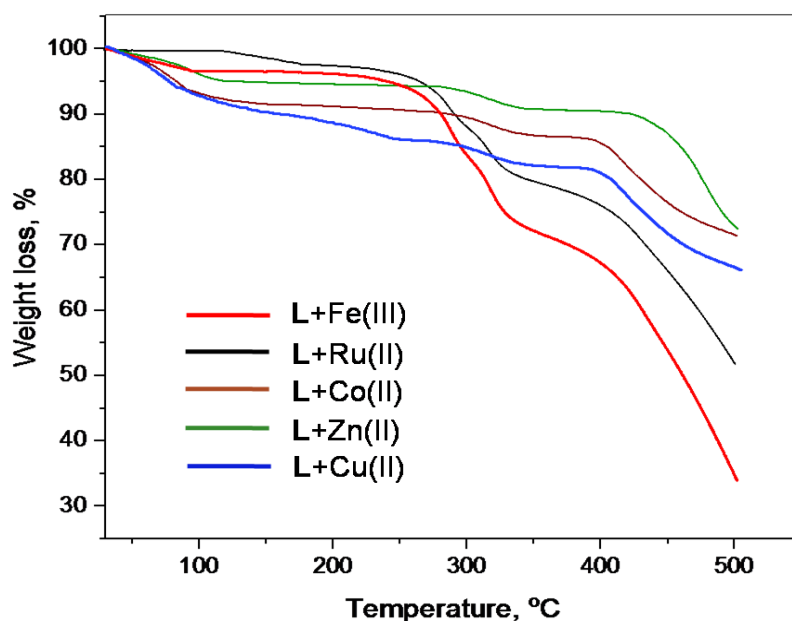


Fig. 5 – TGA curves of the complexes.

### Fluorescence Spectroscopy

Excitation and emission spectra of the compounds were gathered in ethanol at  $\sim 10^{-4}$  M concentration (excitation wavelength: 354 nm). The emission data of the compounds are presented in Experimental section.

Four emission bands of different intensities were observed in the fluorescence spectrum of **L** in ethanol at 489 (broad medium), 422 (broad weak), 395 (weak) and 373 (weak) nm. These bands probably result from, respectively, the normal Stokes shift arising from excited  $\pi^*$  electronic state and intramolecular charge transfer and monocation

protonated of the benzimidazole nitrogen atom with double bond as a result of the interaction such as hydrogen bonding with the solvent (ethanol).<sup>34,35</sup> The fourth one is probably due to the intra-ligand transitions (373 nm).

In the complexes, it was observed that the emission wavelengths move around to lower wavelength values while the fluorescence intensity

decreases, in other words, there is a blue shift as expected. However, while fluorescence intensity decreased in all complexes, no decrease was observed in the Cu(II) complex. This may be due to the fact that Cu(II) ion coordinate to the C=O oxygen atom, unlike other metal ions. For the comparison, the fluorescence spectra of **L** and the Cu(II) complex in ethanol are shown in Fig. 6.

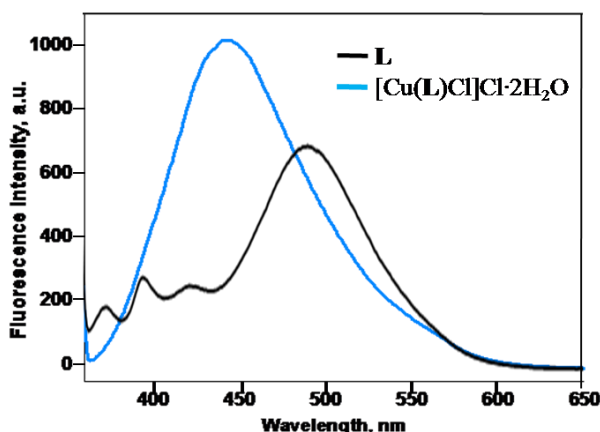


Fig. 6 – The fluorescence spectra of the ligand and its Cu(II) complex in ethanol

In the light of all these data, the structures in Figure 7 can be suggested for the complexes.

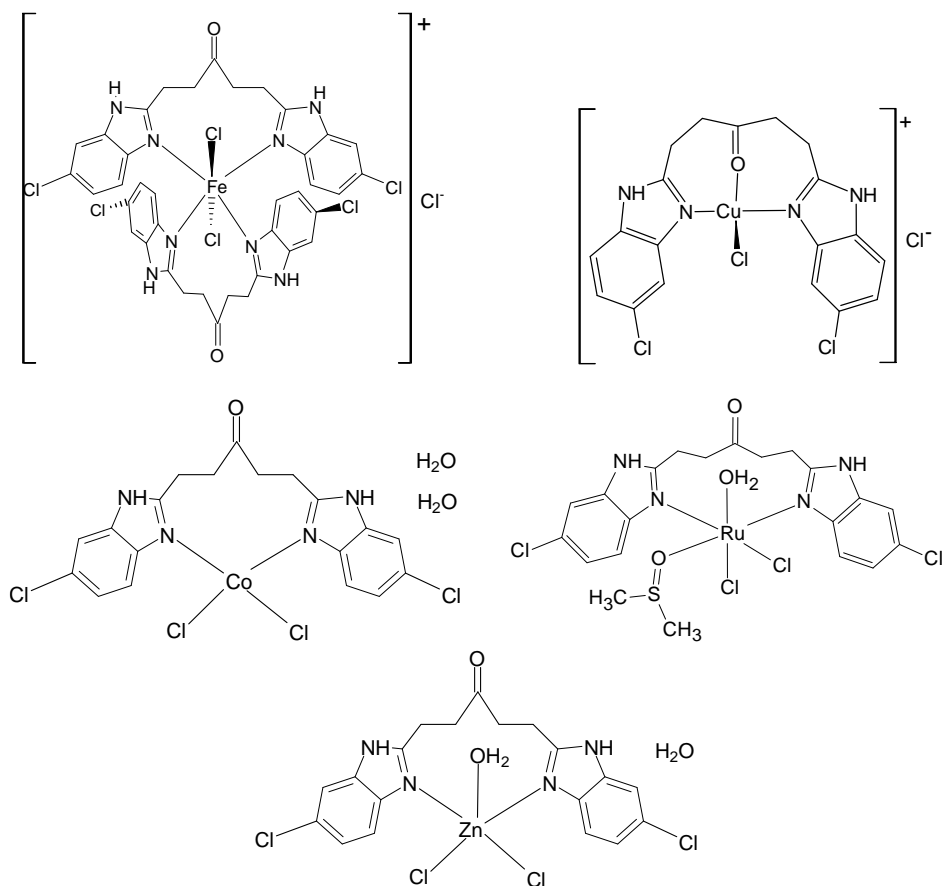


Fig. 7 – The proposed coordination for the Fe(III), Co(II), Cu(II), Zn(II) and Ru(II) complexes.

### Antibacterial Activity

In recent years, there has been an increase in studies on the synthesis of new compounds with biological activity. Especially, the resistance acquired by pathogenic microorganisms to the antibiotics has reached dangerous levels, further increasing antimicrobial studies.<sup>36</sup> In this study, antibacterial activity of the ligand (**L**) and its complexes newly synthesized were tested against Gram-positive (*Bacillus subtilis* ATCC 6633, *Bacillus megaterium* ATCC 19213, and *Enterococcus faecalis* ATCC 29212) and

gram-negative (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 and *Salmonella typhimurium* ATCC 14028) bacteria using disc diffusion method. The test results are shown at Table 1 as inhibition zone (mm).

The Fe(III), Co(II), Cu(II) and Ru(II) complexes showed antibacterial activity at the dose range 250 – 500 µg/mL against both Gram+ and Gram– bacteria whereas the ligand itself has no activity. Especially, it is interesting and valuable that Co(II) and Ru(II) complexes showed activity against all bacteria.

Table 1

Antibacterial activity values of the compounds with compared antimicrobial agent (inhibition zone, mm)

Compounds	Conc. (µg/mL)	Bacteria					
		<i>B. subtilis</i>	<i>B. megaterium</i>	<i>E. faecalis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. typhimurium</i>
<b>L</b> (C <sub>19</sub> H <sub>16</sub> Cl <sub>2</sub> N <sub>4</sub> O)	500	-	-	-	-	-	-
	250	-	-	-	-	-	-
[Fe( <b>L</b> ) <sub>2</sub> Cl <sub>2</sub> ]Cl·2H <sub>2</sub> O	500	-	-	8	-	7	7
	250	-	-	-	-	7	-
[Co( <b>L</b> )Cl <sub>2</sub> ]·2H <sub>2</sub> O	500	7	7	8	8	8	8
	250	7	7	8	7	7	7
[Cu( <b>L</b> )Cl]Cl·2H <sub>2</sub> O	500	7	-	7	8	7	-
	250	7	-	7	7	7	-
[Zn( <b>L</b> )(Cl) <sub>2</sub> (H <sub>2</sub> O)]·H <sub>2</sub> O	500	-	-	-	-	-	-
	250	-	-	-	-	-	-
[Ru( <b>L</b> )(DMSO)(H <sub>2</sub> O)Cl <sub>2</sub> ]	500	8	9	7	8	9	7
	250	-	8	7	8	7	7
Ofloxacin	10 µg/disc	18	20	16	17	16	18

### Antioxidant Activity

In this study, the antioxidant activities of **L** and its complexes were tested by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and ferric reducing / antioxidant power (FRAP) assays (Table 2). The higher trolox equivalent antioxidant capacity (TEAC) values in FRAP test and the lower 50% scavenging concentration (SC<sub>50</sub>) values in DPPH• scavenging test specify better antioxidant activity. According to the literature, the antioxidant activity of the complexes can differ from which were based on the metal ion and the connected groups to it.<sup>37</sup> According to the DPPH• scavenging results, the Ru(II) complex (SC<sub>50</sub>:

0.0169±0.0005 mg/mL) had quite significant radical scavenging activity and the Co(II) complex (SC<sub>50</sub>: 2.6944±0.0179 mg/mL) had lower activity. Among the compounds tested, the DPPH radical scavenging activity of the Ru(II) complex was found almost the same as that of 3,5-di-tert-4-butylhydroxytoluene (BHT), a commercial standard. The FRAP values were found in the range of 2.96±1.79 – 937.59±0.85 µM TEAC. The ferric reducing/antioxidant power of the Ru(II) complex is the highest with a 937.59±0.85 µM TEAC value, while that of the Zn(II) complex is the lowest (2.96±1.79 µM TEAC). In addition, the results of the two antioxidant activity methods exhibited a good positive correlation (R<sup>2</sup>: 0.8802).

Table 2  
Antioxidant activities of the studied compounds and standards\*

Compound	DPPH• (SC <sub>50</sub> , mg/mL)	FRAP (μM, TEAC)
L	2.1588±0.0079	6.85±0.32
[Fe(L) <sub>2</sub> Cl <sub>2</sub> ]Cl·2H <sub>2</sub> O	0.3190±0.0012	28.33±0.56
[Co(L)Cl <sub>2</sub> ]·2H <sub>2</sub> O	2.6944±0.0179	54.63±1.40
[Cu(L)Cl]Cl·2H <sub>2</sub> O	0.0454±0.0008	696.67±2.00
[Zn(L)(Cl) <sub>2</sub> (H <sub>2</sub> O)]·H <sub>2</sub> O	2.5661±0.0181	2.96±1.79
[Ru(L)(DMSO)(H <sub>2</sub> O)Cl <sub>2</sub> ]	0.0165±0.0015	945.4±0.75
BHT	0.0115±0.0001	N.D.
Trolox	0.0028±0.0000	**

\*Each value represents the average of three repetitions. N.D.: Not Detected

\*\*Trolox was used to constructing a calibration curve used for the calculation of TEAC values.

## EXPERIMENTAL

### Chemistry and apparatus

All chemicals and solvents are of reagent grade and were used without further purification.

Elemental analysis data were obtained with a Thermo Finnigan Flash EA 1112 analyzer. Melting or decomposition points of the compounds were determined using a melting point apparatus Buchi M-560 model. Conductivity of the complexes was measured on a WTW Cond315i conductivity meter in DMF at 20±1 °C. <sup>1</sup>H-NMR spectra were run on a Varian Unity Inova 500 NMR spectrometer. FT-IR spectra were recorded on a Bruker Optics Vertex 70 spectrometer using ATR (Attenuated Total Reflection) techniques. Magnetic moment measurements for the paramagnetic complexes were carried out on MK1 Sherwood Scientific apparatus at room temperature by Gouy's method. Diamagnetic corrections were made using Pascal's constants. Fluorescence and UV-Visible spectra were performed on Shimadzu RF-5301 PC Spectrofluorophotometer and Shimadzu UV-1800 Spectrophotometer, respectively. The Electron Spray Ionization-Mass Spectrometry (ESI-MS) analyses were run in positive ion modes using a Thermo Finnigan LCQ Advantage MAX LC/MS/MS. Thermogravimetric studies were made on a TG-60WS Shimadzu, with a heating rate of 10 °C/min and air flowing at the rate of 50 mL/min.

### Synthesis of the ligand (L)

The ligand [1,5-bis(6-chloro-1*H*-benzimidazol-2-yl)pentan-3-one] was synthesized from 0.01 mole of 4-oxopimelic acid (or 4-oxo-heptanedioic acid, 1.74 g) and 0.02 mole of 4-chloro-1,2-phenylenediamine (2.85 g) in 5.5 N HCl (20 mL) according to the literature.<sup>38</sup> Slightly yellow solid. Yield: 56%; Dec.p.: 135 °C. Elemental analysis: calcd. for C<sub>19</sub>H<sub>16</sub>Cl<sub>2</sub>N<sub>4</sub>O (%): C, 58.93; H, 4.16; N, 14.47; Found (%): C, 58.81; H, 4.08; N, 14.63. MW: 387.26 g/mol. UV-vis (λ<sub>max</sub>/nm, in MeCN): 211 m, 228 m, 264 m, 291 m. FT-IR spectroscopy (ATR, cm<sup>-1</sup>): 3311 m ν(NH), 3153 m,br, 3069 m,br ν(CH<sub>arom</sub>), 2915 m ν(CH<sub>aliph</sub>), 1712 s ν(C=O), 1629 m

ν(C=N), 1599 m ν(C=C), 1539 m, 1416 m, 1277 m, 1097 m, 1025 m, 924 m, 854 m, 796 m δ(CH<sub>arom</sub>), 704 m ν(C-Cl), 640 m, 516 m, 427 m. <sup>1</sup>H-NMR (ppm, 500 MHz): 7.50 d (1H, J=1.95 Hz, H4), 7.45 d (1H, J=8.29, H7), 7.13 dd (1H, J=8.79, 1.95, H6), 3.10 m (2H, -CH<sub>2</sub>-C=O), 3.02 m (2H, -CH<sub>2</sub>-CH<sub>2</sub>-C=O). <sup>13</sup>C-NMR (ppm, 125 MHz): 208.51 (C=O), 156.59 (C2), 132.40 (C9), 129.36 (C8), 126.28 (C5), 122.08 (C4), 115.98 (C6), 115.02 (C7), 39.71 (Im-CH<sub>2</sub>-CH<sub>2</sub>-C=O), 23.20 (Im-CH<sub>2</sub>-CH<sub>2</sub>-C=O). ESI-MS (m/z, %): 387.5 (100, M<sup>+</sup>), 389.4 (72.6, [M+2H]<sup>+</sup>), 388.4 (27.8, [M+H]<sup>+</sup>), 390.4 (23.7, [M+3H]<sup>+</sup>). Fluorescence spectra (λ<sub>max</sub>/nm): 373 w, 395 w, 422 w,br, 489 m,br.

### Syntheses of the complexes

#### [Fe(L)<sub>2</sub>Cl<sub>2</sub>]Cl·2H<sub>2</sub>O

The ligand (L; 0.290 g; 0.75 mmol) and FeCl<sub>3</sub>·9H<sub>2</sub>O (0.243 g; 0.75 mmol) were separately dissolved in ethanol (10 mL) and then they were mixed and refluxed for 2 h. The mixture was filtered and kept at room temperature. After approximately one week, the precipitate formed was filtered and allowed to dry at room temperature. Brown solid. Yield: 65%. Decomposition point (Dec.p.): 185 °C. Elemental analysis: calcd. for C<sub>38</sub>H<sub>36</sub>Cl<sub>7</sub>N<sub>8</sub>O<sub>4</sub>Fe (%): C, 46.92; H, 3.73; N, 11.52; Found (%): C, 46.84; H, 3.78; N, 11.10. MW: 972.76 g/mol. Molar conductivity: 184.3 Ω<sup>-1</sup>cm<sup>2</sup>mol<sup>-1</sup>. Magnetic moment (μ<sub>eff</sub>): 6.12 BM. UV-vis (λ<sub>max</sub>/nm, in MeCN): 212 m, 232 m, 279 m, 285 m, 308 m,br, 351 m,br. FT-IR (ATR, cm<sup>-1</sup>): 3250 m,br ν(NH+OH), 3077 m ν(CH<sub>arom</sub>), 3001 m, 2776 m, 2702 m,br, 2680 m,br, 1718 m ν(C=O), 1620 m ν(C=N), 1570 m ν(C=C), 1456 m, 1402 m, 1210 m, 1061 m, 923 m, 836 m, 810 s δ(CH<sub>arom</sub>), 702 m ν(C-Cl), 674 m ν(N-Fe), 596 m, 423 m. Fluorescence spectra (λ<sub>max</sub>/nm): 394 w, 441 w,br. TGA (temp., °C: weight loss, %): 50: 1.2; 75: 2.5; 100: 3.5; 150: 3.5; 200: 3.9; 250: 5.6; 300: 16.6; 350: 28.0; 400: 32.8; 450: 46.4; 500: 65.6.

#### [Co(L)Cl<sub>2</sub>]·2H<sub>2</sub>O

Similar to the Fe(III) complex, a mixture of L and CoCl<sub>2</sub>·6H<sub>2</sub>O (0.179 g; 0.75 mmol) was prepared and refluxed. After reflux the mixture was filtered and allowed to stand at room temperature. A precipitation was formed after one hour and it was filtered and dried at room temperature. Blue solid.



Yield: 76%. Dec.p.: 295 °C. Elemental analysis: calcd. for  $C_{19}H_{20}Cl_4N_4O_3Co$  (%): C, 41.26; H, 3.64; N, 10.13; Found (%): C, 42.07; H, 3.43; N, 9.92. MW: 553.1 g/mol. Molar conductivity:  $51.1 \Omega^{-1}cm^2mol^{-1}$ . Magnetic moment ( $\mu_{eff}$ ) = 4.15 BM. UV-vis ( $\lambda_{max}/nm$ , in MeCN): 212 m, 234 m, 279 m, 307 m, 398 m, 583 sh, 620 sh, 638 m,br. FT-IR (ATR,  $cm^{-1}$ ): 3245 m,br  $\nu(H_2O)$ , 3196 m  $\nu(NH)$ , 3093 m  $\nu(CH_{arom})$ , 2983 m  $\nu(CH_{aliph})$ , 1718 m  $\nu(C=O)$ , 1627 m  $\nu(C=N)$ , 1593 m  $\nu(C=C)$ , 1536 m, 1461 m, 1413 m, 1300 m, 1101 m, 1045 m, 929 m, 807 s  $\delta(CH_{arom})$ , 719 m  $\nu(C-Cl)$ , 681 m  $\nu(N-Co)$ , 652 m, 601 m, 555 m, 507 m, 421 m. Fluorescence spectra ( $\lambda_{max}/nm$ ): 395 w, 446 w,br. TGA (temp., °C: weight loss, %): 50: 1.4; 75: 3.8; 100: 6.4; 150: 8.0; 200: 8.3; 250: 8.8; 300: 9.9; 350: 12.4; 400: 13.6; 450: 22.8; 500: 32.2.

#### [Cu(L)Cl]Cl·2H<sub>2</sub>O

Similar to above complexes, a mixture of **L** and  $CuCl_2 \cdot 3H_2O$  (0.142 g) was prepared and refluxed. Subsequent processes are the same as for the Fe(III) complex. Brown solid. Yield: 81%. Dec.p.: 198 °C. Elemental analysis: calcd. for  $C_{19}H_{20}Cl_4N_4O_3Cu$  (%): C, 40.92; H, 3.61; N, 10.05; Found (%): C, 40.86; H, 2.59; N, 9.54. MW: 557.75 g/mol. Molar conductivity:  $68.0 \Omega^{-1}cm^2mol^{-1}$ . Magnetic moment ( $\mu_{eff}$ ): 1.68 BM. UV-vis ( $\lambda_{max}/nm$ , in MeCN): 253 m, 289 m, 307 m, 336 m, 392 m,br, 420 sh. FT-IR Spectroscopy (ATR,  $cm^{-1}$ ): 3301 m,br  $\nu(OH)$ , 3125 m  $\nu(NH)$ , 3091 m,br  $\nu(CH_{arom})$ , 3040 m,br, 2958 m  $\nu(CH_{aliph})$ , 1695 w,sh  $\nu(C=O)$ , 1665 m  $\nu(C=N)$ , 1617 m  $\nu(C=C)$ , 1583 m, 1447 m, 1328 m, 1209 m, 1064 m, 931 m, 858 m, 805 s  $\delta(CH_{arom})$ , 714 m  $\nu(C-Cl)$ , 668 m  $\nu(N-Cu)$ , 630 m, 598 m, 479 m, 429 m. Fluorescence spectra ( $\lambda_{max}/nm$ ): 441 m. TGA (temp., °C: weight loss, %): 50: 1.7; 75: 4.0; 100: 6.5; 150: 9.1; 200: 10.5; 250: 12.7; 300: 15.4; 350: 19.6; 400: 23.5; 450: 28.1; 500: 35.0.

#### [Zn(L)(Cl)<sub>2</sub>(H<sub>2</sub>O)]·H<sub>2</sub>O

Similar to the other complexes, a mixture of **L** and  $ZnCl_2 \cdot 6H_2O$  (0.184 g; 0.75 mmol) was prepared and refluxed. After two days a precipitate was formed and filtered, washed with ethanol. The filtrate was kept at room temperature for drying. Off-white solid. Yield: 73%. Dec.p.: 235 °C. Elemental analysis: calcd. for  $C_{19}H_{20}Cl_4N_4O_3Zn$  (%): C, 40.78; H, 3.60; N, 10.01; Found (%): C, 40.93; H, 3.53; N, 10.04. MW: 559.6 g/mol. Molar conductivity:  $39.4 \Omega^{-1}cm^2mol^{-1}$ . UV-vis ( $\lambda_{max}/nm$ , in MeCN): 212 m, 230 m, 267 m, 295 m. FT-IR (ATR,  $cm^{-1}$ ): 3274 m,br  $\nu(OH)$ , 3211 m  $\nu(NH)$ , 3066 m  $\nu(CH_{arom})$ , 2962 m  $\nu(CH_{aliph})$ , 1704 s  $\nu(C=O)$ , 1619 m  $\nu(C=N)$ , 1596 m  $\nu(C=C)$ , 1532 m, 1447 m, 1283 m, 1069 m, 928 m, 852 m, 809 m  $\delta(CH_{arom})$ , 716 m  $\nu(C-Cl)$ , 682 m  $\nu(N-Zn)$ , 600 m, 550 m, 500 m, 467 m, 422 m. <sup>1</sup>H-NMR (ppm, 500 MHz): 7.69 s (1H, H4), 7.60 d (1H, J=8.30, H7), 7.21 dd (1H, J=8.79, 1.95, H6), 3.14 m (2H,  $-CH_2-C=O$ ), 3.07 m (2H,  $-CH_2-CH_2-C=O$ ). <sup>13</sup>C-NMR (ppm, 125 MHz): 207.79 (C=O), 157.47 (C2), 127.16, 123.02, 116.01, 115.55, 39.87 (Im- $CH_2-CH_2-C=O$ ), 23.07 (Im- $CH_2-CH_2-C=O$ ). Fluorescence spectra ( $\lambda_{max}/nm$ ): 392 w,br, 435 w,br. TGA (temp., °C: weight loss, %): 50: 0.9; 75: 1.9; 100: 3.1; 150: 5.7; 200: 6.4; 250: 6.6; 300: 8.7; 350: 10.9; 400: 12.7; 450: 15.3; 500: 27.2.

#### [Ru(L)(DMSO)(H<sub>2</sub>O)Cl<sub>2</sub>

Similar to the other complexes, a mixture of **L** and  $Ru(DMSO)_4Cl_2$  (0.363 g; 0.75 mmol) was prepared and refluxed. Then, the mixture was filtered and kept at room temperature. After a few days a darkish precipitate was formed and it was filtered and dried at room temperature. Dark smoked solid. Yield: 65%. Dec.p.: 320 °C. Elemental analysis:

calcd. for  $C_{21}H_{24}Cl_4N_4O_3RuS$  (%): C, 38.48; H, 3.69; N, 8.55; S, 4.89; Found (%): C, 39.63; H, 4.16; N, 8.09; S, 4.96. MW: 655.39 g/mol. Molar conductivity:  $29.0 \Omega^{-1}cm^2mol^{-1}$ . UV-vis ( $\lambda_{max}/nm$ , in MeCN): 223 m, 241 m, 289 m, 353 sh, 575 m,br. FT-IR (ATR,  $cm^{-1}$ ): 3189 m,br  $\nu(H_2O)$ , 3105 m,br  $\nu(CH_{arom})$ , 3012 m  $\nu(CH_{DMSO})$ , 2919 m  $\nu(CH_{aliph})$ , 1715 m  $\nu(C=O)$ , 1622 m  $\nu(C=N)$ , 1566 m  $\nu(C=C)$ , 1453 m, 1413 m, 1307 m, 1064 m, 1018 m, 924 m, 805 m  $\delta(CH_{arom})$ , 714 m  $\nu(C-Cl)$ , 686 m  $\nu(N-Ru)$ , 598 m, 423 m. <sup>1</sup>H-NMR (ppm, 500 MHz): 7.58 s,br (1H, H4), 7.53 s,br (1H, H7), 7.32 s,br (1H, H6), 3.17 s,br (2H,  $-CH_2-C=O$ ), 3.12 s,br (2H,  $-CH_2-CH_2-C=O$ ), 2.54 s (6H, DMSO). <sup>13</sup>C-NMR (ppm, 125 MHz): NMR signal could not be obtained. Fluorescence spectra ( $\lambda_{max}/nm$ ): 394 sh, 448 w,br. TGA (temp., °C: weight loss, %): 50: 0.15; 75: 0.15; 100: 0.15; 150: 1.9; 200: 3.1; 250: 5.6; 300: 6.7; 350: 27.4; 400: 29.4; 450: 46.0; 500: 65.4.

#### Antibacterial activity determination

The solutions of the compounds, **L** and its Fe(III), Co(II), Cu(II), Zn(II) and Ru(II) complexes, were prepared with DMSO at 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL concentrations. The used microorganisms were three Gram-positive (*Bacillus subtilis* ATCC 6633, *Bacillus megaterium* ATCC 19213, and *Enterococcus faecalis* ATCC 29212) and three Gram-negative (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, and *Salmonella typhimurium* ATCC 14028) bacteria. The studied bacteria were diluted with Mueller Hinton Broth (MHB) containing  $10^6$  cfu/mL. DMSO and Ofloxacin (10 µg/disc) were used negative control and positive control, respectively. Mueller Hinton Agar (MHA) was used as the medium. Antibacterial activity was evaluated by disc diffusion method described by Karaçelik *et al.*<sup>39</sup> Each bacterial solution was separately spread on MHA medium in sterile conditions. Then, 6mm standard discs were individually impregnated with 10 µL of each concentration of tested compounds and were placed on MHA. The prepared experimental setups were incubated at 37 °C and after 24 hours the diameters of the transparent inhibition zone around the standard discs, on which the compounds were impregnated, were measured and recorded. Each experiment was performed in triplicate.

#### Antioxidant activity determination

##### 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activity

Radical scavenging activity was tested by using the commonly used DPPH radical scavenging activity.<sup>40</sup> Each of the concentrations of the samples were mixed with 100 µM methanolic DPPH• solution in an equal volume (750 µL) and kept for 50 minutes at room temperature. Then, all experimental setups were spectrophotometrically measured at 517 nm. The concentrations corresponding to the absorbances found were plotted, and the 50% scavenging concentration (SC<sub>50</sub>) values were calculated in mg/mL. Low SC<sub>50</sub> values indicated higher radical cleaning potential.

##### Ferric Reducing / Antioxidant Power (FRAP) Method

In FRAP method, 50 µL of each tested samples was mixed with 1.5 mL of FRAP reagent and after 20 minutes the absorbance was spectrophotometrically measured at 595 nm. The absorbance of all tubes was compiled against pure water. The ferric reducing activity of each sample was determined by the calibration graph obtained using trolox in the range of 31.25-1000 µM and the micromolar trolox equivalent antioxidant capacity (TEAC) was determined.<sup>41</sup>

## CONCLUSIONS

A new bis-benzimidazole ligand, 1,5-bis(6-chloro-1*H*-benzimidazol-2-yl)pentan-3-one (**L**), and its complexes with Fe(III), Co(II), Cu(II), Zn(II) and Ru(II) chlorides are synthesized and characterized by elemental analysis, molar conductivity, magnetic moment, FT-IR, <sup>1</sup>H-NMR, TGA, ESI-MS, UV-visible and fluorescence spectroscopy. The spectral data suggest that the chelating ligand acted as bidentate through both the C=N nitrogen atoms in the complexes except the Cu(II) complex. According to the infrared spectral data, there is a different coordination behavior in the Cu(II) complex compared to the others: C=N nitrogen atoms as well as carbonyl oxygen were coordinated and the ligand showed a tridentate behavior. According to the molar conductance measurements Fe(III) and Cu(II) complexes are 1:1 electrolyte whereas Co(II), Zn(II) and Ru(II) complexes are non-ionic. In addition, antibacterial and antioxidant activity of the compounds were tested. The Ru(II) and Co(II) complexes showed moderate activity against to both Gram+ and Gram- bacteria. According to the DPPH• results, the Ru(II) complex of **L** had quite significant radical scavenging activity (antioxidant) almost the same as that of a commercial standard (BHT).

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