Bull. Chem. Soc. Ethiop. **2010**, 24(3), 391-400. ISSN 1011-3924
Printed in Ethiopia

SPECTRAL CHARACTERIZATIONS AND ANTIBACTERIAL EFFECT OF 2-(5-R-1*H*-BENZIMIDAZOL-2-YL)-4-METHYL/BROMO-PHENOLS AND SOME METAL COMPLEXES

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(Received October 12, 2009; revised July 8, 2010)

ABSTRACT. 2-(5-H/Cl/Me/NO_2-1*H*-benzimidazol-2-yl)-4-Me/Br-phenols (HL_1–HL_5) were synthesized. HL_1 complexes with $Cu(NO_3)_2$, $AgNO_3$, $Zn(ClO_4)_2$ and; HL_4, HL_5 complexes with $Zn(ClO_4)_2$ were prepared. The structures of the compounds were confirmed on the basis of elemental analysis, molar conductivity, magnetic moment, FT-IR, ^{1}H- and ^{13}C-NMR. Antibacterial activity of the ligands and the complexes were evaluated using the disk diffusion method in dimethyl sulfoxide (DMSO) as well as the minimal inhibitory concentration (MIC) dilution method, against nine bacteria, and the results were compared with penicillin–G and oxytetracycline. While HL_1 ligand has considerable antibacterial activity on *B. cereus* only; it's Ag(I) complex show antibacterial effect toward almost all the bacteria. It is highly interesting that HL_5 and $[Zn(HL_5)(L_5)]ClO_4$ exhibit considerable high antibacterial activity toward *K. pneumoniae*, *B. cereus*, *S. epidermidis* and *B. subtilis*.

KEY WORDS: Benzimidazole, Phenol, Metal complexes, Antibacterial activity

INTRODUCTION

It was reported that many benzimidazolylphenol derivatives and their metal complexes exhibit various antimicrobial activities. For example, some benzimidazolyl-phenol type ligands and their Fe(III) complexes showed broad spectrum of antimicrobial activity that were either more active or equipotent as the references [1, 2]. Co(II) and Ni(II) chelates of 2-(2´-hydroxyphenyl)-benzimidazole have antifungal activity against *Alternaria alternata* and *Aspergillus niger* [3, 4]. Fe(III), Ag(I) and Hg(II) complexes of some 5-substituted 2-(2´-hydroxyphenyl)-1*H*-benzimidazoles showed antibacterial and antifungal effects [5].

In our previous works, antimicrobial activity of various 1*H*-benzimidazol-2-yl-phenol derivatives and their some transition metal complexes was investigated. It was reported that 2-methoxy-6-(5-H/chloro-1*H*-benzimidazol-2-yl)-phenols and Cu(II), Zn(II) complexes exhibit antibacterial activity against gram positive bacteria [6]. 4-Methoxy-2-(1*H*-benzimidazol-2-yl)-phenol and its Ag(I) and Cu(II) complexes were effective on *S. epidermidis*, *S. aureus* and *B. subtilis*. Also, 4-methoxy-2-(5-methyl/chloro-1*H*-benzimidazol-2-yl)-phenols display antibacterial activity toward *S. aureus* [7]. It was noticed that while 2-methyl-6-(1*H*-benzimidazol-2-yl)-phenol had no any activity, it's Ag(I) and Zn(II) complexes demonstrate antibacterial effect toward *K. pneumoniae*, *S. epidermidis* and *S. aureus* bacteria [8]. 2-(5-Nitro-1*H*-benzimidazol-2-yl)-4-bromophenol and its Cu(II), Fe(III) complexes show considerable antibacterial activity against *S. epidermidis* as compared to ciprofloxazin [9].

In this study, 2-(5-H/Cl/Me/NO_2-1*H*-benzimidazol-2-yl)-4-Me/Br-phenols (HL_1–HL_5, Figure 1) and some $Cu(NO_3)_2$, $AgNO_3$, $Zn(ClO_4)_2$ complexes are reported. In addition, antibacterial activity of the compounds is evaluated against nine selected bacteria.

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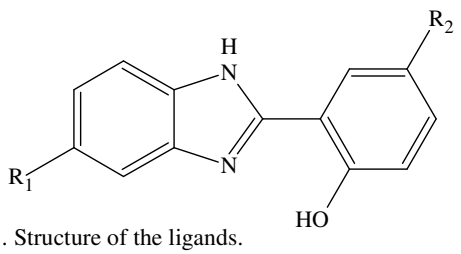


$R_1 = H$, $R_2 = CH_3$ HL_1
$R_1 = CH_3$, $R_2 = CH_3$ HL_2
$R_1 = Cl$, $R_2 = CH_3$ HL_3
$R_1 = Cl$, $R_2 = Br$ HL_4
$R_1 = NO_2$, $R_2 = Br$ HL_5

Figure 1. Structure of the ligands.

EXPERIMENTAL

Materials and apparatus

All chemicals and solvents were of reagent grade (Merck, Fluka and Sigma-Aldrich) and were used without further purification. Melting points were determined using a Gallenkamp melting point apparatus (The Netherlands). C,H,N content was determined on a Thermo Finnigan Flash EA 1112 analyzer (USA–Thermo Fisher Scientific). Molar conductivity of the complexes was measured on a WTW Cond315i conductivity meter (USA–Nova Analytics) in DMSO at 25 ± 1 ºC. ^{1}H- and ^{13}C-NMR spectra were run on a Varian Unity Inova 500 NMR spectrometer (USA) in DMSO-d_6. ^{13}C-NMR spectra were recorded as attached proton test (APT). The residual DMSO-d_6 signal was used as an internal reference. FT-IR spectra were recorded in KBr disks on a Nicolet 380 FT-IR spectrometer (Thermo Fisher Scientific, USA). Magnetic moment measurement of Cu(II) complex was carried out on a Sherwood Scientific apparatus (UK) at room temperature by Gouy's method using $CuSO_4 \cdot 5H_2O$ as the calibrant and were corrected for diamagnetism by applying Pascal's constants.

Synthesis of the ligands

The ligands were prepared according to literature procedures [10, 11], by reacting 2-hydroxy-5-methyl-benzaldehyde (1.36 g, 10 mmol) and an equivalent amount of $NaHSO_3$ (1.04 g, 10 mmol) at room temperature in ethanol (25 mL) for 4-5 hours. For 2-(5-chloro-1H-benzimidazol-2-yl)-4-methylphenol (HL_3) synthesis, this mixture was treated with 4-chloro-1,2-phenylenediamine (1.42 g, 10 mmol) in dimethylformamide (15 mL) and gently refluxed for 2 hours. The reaction mixture was then poured into iced water (500 mL), filtered and crystallized from ethanol.

The other ligands were prepared in a similar manner to ligand HL_3.

Syntheses of the complexes

The synthesis procedures of the complexes are given below.

$[Cu(L_1)_2] \cdot 3H_2O$. HL_1 (112 mg, 0.5 mmol) and $Cu(NO_3)_2 \cdot 3H_2O$ (133 mg, 0.55 mmol) were reacted in methanol (10 mL). After 4 hours reflux a precipitate was formed. It was filtered, washed with methanol (5 mL) and dried at ~80 ºC.

$[Ag(HL_1)_2]NO_3$. HL_1 (112 mg, 0.5 mmol) and $AgNO_3$ (94 mg, 0.55 mmol) were reacted in ethanol (10 mL) at room temperature for 6 hours with stirring. The precipitate was filtered, washed with ethanol (5 mL) and dried at ~80 ºC.

$[Zn(L_1)(H_2O)_2]ClO_4$. HL_1 (112 mg, 0.5 mmol) was suspended in ethyl acetate and $Zn(ClO_4)_2 \cdot 6H_2O$ (205 mg, 0.55 mmol) solution in ethylacetate (15 mL) was added to the ligand solution. The mixture was refluxed for 2 hours. The brick-red precipitate was filtered, washed with ethylacetate (5 mL) and dried under vacuum over calcium chloride.

The other Zn(II) complexes were prepared in a similar manner to $[Zn(L_1)(H_2O)_2]ClO_4$.

Determination of antimicrobial activity

Microorganisms. The antimicrobial activities were evaluated against gram positive (*Staphylococcus aureus* ATCC 29213, *Bacillus cereus* ATCC 11778, *Bacillus subtilis* ATCC 6633, *Staphylococcus epidermidis* ATCC 12228) and gram negative (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enteritidis* KUEN 349, *Proteus mirabilis* CCM 1944) bacteria using disk diffusion method as well as the minimum inhibitory concentration (MIC) dilution method. The strains were obtained from the Centre for Research and Application of Culture Collections of Microorganisms (Istanbul University; KUKENS) and were used for the detection of antibacterial effect of the ligands and their complexes on the strains.

Test media. Mueller-Hinton Agar (Fluka 70191) were used for the detection of the antibacterial effect qualitatively and to maintain the strains. For the detection of the quantitative antibacterial effect as MIC, Mueller-Hinton broth (Fluka 70192) (CAMBH) with $MgCl_2 \cdot 2H_2O$ (10 mg Mg^{2+}/L) and $CaCl_2 \cdot 6H_2O$ (20 mg Ca^{2+}/L) was used as the medium.

Qualitative antibacterial evaluation. Disc diffusion method was used for the detection of the antibacterial effect of the chemical agents qualitatively [12]. For this purpose, filter papers (Whatman No. 1) with 6 mm diameter were autoclaved and dried at 37 °C for overnight. Each chemical agent (21.27 mg) was dissolved in DMSO and 23.5 µL of this solution (containing 500 µg chemical agent) were soaked on to the sterile discs. Bacterial suspension with 1-2 x 10^8 cfu/mL (McFarland 0.5) were prepared from each bacterial strain and streaked onto the agar, chemical agents impregnated discs were placed onto the agar surface and incubated at 37 °C for 24 h. Chemical agents with growth inhibition zones were used for the further examinations.

Quantitative antibacterial evaluation. For the detection of the antibacterial effect of the chemical agents, quantitatively, macro dilution broth method according to Clinical and Laboratory Standards Institute (formerly NCCLS) were performed [13]. Serial dilutions of the chemical agents between 531.75-0.26 µg/mL with CAMBH were prepared within sterile tubes. Bacterial suspension with 10^7 cfu/mL final concentration was inoculated. Positive (without tested chemical agent) and negative (without bacterial suspension) tubes were used at the end of the each organism tested. Tubes were incubated at 37 °C for 24 h. The MIC value was defined as the lowest concentration of the chemical agent giving complete inhibition of visible growth.

RESULTS AND DISCUSSION

Physical properties

The analytical data and physical properties of the ligands and the complexes are summarized in Table 1. The melting points of the ligands HL_2–HL_5 suggested that the methyl substitution on 5-position of the benzimidazole moiety decreases the melting point, and chlorine atom increases it with respect to HL_1 ligand. Also, bromo substituent on 4'-position of the phenol moiety increases the melting point.

Molar conductivity values of the Ag(I) and Zn(II) complexes of HL_1 were 45 and 56 $\Omega^{-1}cm^2mol^{-1}$, respectively. These results are indicative for 1:1 electrolytes. The other complexes have non-ionic character according to the molar conductivity values [14, 15].

Magnetic moment value of $[Cu(L_1)_2]\cdot 3H_2O$ complex was 1.61 BM at room temperature, which is close to the spin value for only one unpaired electron. It is consistent with a square planar structure of Cu(II) complex [16-18].

Zn(II) complexes of HL_4 and HL_5 ligands have 1:2 M:L ratio. According to the analytical and spectral data, these complexes may have the structure as $[Zn(HL)(L)]ClO_4$ in which a hydroxy proton of one of the ligands is removed whereas the others' is not on complexation. There is a similar structure reported in the literature by Tong and Ye [19].

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

Compound	Elemental analysis: found (calcd) % C	H	N	Yield %	M.p. ºC	Color	Λ^a
HL_1 $C_{14}H_{12}N_2O$	75.3 (75.0)	5.7 (5.4)	12.2 (12.5)	76	257	Color-less	--
HL_2 $C_{15}H_{14}N_2O$	75.5 (75.6)	5.8 (5.9)	11.0 (11.8)	65	251	Color-less	--
HL_3 $C_{14}H_{11}ClN_2O$	65.1 (65.0)	4.1 (4.3)	10.6 (10.8)	73	288	Dirty white	--
HL_4 $C_{13}H_8BrClN_2O$	48.4 (48.2)	2.8 (2.5)	8.6 (8.7)	64	309	Dirty white	--
HL_5 $C_{13}H_8BrN_3O_3$	46.4 (46.7)	2.6 (2.4)	12.8 (12.6)	58	277	Dark yellow	--
$[Cu(L_1)_2]\cdot 3H_2O$ [b] $C_{28}H_{28}CuN_4O_5$	58.8 (59.6)	4.6 (5.0)	9.6 (9.9)	75	>350	Dark green	9.5
$[Ag(HL_1)_2]NO_3$ $C_{28}H_{23}AgN_5O_5$	52.1 (54.5)	4.2 (3.8)	11.2 (11.3)	64	228	Grey	45
$[Zn(L_1)(H_2O)_2]ClO_4$ $C_{14}H_{15}ClN_2O_7Zn$	40.0 (39.6)	3.4 (3.6)	6.4 (6.6)	72	>350	Brick-red	56
$[Zn(HL_4)(L_4)]ClO_4$ $C_{26}H_{15}Br_2Cl_3N_4O_6Zn$	37.9 (38.5)	2.2 (1.9)	6.7 (6.9)	75	324	Grey	18
$[Zn(HL_5)(L_5)]ClO_4$ $C_{26}H_{15}Br_2ClN_6O_{10}Zn$	37.2 (37.5)	2.1 (1.8)	10.4 (10.1)	77	>350	Red-brown	22

[a] Λ = molar conductivity, $\Omega^{-1}cm^2mol^{-1}$ (25±1ºC) at DMSO. [b] μ_{eff} value for $[Cu(L)_2]\cdot 3H_2O$ was 1.61 BM.

FT-IR spectra

FT-IR spectral data of the ligands and the complexes are given in Table 2. In the IR spectra of HL_1, the bands at 3330 and 3284 cm^{-1} are due to OH and NH stretching vibration frequencies, respectively. These bands appear closer to each other due to intramolecular hydrogen bonding between the phenoxy hydrogen atom and one of the imine nitrogen atoms [2, 20, 21]. These bands change significantly upon metal complexation indicating deprotonation and subsequent involvement of the phenoxy group in metal coordination.

In the other ligands (HL_2-HL_5), the characteristic ν(O–H) and ν(N–H) vibration frequencies exhibit only a single strong band at *ca.* 3300 cm^{-1} in their IR spectra, caused by double intramolecular hydrogen bonding between the phenoxy hydrogen atom and one of the imine nitrogen atoms (Table 2, Figure 2). This band of HL_4 and HL_5 spectra shifted significantly upon Zn(II) complexation indicating deprotonation and subsequent involvement of the phenoxy group.

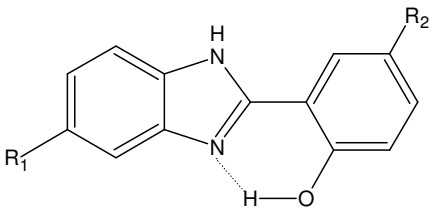


Figure 2. The intramolecular hydrogen bonding in the HL_2–HL_5 ligands.

Table 2. FT-IR spectral data of the compounds under study.

Compound	Frequency (cm^{-1}), KBr pellets
HL_1	3330 s, 3284 s, 3057 w, 2918 w, 1640 m, 1613 m, 1590 m, 1569 s, 1455 m, 1397 m, 1286 s, 1262 s, 1235 m, 808 s, 731 m, 643 m
$[Cu(L_1)_2]\cdot 3H_2O$	3431 m, br, 3038 m, 2915 m, 1631 m, 1623 m, 1554 m, 1492 s, 1383 m, 1308 m, 1262 s, 1138 m, 1046 m, 831 m, 808 m, 600 m
$[Ag(HL_1)_2]NO_3$	3331 s, 3065 m, 2922 w, 1615 m, 1598 m, 1505 s, 1454 m, 1385 s, 1362 m, 1316 m, 1246 m, 1138 m, 829 m, 808 s, 745 s, 680 m
$[Zn(L_1)(H_2O)_2]ClO_4$	3459 m, br, 3309 m, br, 3067 w, 2922 w, 1630 m, br, 1601 m, 1513 s, 1470 m, 1355 m, 1254 m, 1110 s, 1035 m, 824 m, 778 m, 747 m, 623 m
HL_2	3334 s, 3049 w, 2918 w, 1636 m, 1613 m, 1594 m, 1509 s, 1386 s, 1316 m, 1259 s, 1132 m, 808 s, 600 m, 550 m
HL_3	3327 s, 3055 m, 2923 w, 1638 m, 1613 m, 1585 m, 1510 s, 1385 s, 1307 m, 1254 s, 1059 m, 934 m, 810 s, 595 m, 546 m
HL_4	3340 s, 3072 m, 2921 m, 1632 m, 1623 m, 1580 m, 1485 s, 1377 s, 1284 m, 1255 s, 1140 m, 1062 m, 843 m, 812 s, 634 m, 577 m, 550 m
$[Zn(HL_4)(L_4)]ClO_4$	3238 s, 3109 m, br, 1621 m, 1597 m, 1483 s, 1299 m, 1246 m, 1115 s, 1065 s, 937 m, 824 s, 699 m, 632 m
HL_5	3327 m, br, 3085 m, 1630 m, 1597 m, 1583 m, 1518 s, 1483 s, 1339 s, 1322 s, 1249 s, 1065 m, 970 m, 817 m, 737 m, 633 m, 554 m
$[Zn(HL_5)(L_5)]ClO_4$	3240 m, br, 3110 m, br, 1627 s, 1612 s, 1559 m, 1535 m, 1520 m, 1496 m, 1475 m, 1352 m, 1342 m, 1246 m, 1148 m, 1112 s, 1089 s, 885 m, 824 m, 736 m, 628 s, 538 m, 470 m

s = sharp, w = weak, m = medium, br = broad.

In the spectra of all the compounds, the characteristic ν(C–H) and δ(C–H) modes of ring residues were observed in the region 3038–3110 cm^{-1} and 843–731 cm^{-1}, respectively (Table 2). The weak or medium bands near 2920 cm^{-1} at the spectra of HL_1, HL_2, HL_3 ligands and HL_1 complexes are due to stretching vibrations of the methyl group or groups.

The ν(C=C) frequencies for the benzimidazole and phenol rings are expected to appear at *ca*. 1620 cm^{-1} with their own characteristics for the ligands in the IR spectra. These frequencies shift to the higher frequency upon complex formation. Similarly the (C=N) asymmetric stretching frequencies are expected to appear at *ca*. 1590 cm^{-1} [2, 11]. Thus, the IR band, at 1593 cm^{-1} in HL_1 ligand, shifts to the higher frequencies of 1600 cm^{-1} for the complexes. These frequency changes may support the argument that coordination possibly occurs *via* imine nitrogen atom.

Ag(I) complex showed a strong band at 1385 cm^{-1} in the IR spectra, supporting the presence of uncoordinated nitrate ion, which was also confirmed by conductivity data [22, 23]. Zn(II) complexes have strong bands around 1110 cm^{-1} can be assigned to the stretching vibrations of

the uncoordinated perchlorate anion Cl–O [24, 25]. Cu(II) and Zn(II) complexes of HL_1 have broad bands at 3431 and 3459 cm^{-1}, respectively, can be attributed to $\nu(H_2O)$.

NMR spectra

^{1}H-NMR spectral data of the ligands and the Zn(II) complexes, and the assignments of the peaks are present in Table 3. The HL_1 ligand gives two singlets at 13.12 and 12.86 ppm for NH and OH protons, respectively. These signals are very closer to each other because of intra-molecular hydrogen bonding between the OH hydrogen and the C=N nitrogen atoms.

According to the ^{1}H-NMR spectra, HL_2 has two isomeric forms (Figures 3 and 4, Table 3). The isomeric structures are observed for the benzimidazole protons only. The isomerism could theoretically occur for HL_3, HL_4 and HL_5, also. However, isomerism was not observed for these compounds. Reason of this may be the methyl substituent, an electron donating group, at 5-position of benzimidazole moiety of HL_2. On the other hand, HL_3, HL_4 and HL_5 have electron withdrawing groups at the same position, Cl, Cl and NO_2, respectively. The isomerism occurs in only HL_2 ligand probably due to presence of an electropositive group at 5-position of benzimidazole moiety of HL_2 as a difference from the others.

Table 3. ^{1}H-NMR spectral data: the chemical shift values (δ_H, ppm) with coupling constants (J, Hz).

Compound	The benzimidazole protons					The phenolic protons				
	H4	H5	H6	H7	NH	H3'	H5'	H6'	OH	CH_3
HL_1	7.70 m	7.27 s, br	7.27 s, br	7.60 s, br	13.12 s	7.88 d J = 1.5	7.19 dd J = 8.3, 2.4	6.93 d J = 8.3	12.86 s	2.33 s
$[Zn(L_1)(H_2O)_2]ClO_4$	7.95 s, br	7.26 s, br	7.26 s, br	7.63 d, br J = 1.8	13.25 s, br	7.95 s, br	7.57 d, br J = 7.3	7.30 d, br J = 7.3	---	2.30 s
$[Ag(HL_1)_2]NO_3$	7.81 s, br	7.28 d,br J = 4.4	7.28 d, br J = 4.4	7.62 s, br	13.15 s, br	7.90 d J = 1.5	7.25 dd J = 1.8; 8.3	7.15 d J = 8.3	12.53 s, br	2.32 s
HL_2 (Isomer A) 55%	7.49 s	2.46 s[1]	7.08 d J = 7.8	7.46 d J = 7.8	13.00 s	7.85 s	7.17 d J = 8.3	6.92 d J = 8.3	12.90 s	2.32 s
HL_2 (Isomer B) 45%	7.57 d J = 8.3	7.11 d J = 8.3	2.46 s*	7.37 s	12.97 s	7.85 s	7.17 d J = 8.3	6.92 d J = 8.3	12.90 s	2.32 s
HL_3	7.73 s,br	---	7.28 d, br J = 7.8	7.63 d, br	13.21 s, br	7.88 s, br	7.21 dd J = 1.5, 8.3	6.94 d J = 8.3	12.59 s, br	2.33 s
HL_4	7.73 d J = 1.9	---	7.33 dd J = 8.8, 1.9	7.69 d J = 8.8	ND	8.31 d J = 2.4	7.53 dd J = 8.8, 2.4	7.07 d J = 8.8	ND	---
HL_5	8.51 s,br	---	8.14 dd J = 8.9, 1.5	7.78 d J = 8.8	13.41 s, br	8.29 d J = 2.2	7.55 dd J = 8.8, 2.4	7.04 d J = 8.9	12.37 s, br	---
$[Zn(HL_4)(L_4)]ClO_4$	7.81 s,br	---	7.53 d J = 8.0	7.64 s, br	13.35 s, br	8.28 s	7.73 s, br	7.31 d, br	---	---
$[Zn(HL_5)(L_5)]ClO_4$	8.45 d J = 2.0	---	8.23 dd J = 1.9, 8.8	7.61 d J = 8.8	12.58 s, br	8.14 d J = 2.4	7.43 dd J = 1.7, 8.8	6.95 d J = 8.8	---	---

m, multiplet; s, singlet; br, broad; dd, doublet of doublet; t, triplet. *3H (CH_3); ND: not detected.

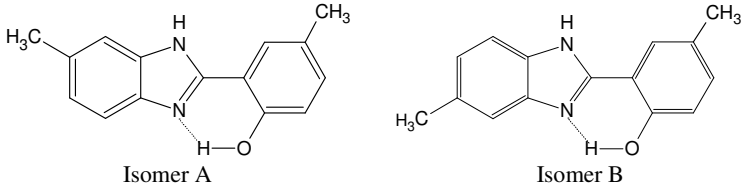


Figure 3. Schematic presentation of the isomeric structures for HL_2.

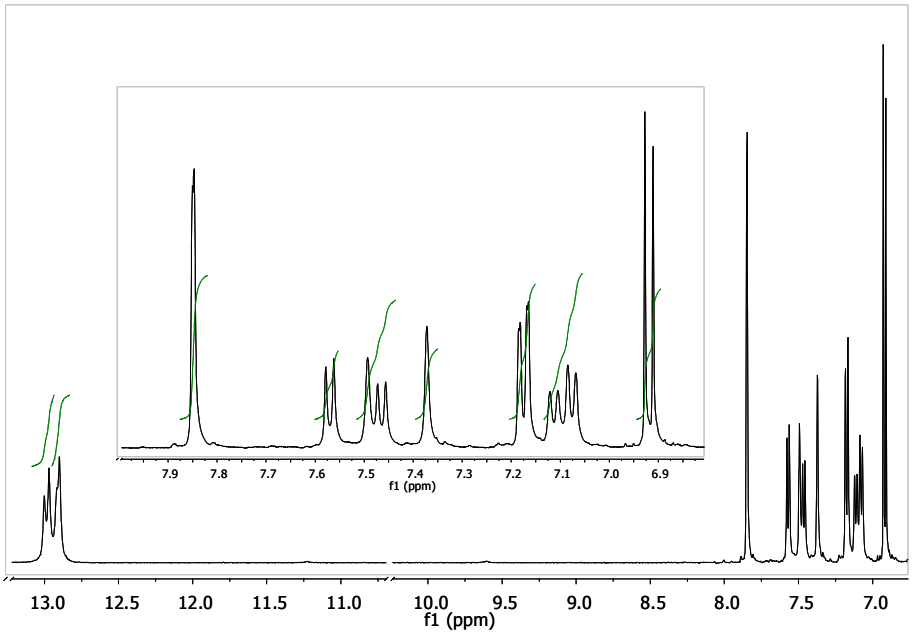


Figure 4. ^{1}H-NMR spectra of HL_2 isomers (A and B) in the 6.5-13.5 ppm region.

In the ^{1}H-NMR spectra of the Zn(II) complexes, some prominent changes were observed in the characteristics regions of the phenolic and benzimidazole benzene ring protons with respect to the ligands. For instance, in the spectra of $[Zn(L_1)(H_2O)_2]ClO_4$ complex, multiplet and doublets change to broad singlets or broad doublets because of the metal ion's strong perturbing effect. It can be said that, on complexation, acidic character of the benzimidazole and the phenol moiety protons is increased.

The OH proton signal in the ^{1}H-NMR spectra of the Zn(II) complexes was removed as expected. This observation is an evidence for the OH hydrogen's eliminating and the phenolic oxygen's coordinating to the Zn(II) ion (Figure 5).

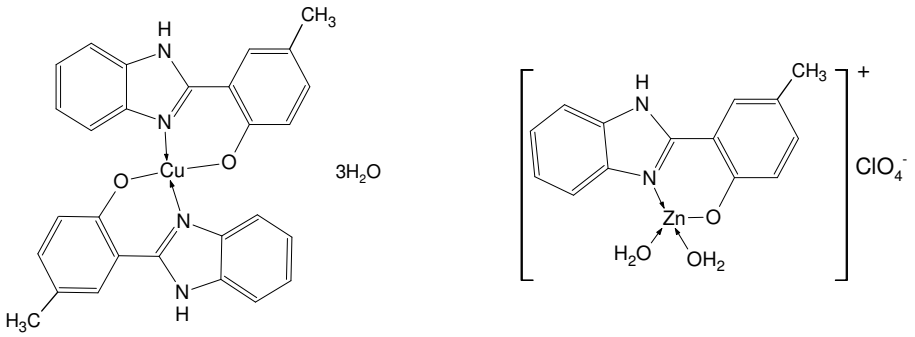


Figure 5. The suggested structures for $[Cu(L_1)_2]\cdot 3H_2O$ and $[Zn(L_1)(H_2O)_2]ClO_4$ according to the spectral and analytical data.

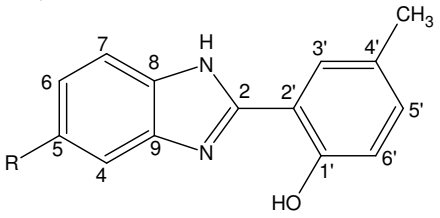
The protons of the methyl group at the benzimidazole moiety gave a singlet at 2.46 ppm while the methyl group at the phenol ring appeared at 2.32 ppm in the ^{1}H-NMR spectra of HL_2 which included two methyl groups.

^{13}C-NMR (attached proton test, APT) spectral data of the ligands and the Ag(I) and Zn(II) complexes are given in Table 4. In the APT spectra of HL_1 and its Ag(I) complex, only eight carbons were determined; in the $[Zn(L_1)(H_2O)_2]ClO_4$ complex spectra all of the carbon atoms were detected. 156.59, 156.01 and 159.87 ppm signals are attributed to C1' carbon atom (OH bonded carbon) in HL_1 and its Ag(I) and Zn(II) complex, respectively. 152.47 (HL_1), 152.20 (Ag(I) complex) and 152.33 (Zn(II) complex) ppm signals are assigned C2 carbon atom (C=N). C8 and C9 carbons are not observed in the ligand and its Ag(I) complex spectra, however they appear separately at 143.74 and 140.58 ppm values at the APT spectra of $[Zn(L_1)(H_2O)_2]ClO_4$, respectively. The low ppm signals of HL_1 and its Ag(I) and Zn(II) complexes, 20.92, 20.84 and 20.28 ppm, are due to methyl carbon atoms, respectively. The other assignments are presented in Table 4.

Table 4. ^{13}C-NMR (APT) spectral data of the HL_1 and its Ag(I) and Zn(II) complexes (δ_C, ppm; in DMSO-d_6).

Compound	Aromatic CH and methyl carbons	Quaternary carbons
HL_1	133.1, 126.9, 117.7, 20.9*	156.6, 152.5, 128.4, 112.9
$[Ag(HL_1)_2]NO_3$	133.3, 127.7, 117.6, 20.8*	156.0, 152.2, 128.6, 113.7
$[Zn(L_1)(H_2O)_2]ClO_4$	133.4, 128.4, 124.5, 123.3, 112.1, 20.3*	159.9, 152.3, 143.7, 140.6, 132.9, 120.6, 117.1
HL_4	135.8, 130.2, 124.7, 120.1, 116.7, 115.1	157.6, 150.6, 137.3, 134.7, 128.8, 114.3, 111.1
HL_5	135.6, 134.6, 132.2, 130.3, 129.4, 120.2, 119.7	160.0, 157.5, 143.8, 122.0, 115.4, 111.2, 110.9
$[Zn(HL_4)(L_4)]ClO_4$	134.9, 129.8, 128.4, 126.6, 123.9, 112.8	160.8, 152.6, 144.8, 119.8, 112.9, 100.7
$[Zn(HL_5)(L_5)]ClO_4$	139.2, 136.1, 130.2, 120.2, 119.4, 115.4, 112.3. 111.2	157.6, 153.8, 143.9, 141.1, 137.1, 114.6,

*CH_3



Antibacterial activity

The results concerning in vitro antibacterial activity of the ligands and the complexes together with the inhibition zone (mm) and MIC values (μg/mL) are presented in Tables 5 and 6, respectively.

As can be seen from Tables 5 and 6, $[Ag(HL_1)_2]NO_3$ complex shows considerable antibacterial effect (higher than that of oxytetracycline) toward all the bacteria except *P. mirabilis*. It is remarkable that HL_5 exhibits superior activity especially against *K. pneumoniae*, *B. cereus* and *S. epidermidis* while HL_2 and HL_4 have no any activity on the bacteria. In addition, Zn(II) complex of HL_5 shows higher antibacterial effect on also *B. subtilis* besides

these three bacteria: 1.04, 2.08, 1.04 and 4.15 μg mL^{-1}, respectively. Similarly, Zn(II) complex of HL_4 was effective on these four bacteria (*K. pneumoniae*, *B. cereus*, *S. epidermidis* and *B. subtilis*) when the ligand itself has no any activity. On the other hand, $[Zn(\mathbf{L_1})(H_2O)_2]ClO_4$ exhibited weak antibacterial effect on *E. coli*, *K. pneumoniae* and *S. aureus* that HL_1 ligand has no any effect on them.

Table 5. *In vitro* antimicrobial activity of the compounds (inhibition zone, mm).

Compound	Micro organisms								
	1	2	3	4	5	6	7	8	9
HL_1	– [a]	–	–	–	–	10	5	–	5
HL_2	–	–	–	–	–	–	–	–	–
HL_3	–	–	–	–	–	x [b]	–	x	–
$[Cu(L_1)_2]\cdot 3H_2O$	–	–	–	–	–	–	–	–	–
$[Ag(HL_1)_2]NO_3$	10	8	10	–	5	10	10	10	8
$[Zn(L_1)(H_2O)_2]ClO_4$	–	–	x	–	<5	10	x	x	10
HL_4	–	–	–	–	–	–	–	–	–
HL_5	–	–	–	–	9	12	12	5	10
$[Zn(HL_4)(L_4)]ClO_4$	–	–	–	–	12	14	14	–	14
$[Zn(HL_5)(L_5)]ClO_4$	–	–	–	–	<8	14	10	–	<8
Penicillin-G	7	–	12	7	–	–	7	30	20
Oxytetracycline	–	18	18	–	18	20	–	18	14

[1] **–** : zone did not form; [2] x : very weak zone.

Table 6. *In vitro* antimicrobial activity of the compounds (MIC, μg/mL).

Compound	Micro organisms								
	1	2	3	4	5	6	7	8	9
HL_1	–[a]	–	–	–	–	16.6	532	–	*[b]
HL_2	–	–	–	–	–	–	–	–	–
HL_3	–	–	–	–	–	*	–	*	–
$[Cu(L_1)_2]\cdot 3H_2O$	–	–	–	–	–	–	–	–	–
$[Ag(HL_1)_2]NO_3$	16.6	66.5	33.2	–	33.2	8.31	4.15	16.6	33.2
$[Zn(L_1)(H_2O)_2]ClO_4$	–	–	*	–	*	532	532	*	*
HL_4	–	–	–	–	–	–	–	–	–
HL_5	–	–	–	–	1.04	1.04	2.08	*	16.6
$[Zn(HL_4)(L_4)]ClO_4$	–	–	–	–	66.5	266	8.31	–	66.5
$[Zn(HL_5)(L_5)]ClO_4$	–	–	–	–	1.04	2.08	1.04	–	4.15

1 = *P. aeruginosa ATCC 27853*, 2 = *S. enteriditis KUEN 349*, 3 = *E. coli ATCC 25922*, 4 = *P. mirabilis CCM 1944*, 5 = *K. pneumoniae ATCC 4352*, 6 = *B. cereus ATCC 11778*, 7 = *S. epidermidis ATCC 12228*, 8 = *S. aureus ATCC 29213*, 9 = *B. subtilis ATCC 6633*. [a]No antibacterial activity qualitatively. [*b]MIC > 532 μg mL^{-1}.

All these results show that Zn(II) ion in the complexes increases the antibacterial effect. HL_5, includes bromo and nitro substituents, has higher antibacterial activity than the other ligands. On the other hand, it can be said that antibacterial activity of the Zn(II) complexes is more effective on gram(+) bacteria as compared to gram(–) bacteria (selective activity).

Superior activities of HL_5 ligand and $[Zn(HL_5)(L_5)]ClO_4$ complex against four bacteria, *K. pneumoniae*, *B. cereus*, *S. epidermidis* and *B. subtilis*, are considered to be valuable contribution to the researches on metal-based drugs field. The results of our study indicated that the compounds, particularly such as $[Ag(HL_1)_2]NO_3$, HL_5 and $[Zn(HL_5)(L_5)]ClO_4$ have the potential to generate novel metabolites, by displaying high affinities towards various receptors.

It can be claimed that the strong antimicrobial activities of these compounds especially against *S. epidermidis* warranted further investigation on these compounds.

ACKNOWLEDGEMENT

This work was supported by Istanbul University Research Fund. Project number: 262/23082004.

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