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Synthesis, characterization, and antibacterial effect of 4-methoxy-2-(5-H/Me/Cl/NO_2-1*H*-benzimidazol-2-yl)-phenols and some transition metal complexes

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4-Methoxy-2-(5-H/methyl/chloro/nitro-1*H*-benzimidazol-2-yl)-phenol (HL_X; X=1-4) ligands and HL_1 complexes with $Fe(NO_3)_3$, $Cu(NO_3)_2$, $AgNO_3$, and $Zn(NO_3)_2$ were synthesized and characterized. The structures of the compounds were confirmed on the basis of elemental analysis, molar conductivity, magnetic moment, FT-IR, UV-visible, and ^{1}H- and ^{13}C-NMR. Antibacterial activity of the free ligands, their hydrochloride salts, and the complexes was evaluated using the disk diffusion method in dimethyl sulfoxide (DMSO) as well as the minimum inhibitory concentration (MIC) dilution method, against 9 bacteria, and the results were compared with penicillin-G and oxytetracycline. It was observed that HL_1, $[Ag(\mathbf{HL}_1)](NO_3)$, and $[Cu(\mathbf{L}_1)_2](H_2O)_2$ are effective on *S. epidermidis*, *S. aureus*, and *B. subtilis* (gram+) organisms compared with the other compounds. All compounds except HL_4 and $[Zn(\mathbf{L}_1)(H_2O)_2]NO_3$ showed antibacterial activity on *S. aureus*.

Key Words: 4-Methoxy-benzimidazolylphenol, metal complexes, antibacterial activity.

Introduction

Benzimidazolyl phenols are strong chelating agents. Various transition metal chelate complexes of benzimidazolyl-phenol type ligands are reported.[1−5]

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It is known that many benzimidazolyl phenol derivatives and their metal complexes have various antimicrobial activities. For example, some benzimidazolyl-phenol type ligands and their Fe(III) complexes showed broad spectrum activities that were either more active or as potent as the references.[2,6] Co(II) and Ni(II) chelates of 2-(2'-hydroxyphenyl)-benzimidazole have antifungal activity against *Alternaria alternata* and *Aspergillus niger*.[7,8] Fe(III), Ag(I), and Hg(II) complexes of some 5-substituted 2-(2'-hydroxyphenyl)-1*H*-benzimidazoles showed antibacterial and antifungal effects.[9]

In our previous work, 2-methoxy-6-(5-H/methyl/chloro/nitro-1*H*-benzimidazol-2-yl)-phenols and some transition metal complexes were synthesized and characterized. Some ligands and Cu(II) and Zn(II) complexes showed antibacterial activity against gram(+) bacteria.[10]

The aim of this study was to obtain and characterize new compounds and investigate their antibacterial effects. Therefore, 4-methoxy-2-(1*H*-benzimidazol-2-yl)-phenol (HL_1), its methyl/chloro/nitro derivatives (HL_X; X=2-4, Figure 1), and Fe(III), Cu(II), Ag(I), Zn(II) nitrate complexes are reported. Their antibacterial activities were evaluated by the disk diffusion method against 9 bacteria. The ligands and the complexes were characterized by elemental analysis, molar conductivity, magnetic moment, FT-IR, UV-visible, and ^{1}H- and ^{13}C-NMR spectroscopy.

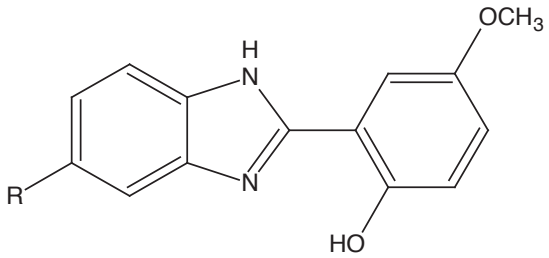


Figure 1. Structure of the ligands. R = H, HL_1; R = CH_3, HL_2; R = Cl, HL_3; R = NO_2, HL_4.

Experimental

Materials: All chemicals and solvents were reagent grade and were used as purchased without further purification. Melting points were determined using an Electrothermal melting-point apparatus. Analytical data were obtained with a Thermo Finnigan Flash EA 1112 analyzer. Molar conductivity of the complexes was measured on a WPA CMD750 conductivity meter in DMSO at 25 °C. ^{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. Magnetic measurements were carried out on a Sherwood Scientific apparatus at room temperature by Gouy's method.

Synthesis of the ligands

4-Methoxy-2-(1*H*-benzimidazol-2-yl)-phenol (HL_1). The ligands were prepared according to literature procedures[4,11] by reacting 2-hydroxy-5-methoxybenzaldehyde (1.52 g, 10 mmol) and an equivalent amount of $NaHSO_3$ (1.04 g, 10 mmol) at room temperature in EtOH (25 mL) for several hours. The mixture was treated with

o-phenylenediamine (1.08 g, 10 mmol) in dimethylformamide (15 mL) and gently refluxed for 2 h. The reaction mixture was then poured into iced water (500 mL), filtered, and crystallized from EtOH.

The other ligands were prepared in a similar manner to ligand HL_1.

Synthesis of the complexes

$[Fe(\mathbf{L}_1)(OH)(H_2O)_2]NO_3$. A hot solution of the ligand HL_1 (120 mg, 0.5 mmol) in i-PrOH (10 mL) was treated with $Fe(NO_3)_3{\cdot}9H_2O$ (222 mg, 0.55 mmol) in *i*-PrOH (5 mL) at ∼60 °C for several hours. The solution mixture was allowed to stand at ∼4 °C for several days to precipitate black solid products, which were collected by filtration and dried at 80 °C.

$[Cu(\mathbf{L}_1)_2](H_2O)$. HL_1 ligand (120 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 h reflux the precipitate was filtered and dried at 80 °C.

$[Ag(\mathbf{HL}_1)](NO_3)$. HL_1 ligand (120 mg, 0.5 mmol) and $AgNO_3$ (94 mg, 0.55 mmol) was treated in EtOH (10 ml) at room temperature for 6 h. with stirring. The precipitate was filtered and dried at 80^oC.

$[Zn(\mathbf{L}_1)(H_2O)_2]NO_3$. HL_1 ligand (120 mg, 0.5 mmol) was suspended in ethyl acetate (15 mL) and $Zn(NO_3)_2{\cdot}6H_2O$ (164 mg, 0.55 mmol) was added to the ligand solution. The mixture was refluxed for 2 h. The reddish-brown precipitate was filtered and dried at 80 °C.

Microorganisms: The antimicrobial activities are evaluated against gram(+) (*Staphylococcus aureus* ATCC 29213, *Bacillus cereus* ATCC 11778, *Bacillus subtilis* ATCC 6633, *Staphylococcus epidermidis* ATCC 12228) and gram(-) (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enteritidis* KUEN 349*, Proteus mirabilis* CCM 1944) bacteria using disk diffusion as well as minimum inhibitory concentration (MIC) dilution. The strains were provided from the Center for Research and Application of Culture Collections of Microorganisms of İstanbul University (KUKENS).

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 by MIC, Mueller-Hinton broth (Fluka 70192) (CAMBH) with Mg^{2+} (10 mg Mg^{2+} mL^{-1}) and Ca^{2+} (20 mg Ca^{2+} L^{-1}) was used as the medium.

Detection of the antibacterial activity

Qualitative antibacterial evaluation. Disk diffusion was used for the detection of the antibacterial effect of the chemical agents qualitatively.[12] For this purpose, filter papers (Whatman No: 1) 6 mm in diameter were autoclaved and dried at 37 °C overnight. Each chemical agent (21.27 mg) was dissolved in DMSO and 23.5 μL of this solution (containing 500 μg of chemical agent) were soaked on to the sterile discs. Bacterial suspensions with 1-2 × 10^8 cfu mL^{-1} (McFarland 0.5) were prepared from each bacterial strain and streaked onto the agar; chemical agent 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 quantitative antibacterial evaluations.

Quantitative antibacterial evaluation. For the detection of the antibacterial effect of the chemical agents, quantitatively, the macro dilution broth method according to the Clinical and Laboratory Standards Institute (formerly NCCLS) was performed.[13] Serial dilutions of the chemical agents between 532 μnd 0.26 μg mL^{-1} with CAMBH were prepared within sterile tubes. Bacterial suspension with 10^7 cfu mL^{-1} final concentration

was inoculated. Positive (without tested chemical agent) and negative (without bacterial suspension) tubes were used after each organism was tested. The 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.

Penicillin-G and oxytetracycline were used as reference antibiotics for bacteria. In addition, as a control, antimicrobial effects of the DMSO were investigated against the test microorganisms. The results were evaluated according to values of the controls.

Results and discussion

Physical properties. The analytical data and physical properties of the ligands and the complexes are summarized in Table 1. According to the melting points, the methyl substitution on 5-position of benzimidazole moiety decreases the melting point, and chloro and nitro substitutions increase it according to HL_1 ligand.

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

Compound	Found (calcd) %			Yield	M.p.	Color	Λ
	C	H	N	%*	dec.		
HL$_1$ $C_{14}H_{12}N_2O_2$	70.2 (70.0)	4.9 (5.0)	11.5 (11.7)	90	272	white	-
HL$_2$ $C_{15}H_{14}N_2O_2$	70.6 (70.8)	5.4 (5.6)	10.8 (11.0)	75	262	white	-
HL$_3$ $C_{14}H_{11}ClN_2O_2$	61.4 (61.2)	4.3 (4.0)	9.9 (10.2)	65	294	dirty white	-
HL$_4$ $C_{14}H_{11}N_3O_4$	58.6 (58.9)	4.2 (3.9)	14.3 (14.7)	75	315	red-brown	-
$[Fe(\mathbf{L}_1)(OH)(H_2O)_2]NO_3$ $C_{14}H_{16}FeN_3O_8$	40.3 (41.0)	3.6 (3.9)	10.7 (10.2)	75	> 350	brown-black	40
$[Cu(\mathbf{L}_1)_2](H_2O)_2$ $C_{28}H_{26}CuN_4O_6$	58.5 (58.2)	4.5 (4.3)	10.0 (9.7)	75	212	dark brown	14
$[Ag(\mathbf{HL}_1)](NO_3)$ $C_{14}H_{12}AgN_3O_5$	41.4 (41.0)	3.3 (2.9)	10.5 (10.2)	65	231	skin color	33
$[Zn(\mathbf{L}_1)(H_2O)_2]NO_3$ $C_{14}H_{15}N_3O_7Zn$	41.1 (41.8)	3.3 (3.7)	10.9 (10.4)	70	338	reddish brown	44

μ_{eff} values for $[Fe(\mathbf{L}_1)(OH)(H_2O)_2]NO_3$ and $[Cu(\mathbf{L}_1)_2](H_2O)_2$ are 3.83 and 1.58 BM, respectively.
Λ, molar conductivity Ω^{-1} cm^2 mol^{-1} (25 °C); dec., decomposed.
*, approximate values.

The molar conductivity values of the complexes are 40, 33, and 44 Ω^{-1} cm^2 mol^{-1} for Fe(III), Ag(I), and Zn(II) complexes, respectively. These results are indicative for the 1:1 electrolyte complexes. The molar conductivity of Cu(II) complex, 14 Ω^{-1}cm^2 mol^{-1}, fits that of a non-electrolyte complex.

The room temperature effective magnetic moment value of Fe(III) complex is 3.83 BM, indicating stabilization of the species having intermediate ferric spin (S = 3/2) state. The occurrence of such an intermediate spin state is typical for the 6 and 5 coordinate ferric complexes (Figure 3).[2,14,15] Magnetic moment value of $[Cu(\mathbf{L}_1)_2](H_2O)_2$ complex is 1.58 BM, which is in the expected range for a typical mononuclear d^9 Cu(II) complex.

FT-IR spectra. FT-IR spectral data of the ligands and the complexes are given in Table 2. The characteristic ν(O–H) and ν(N–H) vibration frequencies of the ligands exhibit only a single strong band at 3314 cm^{-1} in the IR spectra of HL_1, caused by doubly intramolecular hydrogen bonding between the phenoxyl hydrogen atom and one of the imine nitrogen atoms.[6,16,17] However, in the HL_2, HL_3, and HL_4 spectra, there are 2 bands at ca. 3400 and 3300 cm^{-1}, for ν(N–H) and ν(O–H), respectively. The 3314 cm^{-1} band in the HL_1 changes significantly upon metal complexation, indicating deprotonation and subsequent involvement of the phenoxyl group in metal coordination. Fe(III), Cu(II), and Zn(II) complexes have 2 broad bands between 3230 and 3450 cm^{-1} corresponding to ν(NH) and $\nu(H_2O)$, respectively. Appearance of a medium or strong broad band at ca. 3440 cm^{-1} in the Fe(III), Cu(II), and Zn(II) complexes strongly supports the presence of coordinated water molecules.

The characteristic ν(C–H) and δ(C–H) modes of ring residues are observed in the wave region at 3064-3107 cm^{-1} and 835-735 cm^{-1}, respectively (Table 2). The weak bands in the 2918 and 2971 cm^{-1} range are due to stretching vibrations of the methoxy group.

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

Compound	Frequency (cm^{-1})
HL_1	3314 s, 3065 w, 2971 m, 1615 m, 1593 m, 1523 m, 1498 m, 1454 s, 1276 m, 1206 s, 1046 m, 810 m, 742 m
$[Fe(\mathbf{L}_1)(OH)(H_2O)_2]NO_3$	3434 s,br, 3261 sh,br, 3107 w, 2941 w, 1628 m, 1620 m, 1555 m, 1532 m, 1486 m, 1386 s, 1266 m, 1243 m, 1108 m, 1051 m, 935 m, 835 m, 750 m
$[Cu(\mathbf{L}_1)_2](H_2O)_2$	3422 m,br, 3238 m,br, 3064 m,br, 2960 m, 1624 m, 1605 w, 1563 m, 1517 m, 1493 m, 1463 m, 1236 m, 1039 m, 820 m, 758 m
$[Ag(\mathbf{HL}_1)](NO_3)$	3320 s, 3061 w, 2938 w, 1623 m, 1600 w, 1508 m, 1454 m, 1385 s, 1284 m, 1208 m, 1046 m, 815 m, 756 m
$[Zn(\mathbf{L}_1)(H_2O)_2]NO_3$	3448 s,br, 3257 m,br, 2933 w, 1640 sh, 1624 m, 1559 m, 1509 m, 1470 m, 1386 s, 1324 m, 1224 m, 1058 m, 824 m, 773 m, 750 m
HL_2	3407 br, 3322 s, 3072 w, 2956 w, 2914 w, 1620 m, 1597 m, 1528 m, 1497 s, 1439 m, 1281 s, 1208 s, 1051 m, 808 m, 758 m
HL_3	3399 br, 3311 s, 3076 w, 2918 w, 1620 m, 1601 sh, 1524 m, 1462 s, 1440 m, 1274 m, 1216 s, 1024 s, 804 m, 758 m, 600 m
HL_4	3430 br, 3318 s, 3087 m, 2937 m, 1624 m, 1597 m, 1528 s, 1497 m, 1455 m, 1343 s, 1212 m, 1093 m, 1039 m, 812 m, 735 m, 565 m

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 are shifted at higher

frequency upon complex formation. Similarly the (C=N) asymmetric stretching frequencies are expected to appear at ca. 1590 cm^{-1}. 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.

Fe(III), Ag(I), and Zn(II) complexes show strong bands at 1386 cm^{-1} in their IR spectra, supporting the presence of uncoordinated nitrate ion, which was also confirmed by conductivity data.[18–20]

Electronic Spectra. UV-Visible spectral data of the ligands and the complexes are given in Table 3. The electronic spectra of the ligands and the complexes exhibit intense bands in the 200–300 nm region, which may be assignable to n → p* and p → p* transitions.

The electronic spectra of the Fe(III) complex are of little help in the present case, since the d → d transitions are masked by the broad strong charge-transfer bands (570 nm, L→Fe, charge transfer).[18] The broad band at 439 nm is a charge-transfer band in the Zn(II) complex.

The electronic spectra of the Cu(II) complex showed 3 bands at 641, 451, and 353 nm. The 353 nm band is assigned to a metal-ligand charge transfer. The other 2 bands are assigned to $^2B_{1g} \rightarrow {}^2A_{1g}$ and $^2B_{1g} \rightarrow {}^2E_g$ transition, respectively. These assignments are typically characteristic for square-planar geometry for Cu(II) complexes (Figure 3).[21,22]

Table 3. UV-visible spectral data of the compounds.

Compound	Wavelength (λ_{max}, nm)
HL_1	230, 289, 300, 353
$[Fe(\mathbf{L}_1)(OH)(H_2O)_2]NO_3$	252, 315, 362, 375, 570
$[Cu(\mathbf{L}_1)_2](H_2O)_2$	244, 293, 300, 353, 451, 641
$[Ag(\mathbf{HL}_1)](NO_3)$	280, 289, 300, 338
$[Zn(\mathbf{L}_1)(H_2O)_2]NO_3$	271, 298, 328, 380, 439
HL_2	226, 260, 285, 366
HL_3	214, 294, 304, 341
HL_4	273, 286, 337, 358, 394, 409

NMR Spectra. ^{1}H- and ^{13}C-NMR spectral data are given in Tables 4 and 5. According to the ^{1}H-NMR spectra, the ligands HL_2 and HL_3 have 2 isomeric forms (Table 4, Figure 2). The isomer structures are observed for the benzimidazole protons only. OH and NH protons show a broad combination band in the spectra of HL_4 that includes a nitro group. This observation results from strong hydrogen bonding between the imine nitrogen with double bond and phenolic hydrogen atoms. The other ligands, HL_1, HL_2, and HL_3, give 2 broad bands for OH and NH protons because probably they have weaker hydrogen bonding than HL_4 in DMSO-d_6.

The phenolic and benzimidazole protons of the Ag(I) and Zn(II) complexes change their characteristics according to the ligand. All of the phenolic and benzimidazole protons of HL_1 ligand appear as multiplets in the NMR spectra. The multiplets in the ligand spectra change to broad singlets or broad doublets in the complexes because of the metal ion's perturbing effect, especially in Zn(II) complex. It can be said that, on complexation, the acidic character of the benzimidazole and the phenol moiety protons is increased.

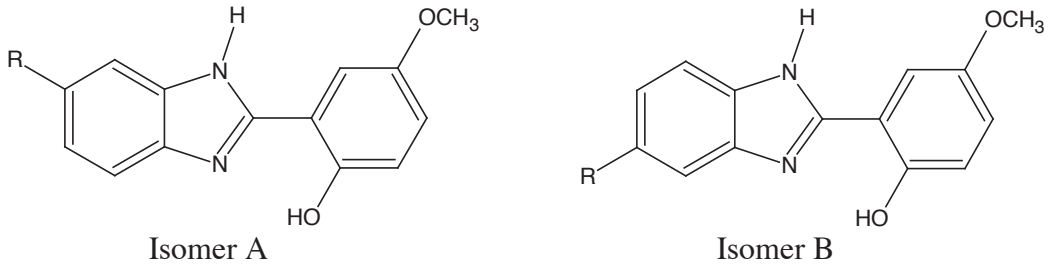
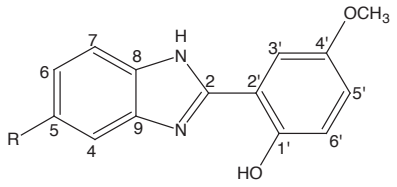


Figure 2. The isomeric structures for HL_2 and HL_3 ligands (R=CH_3, Cl).

Table 4. ^{1}H-NMR spectral data of the ligands and the complexes (in DMSO-d_6).

Compound	Benzimidazole protons					Phenolic protons				
	H4	H5	H6	H7	NH	H3'	H5'	H6'	OH	OCH_3
HL_1	7.66 m	7.28 m	7.28 m	7.66 m	13.08 s,br	7.66 m	6.99 m	6.99 m	12.59 s,br	3.81 s
$[Ag(\mathbf{HL}_1)]NO_3$	7.66 d-d J=1.0, 2.4	7.32 m	7.32 m	7.66 d-d J=1.0, 2.4	12.95 s,br	7.69 d,br J=2.9	7.10 m	7.10 m	12.05 s,br	3.78 s
$[Zn(\mathbf{L}_1)(H_2O)_2]NO_3$	7.80 s,br	7.47 d J=2.7	7.31 d,br J=7.3	7.58 d J=7.3	13.32 s,br	7.95 s,br	7.26 s,br	7.26 s,br	–	3.81 s
HL_2, IsomerA (%60)	7.50 s,br	2.47 s*	7.09 d,br J=8.3	7.58 d,br J=8.0	13.03 s,br	7.63 d J=2.4	6.97 m	6.97 m	12.67 s,br	3.80 s
HL_2, IsomerB (%40)	7.39 s,br	7.13 d,br J=7.8	2.44 s*	7.48 s,br	12.99 s,br	7.63 d J=2.4	6.97 m	6.97 m	12.67 sb,r	3.80 s
HL_3, IsomerA (%50)	7.32 d-d J=1.7,8.5	7.64 d J=8.5	–	7.66 s,br	13.26 s,br	7.67 s	7.01 m	7.01 m	12.23 s,br	3.81 s
HL_3, IsomerB (%50)	7.81 d,br J=1.5	–	7.73 d J=8.5	7.29 d-d J=1.7,8.5	13.22 s,br	7.67 s	7.01 m	7.01 m	12.23 s,br	3.81 s
HL_4	8.53 s	–	8.15 d-d J=8.7,2.1	7.82 d J=8.7	12.50 br	7.69 d J=2.7	7.04 m	7.04 m	12.50 br,s	3.82 s

* 3H (CH_3)



The OH proton signal in the ^{1}H-NMR spectra of the Zn(II) complex is removed as expected. This observation is evidence for the OH hydrogen's eliminating and the phenolic oxygen's coordinating to the metal ions (Figure 3). In the Ag(I) complex, the very broad singlet at 12.05 ppm shows that the phenolic OH

hydrogen is not eliminated; however, the phenolic oxygen atom coordinates to the Ag(I) ion. Shifting the OH hydrogen signal from 12.59 (ligand) to 12.05 ppm (complex) supports the phenolic oxygen atom coordination on complexation.

In the ^{13}C-NMR spectra of the HL_1 and its Ag(I) and Zn(II) complexes the signal around 152.85, 152.50, and 155.16 ppm belongs to imidazole C=N (C-2) carbon atom, respectively. The low ppm signal, 56.41, 56.44, and 57.12 ppm, is due to the methoxy carbon atom in HL_1 and its Ag(I) and Zn(II) complexes, respectively. C8 and C9 carbon atoms of HL_1 ligand show at 152.72 and 152.38 ppm signals, respectively. The other signals belong to the benzimidazole benzene and phenol rings' carbon atoms (Table 5).

Table 5. ^{13}C-NMR spectral data of the HL_1 and its Ag(I) and Zn(II) complexes (in DMSO-d_6).

Compound	**Chemical shifts (δ_C, ppm)**
HL_1	152.85, 152.72, 152.38, 113.04, 119.45, 118.70, 110.63, 56.41
$[Ag(\mathbf{HL}_1)](NO_3)$	152.50, 152.20, 152.01, 113.80, 123.66, 119.48, 118.62, 111.58, 56.42
$[Zn(\mathbf{L}_1)(H_2O)_2]NO_3$	155.16, 150.91, 146.15, 141.00, 114.11, 140.60, 124.60, 123.40, 112.17, 57.12

Antibacterial Effect. The results concerning in vitro antibacterial activity of the ligand, their hydrochloride salts and the complexes together with the inhibition zone (mm) and MIC values are presented in Tables 6 and 7. The MIC value results are compared with the reference antibiotics (Table 7).

Table 6. In vitro antimicrobial activity of the compounds (inhibition zone, mm).

Compound	Microorganisms								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
HL_1	–	–	–	–	–	–	8	8	8
HL_1·HCl	–	–	–	–	8	8	–	–	–
HL_2	10	–	–	–	–	–	–	8	–
HL_2·HCl	–	–	–	–	–	–	8	10	–
HL_3	–	–	–	–	–	–	–	8	–
HL_3·HCl	–	–	–	–	–	–	8	8	–
HL_4	–	–	–	–	–	–	–	–	–
HL_4·HCl	–	–	–	–	–	10	–	–	–
$[Fe(\mathbf{L}_1)(OH)(H_2O)_2]NO_3$	–	–	8	–	7	–	–	8	–
$[Cu(\mathbf{L}_1)_2](H_2O)_2$	–	–	–	–	–	8	12	12	8
$[Ag(\mathbf{HL}_1)](NO_3)$	10	–	–	–	8	–	–	7	7
$[Zn(\mathbf{L}_1)(H_2O)_2]NO_3$	–	–	–	–	–	–	–	–	–

–: zone did not form.

The HL_1 ligand, $[Ag(\mathbf{HL}_1)](NO_3)$ and $[Cu(\mathbf{L}_1)_2](H_2O)_2$ complexes exhibit moderate antibacterial activity. The inhibition zone and MIC values of the HL_1, $[Ag(\mathbf{HL}_1)](NO_3)$, and $[Cu(\mathbf{L}_1)_2](H_2O)_2$ on *S. epidermidis*, *S. aureus*, and *B. subtilis* (gram+) organisms are exceptionally effective compared with the other compounds (Tables 6 and 7, microorganisms 7-9). Of all the test compounds used, HL_1 ligand and its complexes,

especially Cu(II) complex, show antibacterial activities against gram(+) bacteria. On the other hand, it can be said that all compounds are effective on *S. aureus* except HL_4 ligand and $[Zn(\mathbf{L}_1)(H_2O)_2]NO_3$ complex.

Table 7. In vitro antimicrobial activity of the compounds (MIC, μg mL^{-1}).

Compound	Microorganisms								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
HL_1	–	–	–	–	–	–	33	66.5	133
HL_1·HCl	–	–	–	–	*	532	–	–	–
HL_2	*	–	–	–	–	–	–	*	–
HL_2·HCl	–	–	–	–	–	–	*	*	–
HL_3	–	–	–	–	–	–	–	*	–
HL_3·HCl	–	–	–	–	–	–	*	*	–
HL_4	–	–	–	–	–	–	–	–	–
HL_4·HCl	–	–	–	–	–	*	–	–	–
$[Fe(\mathbf{L}_1)(OH)(H_2O)_2]NO_3$	–	–	*	–	*	–	–	*	–
$[Cu(\mathbf{L}_1)_2](H_2O)_2$	–	–	–	–	–	*	66.5	66.5	133
$[Ag(\mathbf{HL}_1)](NO_3)$	66.5	–	–	–	66.5	–	–	66.5	33
$[Zn(\mathbf{L}_1)(H_2O)_2]NO_3$	–	–	–	–	–	–	–	–	–
Penicillin-G	7	–	12	7	–	–	7	30	20
Oxytetracycline	–	18	18	–	18	20	–	18	14

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.*
– No antibacterial activity qualitatively
*: MIC >532 μg mL^{-1}

$[Ag(\mathbf{HL}_1)](NO_3)$ complex shows considerable antibacterial activity on *S. enteriditis*, oxytetracycline shows no antibacterial activity on it and *K. pneumoniae*, and penicillin-G shows no antibacterial activity. Moreover, regarding the antibacterial effect of HL_1 and Cu(II) complex on *S. epidermidis*, oxytetracycline has no effect, which is an important observation.

In conclusion, the proposal structures for the complexes in Figure 3 are in best accord with the experimental data obtained from the analytical data, molar conductivity, magnetic moments, FT-IR, UV-visible and NMR spectroscopic measurements.

Elemental analysis, molar conductivity, magnetic susceptibility, FT-IR, and UV-visible data are in agreement with a 1:1 electrolyte structure that is mononuclear, and 5-coordinate square pyramidal for Fe(III) complex.[2] Zn(II) complex with 4 coordination has tetrahedral, and Cu(II) complex has square-planar geometry as expected.

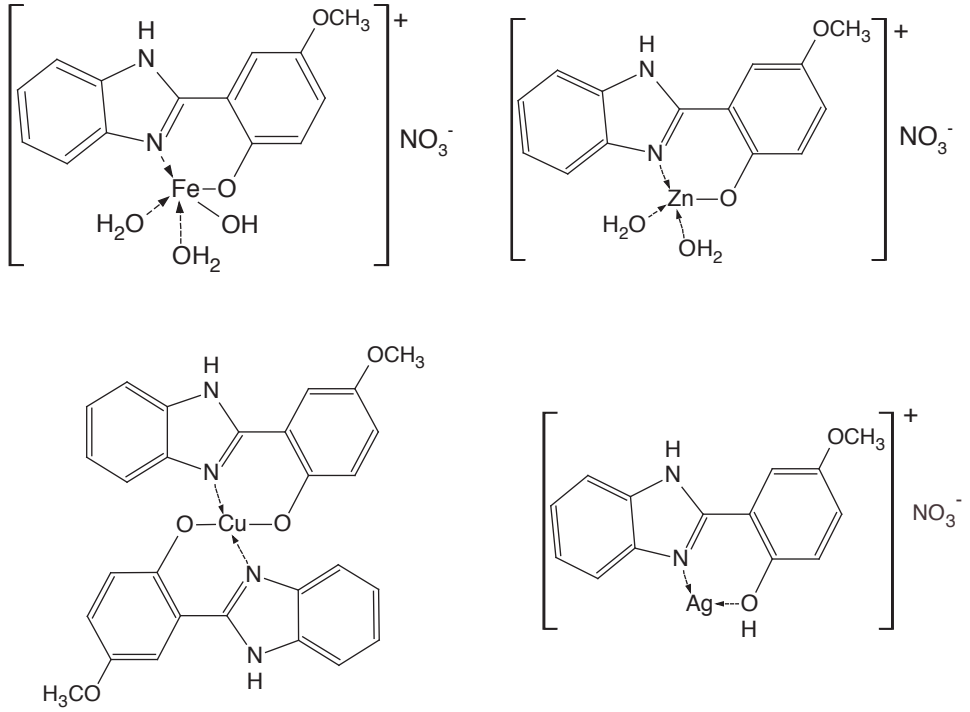


Figure 3. The proposal structures for the complexes under study.

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