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Bioorganometallic Chemistry AOC

Organometallic based biologically active compounds: synthesis of mono- and di-ethanolamine derived ferrocenes with antibacterial, antifungal and cytotoxic properties

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Condensation reactions of 1,1′-diacetylferrocene with ethanolamine were studied. The obtained compounds were further investigated for their ligation and biological properties with Co(II), Cu(II), Ni(II) and Zn(II) metal ions. The synthesized compounds were characterized by their physical, spectral and analytical properties and screened for their antibacterial properties against pathogenic bacterial strains, e.g. *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus* and *Salmonella typhi* and for antifungal activity against *Trichophyton longifusus, Candida albicans, Aspergillus flavus, Microsporum canis, Fusarium solani* and *Candida glaberata* using the agar-well diffusion method. All the compounds have shown good antibacterial and antifungal activity, which increased on coordination with the metal ions, thus introducing a potential class of organometallic-based antibacterial and antifungal agents. Brine shrimp bioassay was also carried out for *in vitro* cytotoxic properties against *Artemia salina*. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS: 1,1′-disubstituted ferrocenes; metal(II) chelates; antibacterial, antifungal, cytotoxic properties

INTRODUCTION

Drug resistance is becoming a globally important medical problem. To address this issue, progression of chelation and its relationship with biological activity composes one promising possibility for the design of novel therapeutic methodologies. A number of studies have highlighted the use of ferrocene and its derivatives for the design of biologically active compounds.[1–5] Some reports have indicated[6,7] that if the aromatic group in penicillin and cephalosporin antibiotics is replaced by the ferrocenyl moiety, bactericidal properties are significantly increased. Some studies have also indicated the bio-activity of ethanolamine against neurodegenerative diseases,[8] its antifungal,[9] cytotoxic,[10] anti-inflammatory,[11] antioxidant[11] and transdermal effects,[12] and its capability of antagonizing calcium-sensing receptors.[13] Keeping in view the biological applications of ferrocene as well as ethanolamine compounds, we thought it worthwhile to combine the chemistry of both the compounds to design organometallic-based biologically active compounds and further probe their biological properties upon chelation.

Acetylferrocene, a typical acylferrocene, undergoes a condensation reaction with amines to form Schiff bases. In a successful attempt to investigate such transformation, we wish to report herein mono- and di-ethanolamine derived ferrocene derivatives L^1 and L^2 (Fig. 1) and their Co(II), Cu(II), Ni(II) and Zn(II) metal complexes (Fig. 2). These prepared compounds were screened for their antibacterial activity against bacterial strains such as *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtillis, Staphylococcus aureus* and *Salmonella typhi*, and, for antifungal activity against *Trichophyton longifusus, Candida albicans, Aspergillus flavus, Microsporum canis, Fusarium solani* and *Candida glaberata* using the agar-well diffusion method.[14,15] All the prepared ferrocene-containing ligands have shown a good affinity as antibacterial/antifungal agents, which increases upon chelation/coordination with the metal ions.

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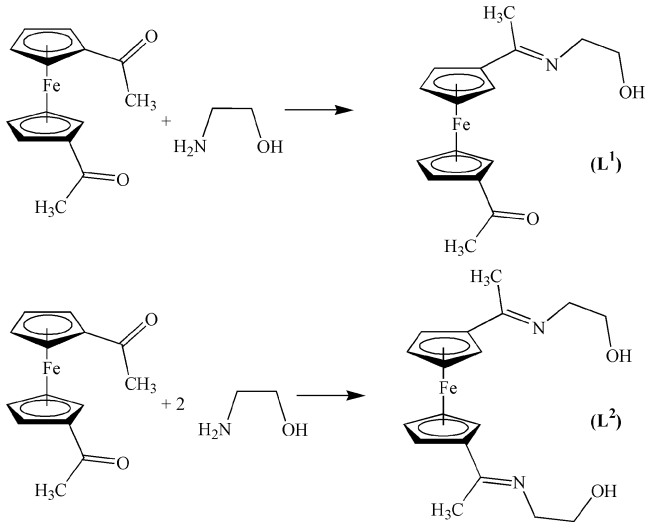

Figure 1. Scheme showing preparation of ligands L^1 and L^2.

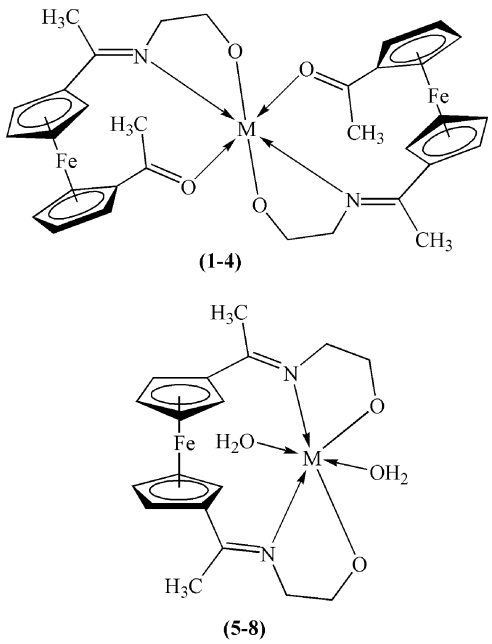

Figure 2. Showing proposed structures of metal(II) complexes **1–8**. M = Co(II), Cu(II), Ni(II), Zn(II).

MATERIAL AND METHODS

All solvents used were of analytical grade. 1,1′-Diacetylferrocene and ethanolamine were obtained from Aldrich. All metals were used as their chloride salts. IR and NMR spectra were recorded on Perkin Elmer 283B and 300 MHz Varian XL-300 instruments. UV–visible spectra were obtained on a Baush and Lomb spectronic 1001. Conductances of the metal complexes were determined in DMF using a YSI-32 model conductometer. Magnetic measurements were done on solid complexes using the Gouy method. Melting points were recorded on a Gallenkamp apparatus and are uncorrected.

Synthesis of ligands

Preparation of 1-acetyl,1′-ethanolamine derived ferrocene L^1

A solution of ethanolamine (0.61 g, 0.01 mol) in ethanol (10 ml) was added drop-wise to a solution of 1,1′-diacetylferrocene (2.70 g, 0.01 mol) taken in slight excess in ethanol (25 ml). The reaction mixture was heated under reflux for 6 h. After cooling to room temperature, the precipitates formed were filtered and washed several times with ethanol followed by diethyl ether. After drying the product was recrystallized from hot ethanol to obtain brown crystalline desired product. These were dried under vacuum over P_4O_{10}. The purity of the product was confirmed by TLC (plastic sheets of a silica gel F_{254} with 0.2 mm layer thickness).

A similar method was used for the preparation of the ligand L^2 using an exactly 1:2 molar ratio (acetylferrocene:ethanolamine) to obtain a dark brown crystalline solid product.

Synthesis of the metal(II) complex

*Preparation of Co(II) complex with 1-acetyl,1′-ethanolamine derived ferrocene $[Co(L^1\text{-}H)_2]$ (**1**)*

A solution of Co(II) $Cl_2.6H_2O$ (0.24 g, 0.001 mol) in absolute ethanol (10 ml) was added drop-wise with stirring to a solution of the 1-acetyl,1′-ethanolamine derived ferrocene (0.63 g, 0.002 mol) in the same solvent (15 ml).The reaction mixture was heated under reflux for 3 h and then cooled to room temperature. The isolated solid was removed by filtration, washed several times with hot ethanol followed by diethyl ether and dried under vacuum. The purity of the product was confirmed by TLC. All other complexes were prepared similarly using the same method. However, 1:1 molar ratio (ligand:metal) was used in complexes **4**– **8**.

Biological properties

Antibacterial bioassay (in vitro)

The synthesized compounds L^1 and L^2 and metal(II) complexes **1–8** were screened *in vitro* for their antibacterial activity against *E. coli*, *P. aeruginosa*, *B. subtillis*, *S. aureus* and *S. typhi* bacterial strains by the agar-well diffusion method.[14,15] The wells (6 mm in diameter) were dug in the media with the help of a sterile metallic borer with centers at least 24 mm apart. Two- to eight-hours-old bacterial inocula containing approximately 10^4–10^6 colony-forming units (CFU/ml) were spread on the surface of the nutrient agar with the help of a sterile cotton swab. The recommended concentration of the test sample (1 mg/ml in DMSO) was introduced in the respective wells. Other wells supplemented with DMSO and reference antibacterial drug, imipenum, served

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as negative and positive controls, respectively. The plates were incubated immediately at 37 °C for 24 h. Activity was determined by measuring the diameter of zones showing complete inhibition (mm). In order to clarify any participating role of DMSO in the biological screening, separate studies were carried out with the solutions alone of DMSO and they showed no activity against any bacterial strains. Also, the metal salts used for complexation were evaluated for antibacterial activity against the same bacterial strains under the same conditions but none of them showed any significant and/or moderate activity, only insignificant activity was observed.

Antifungal activity (*in vitro*)

All compounds were studied against six fungal cultures for antifungal activities. Sabouraud dextrose agar (Oxoid, Hants, UK) was seeded with 10^5 (cfu) ml^{-1} fungal spore suspensions and transferred to Petri plates. Disks soaked in 20 ml (200 μg/ml in DMSO) of all compounds were placed at different positions on the agar surface. The plates were incubated at 32 °C for seven days. The results were recorded[16] by measuring the diameter of zones showing complete inhibition (mm). Miconazole and amphotericin B were used as standard drugs for this bioassay.

Minimum inhibitory concentration

Compounds containing high antibacterial activity (over 80%) were selected for minimum inhibitory concentration (MIC) studies. The minimum inhibitory concentration was determined using the disk diffusion technique by preparing disks containing 10, 25, 50 and 100 μg/ml of the compounds and applying the protocol.[17]

Cytotoxicity (*in vitro*)

Brine shrimp (*Artemia salina* leach) eggs were hatched in a shallow rectangular plastic dish (22 × 32 cm), filled with artificial seawater, which was prepared with commercial salt mixture and double distilled water. An unequal partition was made in the plastic dish with the help of a perforated device. Approximately 50 mg of eggs were sprinkled into the large compartment, which was darkened while the matter compartment was opened to ordinary light. After two days nauplii were collected by a pipette from the lighted side. A sample of the test compound was prepared by dissolving 20 mg of each compound in 2 ml of DMF. From this stock solutions 500, 50 and 5 μg/ml were transferred to nine vials (three for each dilutions were used for each test sample and LD_{50} is the mean of three values) and one vial was kept as control having 2 ml of DMF only. The solvent was allowed to evaporate overnight. After two days, when shrimp larvae were ready, 1 ml of sea water and 10 shrimps were added to each vial (30 shrimps/dilution) and the volume was adjusted with sea water to 5 ml per vial. After 24 h the number of survivors was counted. Data was analyzed by Finney computer program to determine the LD_{50} values.[18,19]

RESULTS AND DISCUSSION

Chemistry

The ligand L^1 was prepared by refluxing ethanol solution of 1,1′-diacetylferrocene (taken in slight excess) with ethanolamine in 1:1 molar ratio. It has been previously observed[20,21] that, in synthesizing similar 1,1′-monosubstituted ferrocene derivatives, mixtures of mono- as well as di-substituted products were achieved and the desired mono-substituted product was recovered by column chromatography. In the present studies, use of slightly excess amount of 1,1′-diacetylferrocene predominantly helped the formation of mono-substituted ethanolamine-derived ferrocene as ligand L^1. The ligand L^2 was prepared by using an exactly 1:2 molar ratio of 1,1′-diacetylferrocene:ethanolamine. The structures of the synthesized ligands were established with the help of their IR, NMR and microanalytical data (Tables 1 and 2). All metal complexes (**1**–**8**; Table 3) of these ligands were prepared by the stoichiometric reaction of the corresponding ligand with the respective metal salt as chloride in a molar ratio M:L of 1:2 and/or 1:1. They are all air- and moisture-stable and are intensely colored amorphous solids that decompose without melting. Molar conductance values of the Co(II), Cu(II), Ni(II) and Zn(II) complexes (19–24 Ω cm^2 mol^{-1}) in DMF showed them to be non-electrolytic in nature.[22]

The elemental analyses data agree well with the proposed formulae for the ligands and also confirmed the $[M(L^1\text{-}H)_2]$ and $[M(L^2\text{-}H_2)_2(H_2O)_2]$ composition (Figs 1 and 2) for the cobalt(II), copper(II), nickel(II) and zinc(II) complexes in an octahedral environment. Only microcrystalline powder of these compounds could be obtained, which was impossible to use for X-ray structural determinations.

IR spectra

IR frequencies of the ligands and their metal complexes are reported in Tables 1 and 3. In the IR spectra of the ligands L^1 and L^2, a sharp band observed at 1615 cm^{-1} was assigned to the ν(C═N) linkage, suggesting[23,24] condensation and formation of the proposed ligands. The shifting of this band to the lower frequency side (8–10 cm^{-1}) provided further evidence in support of the involvement of this nitrogen in coordination to the metal atoms. Furthermore, a characteristic band due to $\nu(NH_2)$ in the spectra of all the ligands was not observed, suggesting condensation of amino-NH_2 moiety with the acetyl-C═O. A broad band at 3415 in ligand L^1 and 3420 in ligand L^2, respectively assigned to—OH, disappeared in the spectra of its metal complexes and, instead, a new band at 1530 cm^{-1} assigned to C–O appeared that gave a clue to the deprotonation and coordination of the C–O oxygen with the metal ions. Moreover, in the far infrared region bands at 405 and 490 cm^{-1} attributed to ν(M–N) and ν(M–O) were observed for all the complexes (Table 3) and not found in the spectra of the free ligands, suggesting coordination

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Table 1. Physical, spectral and analytical data of the ligands

Ligand/Molecular formula	m.p. (°C)	IR (cm^{-1})	Calcd (found) (%) C H N	Yield (%)
L^1 $C_{16}H_{19}FeNO_2$	196	3415 (OH), 1725 (C═O), 1615 (C═N)	61.36(61.52),6.11(6.24),4.47(4.23)	59
L^2 $C_{18}H_{24}FeN_2O_2$	208	3420 (OH), 1615 (C═N).	60.69(60.92),6.79(6.55),7.86(7.98)	56

Table 2. ^1H NMR and ^{13}C NMR data of the ligands and Zn(II) complexes **4** and **8**

No.	^1H NMR (DMSO-d_6) (ppm)	^{13}C NMR (DMSO-d_6) (ppm)
L^1	2.5 (s, 3H, CH_3), 2.6 (s, 3H, CH_3), 4.3–4.4 (m, 2H, ferrocenyl), 4.6–4.7 (m, 2H, ferrocenyl), 4.8–4.9 (m, 2H, ferrocenyl), 5.0–5.1 (m, 2H, ferrocenyl), 6.3 (s, 1H, CH═N), 3.4 (dd, 2H, CH_2), 3.6 (t, 2H, CH_2), 10.3 (s, 1H, OH).	15.7 (CH_3), 15.9 (CH_3), 68.6, 69.6, 83.6, 68.7, 69.7, 83.8 (ferrocenyl-*C*), 150.6 (*C*═N), 56.7, 57.4 (CH_2), 201.3 (C═O).
L^2	2.5 (s, 6H, CH_3), 4.4–4.6 (m, 4H, ferrocenyl), 4.9–5.2 (m, 4H, ferrocenyl), 6.4 (s, 1H, CH═N), 3.6 (dd, 4H, CH_2), 3.7 (t, 4H, CH_2), 10.2 (s, 2H, OH).	15.7 (CH_3), 68.6, 69.6, 83.6 (ferrocenyl-*C*), 150.7 (*C*═N), 56.7, 57.4 (CH_2).
4	2.6 (s, 6H, CH_3), 2.7 (s, 6H, CH_3), 4.4–4.5 (m, 2H, ferrocenyl), 4.6–4.7 (m, 2H, ferrocenyl), 4.9–5.1 (m, 2H, ferrocenyl), 5.2–5.3 (m, 2H, ferrocenyl), 6.5 (s, 1H, CH═N), 3.5 (dd, 4H, CH_2), 3.8 (t, 4H, CH_2).	15.9 (CH_3), 16.2 (CH_3), 68.6, 69.6, 83.8, 68.7, 69.7, 83.8 (ferrocenyl-*C*), 152.8 (*C*═N), 56.9, 57.7 (CH_2), 205.5 (*C*═O).
8	2.6 (s, 6H, CH_3), 4.6–4.7 (m, 4H, ferrocenyl), 5.2–5.3 (m, 4H, ferrocenyl), 6.5 (s, 1H, CH═N), 3.5 (dd, 4H, CH_2), 3.8 (t, 4H, CH_2), 10.5 (s, 4H, H_2O).	15.9 (CH_3), 68.6, 69.6, 83.8 (ferrocenyl-*C*), 150.9 (*C*═N), 56.9, 57.7 (CH_2).

Table 3. Physical, analytical and spectral data of the metal complexes

Complex/ molecular formula	Decom- position (°C)	IR (cm^{-1})	λ_{max} (cm^{-1})	B.M. (μ_{eff})	Calcd (found) (%) C	H	N
(**1**) $[Co(L^1\text{-}H)_2]$ $C_{32}H_{36}Fe_2CoN_2O_4$	225–227	1605 (C═N), 405 (M–N), 490 (M–O), 510 (M–O)	8,265, 17,425, 20,505, 29,955.	4.71	56.25 (56.33)	5.31 (5.16)	4.10 (4.34)
(**2**) $[Cu(L^1\text{-}H)_2]$ $C_{32}H_{36}Fe_2CuN_2O_4$	235–237	1607 (C═N), 405 (M–N), 490 (M–O), 510 (M–O)	14,760, 19,135, 30,355.	1.78	55.87 (55.62)	5.27 (5.19)	4.07 (4.34)
(**3**) $[Ni(L^1\text{-}H)_2]$ $C_{32}H_{36}Fe_2NiN_2O_4$	229–231	1607 (C═N), 405 (M–N), 490 (M–O), 510 (M–O)	10,315, 16,345, 29,295.	3.58	56.27 (56.36)	5.31 (5.54)	4.10 (4.36)
(**4**) $[Zn(L^1\text{-}H)_2]$ $C_{32}H_{36}Fe_2ZnN_2O_4$	220–222	1605 (C═N), 405 (M–N), 490 (M–O), 510 (M–O)	29,235.	Dia	55.72 (55.68)	5.26 (5.09)	4.06 (4.28)
(**5**) $[Co(L^2\text{-}H_2)_2(H_2O)_2]$ $C_{18}H_{26}FeCoN_2O_4$	240–242	1605 (C═N), 405 (M–N), 490 (M–O), 495 ($M–OH_2$),	8,290, 17,465, 20,515, 29,995.	4.75	48.13 (48.36)	5.83 (5.56)	6.24(6.19)
(**6**) $[Cu(L^2\text{-}H_2)_2(H_2O)_2]$ $C_{18}H_{26}FeCuN_2O_4$	246–248	1607 (C═N), 405 (M–N), 490 (M–O), 495 ($M–OH_2$),	14,780, 19,165, 30,385.	1.83	47.64 (47.71)	5.77 (5.93)	6.17 (6.03)
(**7**) $[Ni(L^2\text{-}H_2)_2(H_2O)_2]$ $C_{18}H_{26}FeNiN_2O_4$	235–237	1607 (C═N), 405 (M–N), 490 (M–O), 495 ($M–OH_2$),	10,355, 16,375, 29,325.	3.61	48.16 (48.39)	5.84 (5.92)	6.24 (6.41)
(**8**) $[Zn(L^2\text{-}H_2)_2(H_2O)_2]$ $C_{18}H_{26}FeZnN_2O_4$	230–232	1605 (C═N), 405 (M–N), 490 (M–O), 495 ($M–OH_2$),	29,275.	Dia	47.45 (47.13)	5.75 (5.89)	6.15 (6.22)

of the ν(N–M) and ν(O–M) to the metal atoms.[25,26] Also, a weak band at 510 cm^{-1} due to the ν(M–O) mode was observed indicating the participation of acetyl carbonyl in coordination for complexes **1–4** and at 495 cm^{-1} due to ν(M–OH_2) for complexes **5–8**, strongly suggesting[27] their octahedral geometry (Figs 3 and 4).

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^{1}H NMR and ^{13}C NMR spectra

The ^{1}H NMR and ^{13}C NMR spectra of the free ligands and their metal(II) chelates were taken in DMSO-d_6. The ^{1}H NMR spectra of all the ligands showed strong signals, suggesting 1,1′ disubstitution on cyclopentadienyl moiety of the ferrocenyl group.[28,29] The data reported along with the possible assignments in Table 2 showed that the ligand L^1 displayed all expected signals at δ 2.5, 2.6, 4.3–5.1, 3.4, 3.6, 6.3 and 10.3 ppm assigned to CH_3, ferrocenyl, CH_2, azomethine-H and –OH respectively. Also, the ligand L^2 displayed the same expected signals at their respective ppm values. In the spectra of their diamagnetic Zn(II) complexes **4** and **8** protons shifted downfield by 0.1–0.2 ppm due to the increased conjugation and extension of the delocalized π-system. The number of protons calculated from the integration curves, and those obtained from the values of the expected CHN analyses agreed well with each other. In the ^{13}C NMR spectra, the ligands displayed signals assigned respectively to $-CH_3$, ferrocenyl, *C*═N and *C*═O carbons. These signals appeared 0.2–4.2 ppm downfield in comparison with the corresponding signals of the free ligands with their metal complexes, indicating coordination with the central metal atom. It was observed that DMSO did not have any coordinating effect either on the spectra of the ligands or on its metal complexes (as evidenced by measuring some of the spectra in $CDCl_3$ for which the data is not shown).

Magnetic moments and electronic spectra

The nature of the ligand field around the metal ions and the geometry of the metal complexes were deduced from the electronic spectra and magnetic moment data of the complexes (Table 3). The room temperature magnetic moment of the solid cobalt(II) complexes was found to be in the range 4.71–4.75 B.M., indicative[30] of three unpaired electrons per Co(II) ion in an octahedral environment. The magnetic moment values (1.78–1.83 B.M.) measured for the copper(II) complexes lay in the range expected for a d^9-system, which contains one unpaired electron in an octahedral geometry. The nickel(II) complexes showed μ_{eff} values (3.58–3.61 B.M), corresponding[30] to two unpaired electrons per Ni(II) ion for their octahedral configuration. The zinc(II) complexes were found to be diamagnetic as expected.

The electronic spectra of the Co(II) complexes showed three bands observed at 8265–8290, 17 425–17 465, 20 505–20 515 and 29 955–29 995 cm^{-1}, which may be assigned to $^4T_{1g} \rightarrow {}^4T_{2g}(F)$, $^4T_{1g} \rightarrow {}^3A_{2g}(F)$ and $^4T_{1g} \rightarrow {}^4T_{1g}(P)$ transi tions, respectively, and are suggestive[31] of the octahedral geometry around the cobalt ions. The high energy band at 29 955–29 995 cm^{-1} is assigned to the metal → ligand charge transfer band. The electronic spectra of the Cu(II) complexes showed two low-energy weak bands at 14 760–14 780 and 19 135–19 165 cm^{-1} and a strong high-energy band at 30 355–30 385 cm^{-1}. The low-energy bands in this region are expected for its octahedral configuration and may be assigned to $^2B_{1g} \rightarrow {}^2A_{1g}$ and $^2B_{1g} \rightarrow {}^2E_g$ transitions, respectively. The strong high-energy band, in turn, is assigned to a metal → ligand charge transfer. The Ni(II) complexes exhibited three spin-allowed bands at 10 315–10 355, 16 345–16 375 and 29 295–29 325 cm^{-1} assignable,[32] respectively, to the transitions $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)(\nu_1)$, $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)(\nu_2)$ and $^3A_{2g}(F) \rightarrow {}^3T_{2g}(P)(\nu_3)$, which were characteristic of their octahedral geometry. The electronic spectra of the Zn(II) complexes showed a high intensity band at 29 235–29 275 cm^{-1} due to ligand → metal charge transfer in a distorted octahedral environment.[33]

On the basis of the above observations, it is suggested that the ligand L^1 acts as a tridentate coordinating through the nitrogen of azomethine ν(C═N), the deprotonated oxygen of ν(OH) moiety and oxygen of acetyl group ν(C═O) whereas, the ligand L^2 acts as tetradentate via coordination through two nitrogens of azomethine ν(C═N) and two deprotonated oxygens of ν(OH) moieties. The Co(II), Cu(II), Ni(II) and Zn(II) complexes **5–8** showed an octahedral geometry involving two water molecules coordinated with each metal atom.

Biological activity

Antibacterial bioassay (in vitro)

All compounds were tested against *E. coli*, *P. aeruginosa*, *B. subtillis*, *S. aureus* and *S. typhi* bacterial strains (Table 4) according to the literature protocol.[14,15] The results were compared with those of the standard drug imipenum (Fig. 3). All compounds

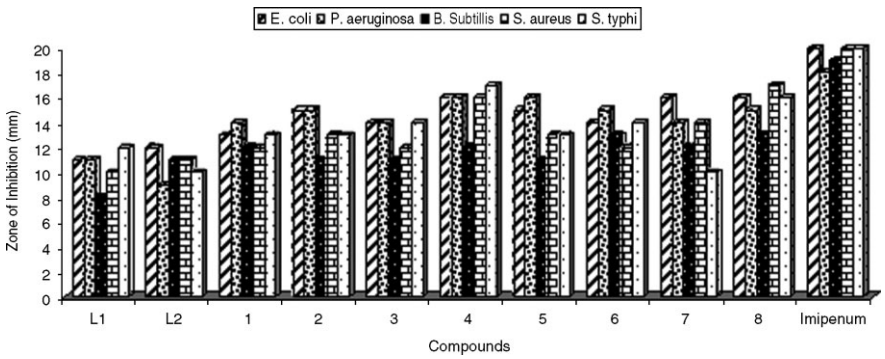

Figure 3. Comparison of antibacterial activity.

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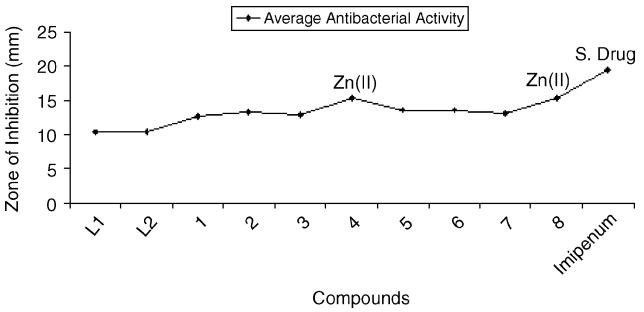

Figure 4. Average antibacterial activity of ligands vs metal(II) complexes.

(ligands as well as metal complexes) showed moderate to significant activity against all bacterial strains except the activity of all compounds against *B. subtillis*, where all compounds showed moderate activity. Compound **1** exhibited overall a moderate activity against all bacterial strains. Compounds **2–8** showed significant activity against *E. coli* and *P. aeruginosa*. Compounds **4, 7** and **8** showed significant activity against *S. aureus*. Compounds **3, 4, 6** and **8** showed significant activity against *S. typhi*. The antibacterial activity was overall enhanced on complexation of the ligands with the metals. It was, however, interesting to note that the zinc(II) complexes were found to be the most active against all species (Fig. 4).

Antifungal bioassay (in vitro)

The antifungal screening of all compounds was carried out against *T. longifusus*, *C. albicans*, *A. flavus*, *M. canis*, *F. solani* and *C. glaberata* fungal strains according to the literature protocol.[16] All synthesized compounds showed good antifungal activity against different fungal strains. All compounds (ligands as well as metal complexes) showed significant antifungal activity against all the fungal strains except *C. albicans*, in which moderate activity was observed by ligands and significant activity was observed by all the metal complexes. The results of inhibition were compared (Fig. 5) with the results of inhibition of standard drugs miconazole and amphotericin B (Table 5). Compound **8** was found to be the most antifungal (Fig. 6).

Minimum inhibitory concentration for antibacterial activity

The preliminary antibacterial screening showed that compounds **2, 4–8** were the most active ones (above 80%). These compounds were, therefore, selected for antibacterial minimum inhibitory concentration studies (Table 6).

Cytotoxic bioassay (in vitro)

All the synthesized compounds were screened for their cytotoxicity (brine shrimp bioassay) using the protocol of Meyer *et al*.[18] From the data recorded in Table 7, it is evident

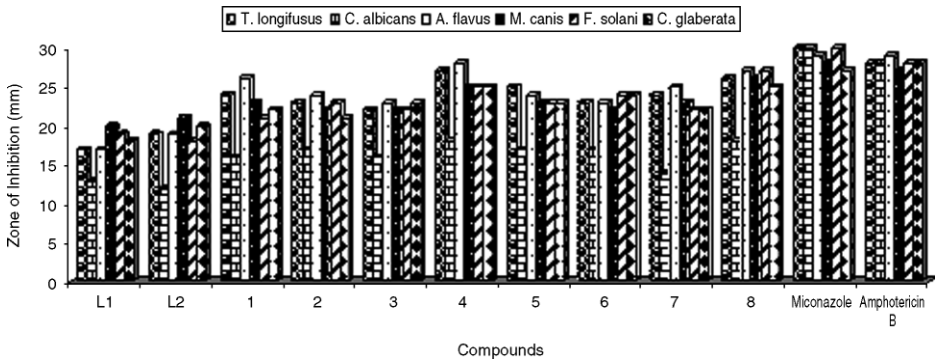

Figure 5. Comparison of antifungal activity.

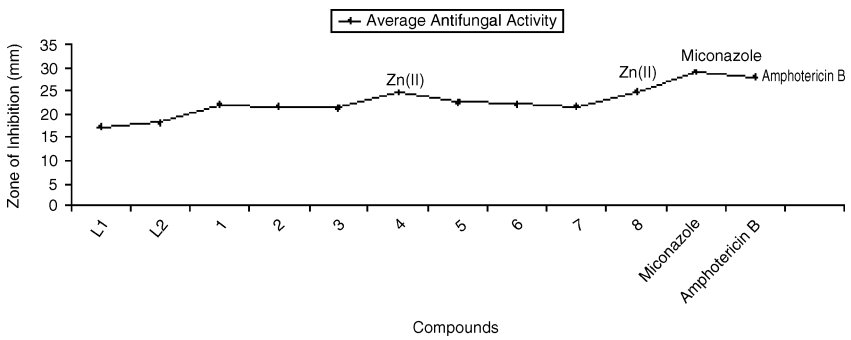

Figure 6. Average antifungal activity in ligands vs metal(II) complexes.

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Table 4. Antibacterial activity data of compounds L^1 and L^2 and **1–8**

	Diameter of zones showing complete inhibition of growth (mm)				
Compound	*E. coli*	*P. aeruginosa*	*B subtillis*	*S. aureus*	*S. typhi*
L^1	11	11	08	10	12
L^2	12	09	11	11	10
1	13	14	12	12	13
2	15	15	11	13	13
3	14	14	11	12	14
4	16	16	12	16	17
5	15	16	11	13	13
6	14	15	13	12	14
7	16	14	12	14	10
8	16	15	13	17	16
DF	05	04	05	04	04
EA	06	05	07	05	06
Imipenum	20	18	19	20	20

14–20 mm = significant activity; 7–13 mm = moderate activity; <7 mm = weak activity.
DF = 1, 1′-diacetylferrocene; EA = ethanolamine.

that two compounds, **2** and **6** displayed potent cytotoxic activity against *Artemia salina*, while the other compounds were almost inactive for this assay. Compound **2** showed activity ($LD_{50} = 0.611$ M) and compound **6** showed activity ($LD_{50} = 0.877$ M) in the present series of compounds. It was interesting to note that only copper complexes showed potent cytotoxicity whereas the other metal complexes did not. This activity relationship may help to serve as a basis for future direction towards the development of certain cytotoxic agents for clinical applications.

The enhancement of antibacterial/antifungal activity in ligands L^1 and L^2 upon chelation is rationalized on the basis of their structures and the mode of coordination/chelation. It has been observed that metal ion upon chelation reduce their polarity due to partial sharing of its positive[34–37] charge with the donor groups of the ligands. This course of action increases the lipophilic nature of the metal atom, which favors[38–43] permeation through the lipid layer of the cell membrane of the micro-organism, thus killing them more effectively as compared with the unchelated ligands. It has also been suggested that functional groups such as azomethine or heteroaromatics present in these compounds display[44–47] broader biological activities that may be responsible for the

Table 5. Antifungal activity data of the compounds L^1 and L^2 and **1–8**

	Diameter of zones showing complete inhibition of growth (mm)					
Compound	*T. longifusus*	*C. albicans*	*A. flavus*	*M. canis*	*F. solani*	*C. glaberata*
L^1	17	13	17	20	19	18
L^2	19	12	19	21	18	20
1	24	16	26	23	21	22
2	23	17	24	22	23	21
3	22	16	23	22	22	23
4	27	18	28	25	25	25
5	25	17	24	23	23	23
6	23	17	23	22	24	24
7	24	14	25	23	22	22
8	26	18	27	26	27	25
Miconazole	30	30	29	28	30	27
Amphotericin B	28	28	29	27	28	28

14–30 mm = significant activity; 7–13 mm = moderate activity; <7 mm = weak activity.

Table 6. Minimum inhibitory concentration (M) of the selected compounds **2, 4–8** against selected bacteria

Compound	*E. coli*	*P. aeruginosa*	*S. aureus*	*S. typhi*
2	—	1.454×10^{-4}	—	—
4	7.249×10^{-5}	1.450×10^{-4}	3.625×10^{-5}	7.249×10^{-5}
5	—	2.226×10^{-4}	—	—
6	—	2.204×10^{-4}	—	—
7	2.227×10^{-4}	—	—	—
8	1.097×10^{-4}	2.195×10^{-5}	1.097×10^{-4}	5.487×10^{-5}

(—) not determined.

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Table 7. Brine shrimp bioassay data of the ligands L^1 and L^2 and their metal(II) complexes **1–8**

Compound	LD_{50} (M)
L^1	>3.193
L^2	>2.807
1	>1.464
2	0.611
3	>1.464
4	>1.450
5	>2.226
6	0.877
7	>2.227
8	>2.195

increase of hydrophobic character and liposolubility of the molecules. It, in due course, enhances activity and biological utilization ratio of the compounds.

CONCLUSION

The results of this investigation support the suggested structures of ethanolamine-derived ferrocenes and their metal(II) complexes. The geometry of Co(II), Cu(II), Ni(II) and Zn(II) complexes is suggested to be octahedral. In the metal complexes of **1–4**, the two L^1 ligands acting as tridentate are coordinated with the metal atom, whereas in the metal complexes of **5–8**, one L^2 ligand and two water molecules are involved in coordination, indicating L^2 to behave as tetradentate (Fig 2). The synthesized ethanolamine-derived ferrocenes and their metal(II) complexes have moderate to significant antibacterial and antifungal properties which increase upon chelation.

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