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Antibacterial copper(II) complexes of 1,1′-symmetric ferrocene-derived Schiff-base ligands: studies of the effect of anions on their antibacterial properties

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Symmetrically substituted 1,1′-dimethylferrocene-derived pyrazine, pyridine and thiazole Schiff-base ligands and their copper(II) complexes have been prepared. The effect of anions on the antibacterial activity of these synthesized metal(II) complexes has been determined. Copyright © 2001 John Wiley & Sons, Ltd.

KEYWORDS: ferrocene Schiff-base ligands; metal chelates; anions; antibacterial

INTRODUCTION

The development and design of new products with the potential for use as biologically active compounds[1–5] has recently become a burgeoning topic within the biological sciences[6–15] and chemistry in particular.[16–19] The antibacterial activity of various heterocyclic systems is well known,[20–24] whereas the application of ferrocene and ferrocene-containing compounds to medicinal chemistry has not gained much attention. However, a few reports[25–28] have indicated that replacement of aromatic groups by the ferrocenyl moiety in penicillin and cephalosporin antibacterials has drastically improved their antibacterial activity. Owing to this improved bactericidal activity upon replacement with the ferrocene or ferrocene-derived compounds, these studies also attracted the attention of the author to combine together the chemistry of ferrocene and such heterocycles that could give a new class of ferrocene-based antibacterial compounds. In an effort to design the chemistry of such compounds, some previously[27,28] synthesized antibacterial ferrocene-based symmetric and unsymmetrical Schiff-base derivatives and their transition metal chelates having different metal ions (cations) but with the same anions have been studied and their antibacterial activity reported (Fig. 1).

In an extension to this, the author now wishes to report some novel symmetrically substituted copper(II) complexes of ferrocene-derived Schiff base ligands having the same copper(II) metal ion but different anions (sulfate, nitrate, acetate or oxalate) and has evaluated the effect of anions on the antibacterial properties against the bacterial species *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumonae*. These studies, have also introduced a new class of potential organometallic-based antibacterial compounds.

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EXPERIMENTAL

Methods and materials

All the solvents used were Analar grade. 1,1-Diacetylferrocene, 2-aminopyrazine, 2-aminopyridine and 2-aminothiazole were obtained from Merck. The copper(II) salts used were the sulfate, nitrate, acetate and oxalate. IR, ^{1}H NMR and ^{13}C NMR spectra were recorded on Perkin Elmer 283B and 300 MHz Varian XL-300 instruments. UV–Vis spectra were obtained on a Baush and Lomb spectronic 1001. Conductance of the metal complexes was determined in dimethylformamide (DMF) on a YSI-32 model conduct-

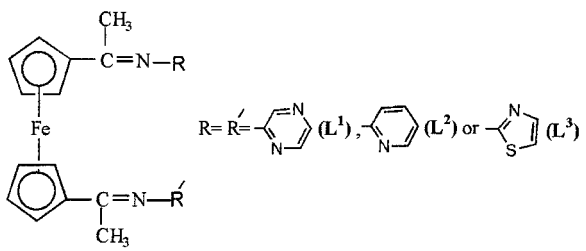

Figure 1. Structure of the Schiff-base ligands.

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Table 1. Physical, analytical and spectral data of metal complexes

Complex [mol. formula]	M. p. (°C)	IRν (cm^{-1})	λ_{max} (cm^{-1})	μ_{eff} (BM)	Calc. (Found) (%)		
					C	H	N
1 $[Cu(L^1)](NO_3)_2$ [611.38]	238–240	1560 (C═N), 1635 (C═N), 340 (M—N)	15 270, 19 610, 22 415, 30 420	1.7	43.2 (43.5)	3.3 (3.2)	18.3 (18.4)
2 $[Cu(L^1)](SO_4)$ [583.44]	230–232	1565 (C═N), 1635 (C═N), 340 (M—N)	15 290, 19 585, 22 415, 30 410	1.9	45.2 (45.4)	3.4 (3.8)	14.4 (14.1)
3 $[Cu(L^1)](C_2O_4)$ [575.38]	235–237	1560 (C═N), 1635 (C═N), 340 (M—N)	15 285, 19 590, 22 415, 30 365	1.8	50.1 (50.3)	3.5 (3.0)	14.6 (14.9)
4 $[Cu(L^1)](CH_3CO_2)_2$ [605.38]	240–242	1560 (C═N), 1635 (C═N), 340 (M—N)	15 270, 19 610, 22 415, 30 385	1.6	51.5 (51.7)	4.3 (4.2)	13.9 (14.1)
5 $[Cu(L^2)](NO_3)_2$ [609.38]	226–228	1565 (C═N), 1635 (C═N), 340 (M—N)	15 270, 19 605, 22 415, 30 390	1.7	47.3 (47.7)	3.6 (3.2)	13.8 (13.9)
6 $[Cu(L^2)](SO_4)$ [581.44]	222–224	1565 (C═N), 1635 (C═N), 340 (M—N)	15 270, 19 595, 22 415, 30 410	1.7	49.5 (49.3)	3.8 (3.6)	9.6 (9.3)
7 $[Cu(L^2)](C_2O_4)$ [573.38]	225–227	1560 (C═N), 1635 (C═N), 340 (M—N)	15 290, 19 590, 22 415, 30 415	1.9	54.4 (54.7)	3.8 (3.6)	9.80 (15.3)
8 $[Cu(L^2)](CH_3CO_2)_2$ [603.38]	228–230	1565 (C═N), 1635 (C═N), 340 (M—N)	15 285, 19 585, 22 415, 30 370	1.7	55.7 (55.5)	4.6 (4.8)	9.3 (9.5)
9 $[Cu(L^3)](NO_3)_2$ [621.50]	218–220	1565 (C═N), 1635 (C═N), 340 (M—N)	15 270, 19 610, 22 415, 30 385	1.8	38.6 (38.7)	2.9 (3.2)	13.5 (13.3)
10 $[Cu(L^3)](SO_4)$ [593.56]	215–217	1560 (C═N), 1635 (C═N), 340 (M—N)	15 290, 19 605, 22 415, 30 420	1.6	40.4 (40.5)	3.0 (3.2)	9.4 (9.3)
11 $[Cu(L^3)](C_2O_4)$ [585.50]	220–222	1565 (C═N), 1635 (C═N), 340 (M—N)	15 285, 19 580, 22 415, 30 425	1.7	45.1 (45.0)	3.1 (3.2)	9.6 (9.3)
12 $[Cu(L^3)](CH_3CO_2)_2$ [615.50]	223–225	1565 (C═N), 1635 (C═N), 340 (M—N)	15 270, 19 585, 22 415, 30 365	1.8	46.8 (46.9)	3.9 (3.5)	9.1 (9.3)

ometer. Magnetic measurements were done on solid complexes using the Gouy method. Microanalyses were carried out by Butterworth Laboratories Ltd. Melting points were recorded on a Gallenkamp apparatus and are uncorrected.

Antibacterial studies were carried out with the help of the Microbiology Department, Qaid-e-Azam Medical College, Bahawalpur, Pakistan. These studies were done on wild pathogenic bacterial species collected from urine and blood samples of infected patients admitted to Bahawal Victoria Hospital, Bahawalpur, Pakistan.

Synthesis of ligands

All the ferrocene-derived Schiff base ligands were prepared and characterized by a reported method.[27]

Synthesis of metal complexes

To a magnetically stirred and warmed solution of the ferrocene-derived Schiff base ligand (1.0 mmol) in ethanol (30 cm^3) was added a solution of the respective metal(II) salt [copper(II) sulfate, copper(II) nitrate, copper(II) acetate and copper(II) oxalate] (1.0 mmol) in ethanol (20 cm^3). The mixture was refluxed for 3 h. During this time a complex was precipitated; upon cooling, this was filtered, washed several times with warm ethanol and diethyl ether and then dried over anhydrous $CaCl_2$. All the complexes were prepared using the same method (Table 1).

Preparation of disc

The ligand/complex (30 μg) in DMF (0.01 cm^3) was applied to a paper disc [prepared from blotting paper (3 mm diameter)] with the help of a micropipette. The discs were left in an incubator for 48 h at 37 °C and then applied to the bacteria-containing agar plates.

Preparation of agar plates

Minimal agar was used for the growth of specific bacterial species. For the preparation of agar plates for *E. coli*, MacConkey agar (50 g), obtained from Merck Chemical Company, was suspended in freshly distilled water (1 l). It was allowed to soak for 15 min and then boiled on a water bath until the agar was completely dissolved. The mixture was autoclaved for 15 min at 120 °C and then poured onto previously washed and sterilized Petri dishes and stored at 40 °C for inoculation.

Inoculation procedure

Inoculation was done with the help of a platinum wire loop

Table 2. Antibacterial activity data

Ligand/complex	*E. coli*	*S. aureus*	*P. ceruginosa*	*K. pneumonae*
L^1	+++	++	++	++
L^2	+	++	-	++
L^3	+++	++	+++	++
1	++++	+++	+++	++
2	+++	+++	+++	++
3	+++	+++	++	+++
4	+++	++	+++	++
5	+++	++	+++	+++
6	++	+++	+	++
7	+++	+++	+++	+++
8	++	+++	++	+++
9	++++	+++	++	+++
10	+++	+++	+++	++
11	++	+++	++	+++
12	+++	+++	++++	+++

Inhibition zone diameter (% inhibition): +, 6–10 mm (27–45%); ++, 10–14 mm (45–64%); +++, 14–18 mm (64–82%); ++++, 18–22 mm (82–100%). Percent inhibition values are relative to inhibition zone (22 mm) of the most active compound with 100% inhibition.

that was made red hot in a flame, cooled and then used for the application of bacterial strains.

Application of discs

A sterilized forceps was used for the application of the paper disc to the already inoculated agar plates. When the discs were applied, they were incubated at 37 °C for 24 h. The diameter of the zone of inhibition was then measured around the disc (Table 2).

RESULTS AND DISCUSSION

Physical properties

The ligands are soluble in methanol and ethanol. All metal complexes dissolve only in DMF and dimethylsulfoxide. All of them are amorphous solids. Molar conductance values of metal complexes (73–78 $\Omega\,cm^2\,mol^{-1}$) in DMF solution show all the complexes to be electrolytic[29] in behavior.

IR spectra

The important IR frequencies of the ligands and some metal chloride complexes, along with their assignments, have already been reported[27] by us. The IR spectrum of the ligand is almost identical to those of its metal complexes in the region 640–1500 cm^{-1}, except that the band due to pyrazine, pyridine and thiazole ring vibration at ~1555 cm^{-1} assigned to C=N in the ligand moved to slightly higher frequency (5–10 cm^{-1}) in the spectra of its metal complexes. This, in turn, suggested[30,31] that coordination of the ligands took place through its pyrazine, pyridine and thiazole ring nitrogen atoms to the metal atom. Also, the shifting of the azomethine band to the higher frequency side (10–15 cm^{-1}) provided support to the involvement of an azomethine nitrogen to the metal atom. Moreover, in the far infrared region, the band at ~340 cm^{-1} attributed to ν(M—N) was observed for all the complexes (Table 1), but was not found in the spectra of the free ligands. This suggests that the ring nitrogen and azomethine nitrogen atoms are involved in the complex formation.

Electronic spectra and magnetic moments

The electronic spectra of the copper(II) complexes (Table 1) showed two low-energy weak bands at 15 270–15 290 and 19 580–19 610 cm^{-1} and a strong high-energy band at 30 365–30 425 cm^{-1}. The low-energy bands in this position typically are expected[33,34] for its square planar configuration and may be assigned to the $^2B_{1g} \rightarrow {}^2A_{1g}$ and $^2B_{1g} \rightarrow {}^2E_g$ transitions respectively.[35] The strong high-energy band, in turn, is assigned to a metal → ligand charge transfer. Also, the magnetic moment values (1.6–1.9 BM) (Table 1) for copper(II) complexes were found to be consistent[36] with the proposed square planar structure of copper(II) complexes (Fig. 2).

Furthermore, a broad band centered at 22 415 cm^{-1} observed for every complex was assigned to the transition $^1A_{1g} \rightarrow {}^1E_{1g}$ in the iron atom of the ferrocenyl group, which indicated[34] that there is no magnetic interaction between the copper (II), and the diamagnetic iron (II) ion of the ferrocenyl group. Similar structures were proposed for metal(II) chelates of L^2 and L^3, as shown in Fig. 2.

Antibacterial properties

Antibacterial properties of the ligands and their metal complexes were studied against bacterial species *E. coli, P. aeruginosa, S. aureus* and *K. pneumonae*. These were tested at a concentration of 30 μg/0.01 ml in DMF solution using a paper disc diffusion method devised and reported[37,38] earlier. The results of these studies (shown in Table 2) indicate that both the Schiff-base ligands and their metal complexes individually exhibited varying degrees of inhibitory effects on the growth of the bacterial species tested. The antibacterial results show that the activity of the compounds became more pronounced after chelation with the metal ion. When the same metal chelate having different anions was

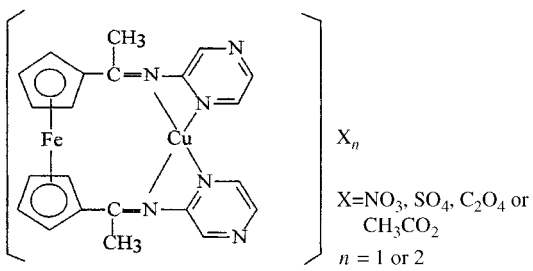

Figure 2. Proposed structure for metal(II) chelate of L^1.

individually screened, the degree of bactericidal activity/potency also varied. From the data obtained, it was generally observed that the order of potency of the Metal chelates with the various anions compared with the results for the metal chelate having a chloride anion, which were evaluated and reported previously[27] using the same bacterial species and conditions is as follows:

$$NO_3 > C_2O_4 > CH_3CO_2 > Cl > SO_4$$

On the basis of these results, it is suggested, therefore, that different anions affect the biological behavior of the metal chelates. It is expected, however, that factors such as solubility, conductivity, dipole moment and cell permeability mechanisms are also influenced by the presence of these anions in the chelate and may contribute in increasing this activity. These studies provide useful information about the biological activity of ferrocene-containing compounds. Also, the knowledge that this activity could become more pronounced by using more potent compounds coupled to the ferrocene molecule, and that anions which stay outside the coordination sphere do play an important role, thus introduces a further class of biologically active compounds.

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