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SYNTHESIS, SPECTROSCOPIC AND BIOLOGICAL STUDIES OF SOME MONO- AND DI-SUBSTITUTED HYDRAZINE-DERIVED FERROCENES

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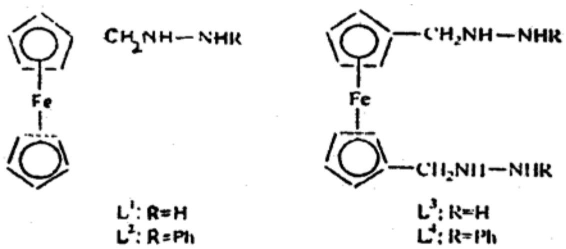
ABSTRACT

Some mono-and disubstituted hydrazine-derived ferrocenes have been synthesized and studied for their antibacterial properties. All these synthesized compounds have been characterized by their physical, analytical and spectral data, which introduces a novel class of ferrocene compounds. The antibacterial properties of these compounds have also been evaluated and thus a significant use of such compounds as antibacterial agent is reported.

INTRODUCTION

The versatile substituent chemistry of ferrocene is well documented (Singh and Singh, 1990; Hulton et al., 1990; Yongxiang and Gang, 1988). It has attracted the attention of many researchers and was used as a precursor for the preparation of coordination and biologically active compounds (Gang et al, 1988; Xiaoxian et al, 1992: Patil et al, 1983; Chohan and Praveen, 1990; Edwards et al, 1975). The strong relationship between metals or their complexes, and the biological activity (William, 1970; Williams, 1972; Srivastave, 1990) is already known. Many studies (Reslova, 1971; Seven and Johnson, 1960) have listed the metal complexes used in cancer therapy. An active research is, therefore, carried out in the area of designing the chemical structures of such analogously used compounds and their metal chelates. The metal complexes with organometallic compounds including the ferrocenyl ligands are of current interest (Patil et al; 1982; Iami and Ota, 1974). The enzyme inhibiting properties of hydrazine and hydrazones are well re-ported (Craiz et al, 1955). In an effort to combine the chemistry of ferrocene and hydrazine, we wish to report some mono-substituted (L^1 and L^2) and 1.1'-disubstituted (L^3 and L^4) hydrazine-derived ferrocenes which can be potentially used in further designing and preparation of many other such useful compounds. These prepared frrocenyl compounds have been characterized by their physical, analytical and spectral data. Enhanced antibacterial activity of penicillin and cephalosporine has been reported by replacing the aromatic group with ferrocenyl moiety. In the light of which this title compounds have been studied for their antibacterial properties in comparison to the effect of their substitution as mono- and di- substituents, against bacterial strains *Escherichia coli, Staphylococcus aureous* and *Pseudomonos aeruginosa.*



(Fig 1) Structure of the Compounds

EXPERIMENTAL

Material and Methods:

All chemicals and solvents used were of analytical grade. IR and NMK spectra were recorded on Philips Analytical PU 9800 FTIR spectrophotometer and on a Brucker 250 MHz spectrometer. UV- Visible spectra were obtained on a Hitachi U-2000 double-beam spectrophotometer. Conductance of the metal complexes was determined in DMF on 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:

1-Hydraztnomethyljerrocene (L^1)

1-Hydroxymethylferrocene (0.64 g, 3.0 mmol) was converted into its chloro derivative by a literature method (Gronowitz and Liljefors, 1979). 1-Chloromethylfcrrocene in anhyrous dichloromethance (20 ml) was added to anhydrous hydrazine (0.1 g, 3.0 mmol) in dichloromethane (20 ml). The reaction mixture was refluxed for 6 h under N_2. After allowing to cool at room temperature, the solvent was evaporated to give an orange solid which was chromatographed over a silicagel column using dischloromethane/petroleum ether (b.p.40-60°C) (70 : 30) as eluent. After removing the solvent, orange crystals were obtained which were recrystalized from dichloromethane to yield L^t (72%). M.P. I78°C. IR (KBr, cm^{-1}).3215 (NW), 3080 (NH), 1695, 1575, 1550 (C=C), 1425, 1325, 1110, 1015, 955 (N-N). ^1H NMR (DMSO-d_6, 250 MHz) δ 3.5 (s, 2H, CH_2N), 3.9 (s, 5H, unsubstituted Cp), 4.4.4.5 (m, 2H, ferrocenyl), 4.6-4.8 (m, 211, ferrocenyl), 5.1 (s, 1H, NH), 8.8 (s, 2H, NH2). ^{13}C NMR (DMSO-d_6, 63 MI Iz) δ 52.4 CH_2), 58.6, 69.7, 70.3, 84.4 (ferrocenyl). Analysis Found C, 57.4; H, 6.0; N, 12.2 Calculated for $C_{11}H_{14}FeN_2$ C, 57.6; H, 5.8; N, 12.4%.

1-Phenylhydrazinomethylferrocene (L^2)

1-Phenylhdrazinomethylferrocene (L^2) was prepared analogously: 1! (78%). M.P. 183°C. IR (KBr, cm^{-1}) 3215 (NW), 3080 (NH), 1695, 1575. 1550 (C=C), 1425, 1325, 1250.1110, 1075, 1015, 955 (N-N). ^1H NMR (DMSO-d_6, 250 MHz) ^1HNMR (DMSO-d_6, 250 MHz) δ 3.6 (s, 2H, CH_2N), 3.9 (s, 5H, unsubstituted Cp), 4.5-4.6 (m, 2H, ferrocenyl), 4.7-4.9 (m, 2H, ferrocenyl), 5.3 (s, 1H NH), 6.7-6.9 (m, 2H, aromatic), 7.4-7.5 (m, 2H, aromatic), 7.8-7.9 (m, 1H, aromatic). ^{13}C NMR (DMSO-d_6, 63 MHz) δ 52.5 (CH_2), 58.7, 69.8, 70.3, 84.5 (ferrocenyl, 125.7, 130.4, 135.6, 144.3 (aromatic). Analysis Found C, 67.8; H, 5.8; N, 13.5 Calculated for $C_{24}H_{26}FeN_4$ C, 67.6; H, 6.1; N. 13.2%.

1,1 -Bis(hydrazinomethy/) ferrocene (L^3)

1, 1'-Bis (hydroxymethyl)ferrocene (0.73 g, 3.0 mmol) was convened analogously to 1,1'-bis (hydrazinomethyl)ferrocene (L^3): (82%). M.P. 197°C. IR (KBr, cm^{-1}) 3215 (NH_2), 3080 (NH), 1685, 1585, 1550 (C=C), 1425, 1110, 1015, 955 (N-N). ^{1}H NMR (DMSO-d_6, 250 MHz) δ 3.5 (s, 4H,CH_2N), 4.5 (dd, 4H, ferrocenyl), 4.8-4.9 (m. 4H, ferrcenyl), 5.1, (s, 2H,NH), 8.8 (s, 2H, NH_2). ^{13}C-NMR (DMSO-d_6, 63 MHz) δ 52.4 (CH_2), 69 7, 70.3. 84.4 (ferrocenyl). Analysis Found C, 52.8; H, 6.9, N, 20.1 Calculated for $C_{12}H_{18}FeN_4$ C, 52.6; 6.6; N, 20.4%.

1,1'-Bis(pheny1hydrazinomethvl)ferrocene (L^4)

1,1'-Bis(phenylhdrazinomethyl) ferrocene (L^4) was prepared similarly to yield L^4: (80%). P. 193°C. IR (KBr, cm^{-1}) 3085 (NH), 1680, 1585, 1555, (C=C). 1425, 1265, 1110, 1015, 915, 955 (N-N). ^{1}H NMR (DMSO-d_6 250 MHz) δ 3.6 (s, 4H, CH_2N), 4.6 (dd, 4H, ferrocenyl), 4.8-4.9 (m, 4H, ferrocenyl), 5.2 (s, 2H NH), 6.7, 6.9 (m, 4H, aromatic), 7.3-7.5 (m, 2H, aromatic), 7.8-7.9 (m, 4H, aromatic). ^{13}C-NMR (DMSO d_6, 63 MHz) δ 52.5 (CH_2). 69.8, 70.3, 84.5 (ferrocenyl, 125.7, 130.4, 135.6, 144.3 (aromatic). Analysis Found C, 67.8; H, 5.8; N, 13.5 Calculated for $C_{24}H_{26}FeN_4$ C. 67.6; H, 6.1; N. 13.2%.

ANTIBACTERIAL STUDIES

Preparation of a Disc:

The ligand/complex (30 μg) in DMF (0.01 ml) was applied on 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 on the bacteria grown agar plates.

Preparation of Agar Plate:

Minimal agar was used for the growth of specific bacterial species. For the preparation of agar plates for *Escherichia coli,* MacConkey agar (50 g), obtained from the Merck Chemical Company, was suspended in freshly prepared distilled water (I L). It was allowed to soak for 15 minutes 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 into previously washed and sterilized Petri dishes and stored at 40°C for inoculation.

Procedure of Inoculation:

Inoculation was done with the help of a platinum wire loop which was made red hot in a flame, cooled and then used for the application of bacterial strains.

Application of Disc:

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

RESULTS AND DISCUSSION

1-Hydroxymethylferrocene and 1,1',-bis(hydroxymethyl) ferrocene were converted to their corresponding 1-chloromethylferrocene and I,1'-bis (chloromethyl) ferrocene derivatives using thionyl chloride in trimethylamine. This was followed by an *in situ* reaction with the respective hydrazines. The hydrazine-derived products were obtained as orange needles after purification by chromatography in dichloromethane/pet.ether on a silicagel column followed by recrystallization in dichloromethane.

The structural assignment of the synthesized hydrazine-derived ferrocenes was done on the basis of their IR, ^{1}H NMR, ^{13}C NMR and analytical data. The title compounds are all air and moisture stable amorphous solid under laboratory conditions. They decompose without melting. They are soluble in polar solvents such as methanol, ethanol, acetonitrile and DMF, but insoluble in weakly polar or non-polar solvents. The elemental analysis data agree well with their expected formulation.

Infrared Spectra:

Some tentative assignments (Yongxiang et al, 1989; Burger et al, 1965) and important chacteristic bands of the title compounds were observed at -3215, 3080, 1545 and 955 cm^{-1} assigned to v (- NH_2), v(NH), v(-C=C) and v(N-N) frequencies. The characteristic bands for the ferrocenyl group appearing in the mono-and di- substituted compounds remained almost unchanged (Pelizzi and Pelizzi, 1976; Nakamoto, 1970) or a very title shift was only noticed.

NMR Spectra

The ^{1}H NMR and ^{13}C NMR spectra of the title compounds have been recorded in DMSO-d6 with TMS as internal reference. The present data on comparison support the conclusions derived from the reported similar)/substituted ferrocene compounds. All these compounds exhibit two peaks in the ^{1}H NMR spectra at δ~3.5 ppm (s) assigned to proton signals of methylene substituted to cyclopentadienyl ring system of ferrocene moiety. Furthermore, two peaks were found at δ~4.5 ppm (singlet) and δ~4.8 ppm (singlet) assigned to substituted cyclopentadienyl rings of ferrocene and at δ~3.9 ppm (singlet) for unsubstituted cyclopentadiene ring in mono-substituted compounds (Rausch and Siegel, 1969). Aromatic proton signals appeared at 6-7.1-7.8 ppm as multiplet. Signals of the NH_2 protons were found at δ~8.8 ppm (singlet) and for NH protons at δ~5.1 ppm (singlet). Similarly, ^{13}C NMR exhibited signals at δ~52.5 due to methylene carbon and at δ ~69.8, 70.4 and 84.3 ppm assigned (Pasto and Johnsons, 1969) to ferrocenyl carbons. Aromatic carbon signals were also found to appear at δ~125.7, 130.2. 135.6 and 144.2 ppm values.

Antibacterial Properties:

Antibacterial properties of the title hydrazine-derived ferrocenes were studied against bacterial species *Escherichia coli, Pseduomonas aeruginosn, Staphylo*coccus *aureus* and *Klebsiella pneumonae.* These were tested at a concentration of 30 µg/0.01 mL in DMF using a paper disc diffusion method devised and reported (Mercimek and Irez, 1995; Chohan, 1999, Chohan and Praveen, 1999; Chohan et al, 1989) earlier. The results of these studies reproduced in Table 1 indicates that title compounds showed variable activity against one or more bacterial strains. On comparison to the mono-substituted compounds (L^1 and L^2) the di-substituted (L^3 and L^4) were found to be more antibacterial obviously because of the attachment of two hdrazine groups to the single ferrocene moiety.

Table 1
Antibacterial Activity Data of the Ligands

Ligand/	Microbial Species			
	a	b	c	d
L^1	++	+	-	++
L^2	++	+	+	++
L^3	++++	+++	++	+++
L^4	+++	++	++	+++

a=Escherichia coli b-*Staphylococcus aureus*
c=Pseduomonas aeruginosa *d-Klebsiella pneumonae*

Inhibition zone diameter mm/(% inhibition): +5-10 (25-45%); ++, 10-15 (45-65%); +++, 14-18(65-85%); ++++, 18-22 (85-100%). Percent inhibition values are relative to inhibition zone (22 mm) of the most active compound with 100% inhibition.

It is evident that it may enhance the lock geometry system of the di-substiuted compounds more effectively to kill more bacteria. These studies however, provided a useful information about the biological activity of ferrocene-containing compounds and the knowledge that this activity could become more pronounced when potent compounds are coupled with ferrocene using both cyclopentadienyl ring systems.

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