

In-vitro Antimicrobial Screening Of Ferrocene Derived Compounds

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Abstract: Two ferrocene containing compounds have been prepared and characterized by elemental analysis, ¹H NMR and UV-Vis spectra and electrochemical measurements. The antimicrobial properties of these compounds have also been evaluated against the test strains (*Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*) and thus a significant use of such compounds as antibacterial agent is reported. The activity data show that the present compounds are found to have greater antibacterial and antifungal activity than the respective standards.

Keywords: ferrocene, antimicrobial, antifungal, test strains.

1. INTRODUCTION

The versatile substituent chemistry of ferrocene has attracted the attention of many researchers and was used as a precursor for the preparation of coordination and biologically active compounds. Medicinal chemists are also open to the inclusion of ferrocene into their drug design strategies because of the novelty introduced by its presence. Ferrocene is a stable, nontoxic compound and having good redox properties. Now research is going on to design new compounds which are active against a wide range of cancers and have lesser side effects. Ferrocenes are also known to exhibit a wide range of biological activity and also ferrocene has attracted special attention since it is a neutral chemically stable and

non-toxic molecule. Incorporation of a ferrocene fragment into a molecule of an organic compound often obtained unexpected biological activity, which is rationalized as being due to their different membrane permeation properties and anomalous metabolism¹. Moreover, the stability and nontoxicity of the ferrocenyl moiety is of particular interest rendering such drugs compatible with other treatment². In this sense, the integration of one or more ferrocene units into a heterocyclic ring molecular has long been recognized as an attractive way to endow a novel molecule functionally. Many ferrocenyl compounds display interesting, cytotoxic, antitumor, antimalarial, antifungal and DNA cleaving activities. Many studies have highlighted³⁻⁶ an extensive use of ferrocene

and ferrocene-containing molecules in substituent and supramolecular chemistry, while the application of ferrocene compounds in medicinal chemistry has not been investigated at a large scale although few reports⁷⁻⁸ have indicated that the replacement of aromatic group by the ferrocenyl moiety in penicillin and cephalosporines improve their antibiotic activity. Considering that interesting redox-active properties⁹⁻¹⁰ due to Fe^{II}-Fe^{III} already exist in ferrocene molecules, we thought it now, worthwhile to combine both the chemistry of ferrocene and already known¹¹⁻¹³ biologically active imidazole containing compounds and explore their biological properties induced by coupling with ferrocenyl group. For this purpose we have synthesized and characterized some ferrocene-derived compounds (1 and 2) (Scheme. 1) and wish to report their biological properties in this paper, which may provide a useful information and may serve as a novel potential area of research which has been ignored before.

2. EXPERIMENTAL SECTION

Materials and methods

Ferrocene carboxaldehyde, biacetyl, piperidine, salicylaldehyde and 3,5-dichlorosalicylaldehyde were purchased from Sigma-Aldrich. Acetic acid, Ammonium acetate, methanol, acetonitrile and ethanol were purchased from SD Fine chemicals. Absorption spectra were recorded on Shimadzu UV-160A UV-Visible spectrophotometer; emission spectra were obtained by using Varian Cary Eclipse spectro fluorometer. ¹H NMR spectra were measured on a JEOL 500 MHz spectrometer. Cyclic voltammetry (CV) and Differential Pulse Voltammeteries (DPV) were performed by using CH instrument (USA) model CH-620 B electrochemical analyzer. A conventional three electrode system consisting of platinum disc as a working electrode, platinum wire as an auxiliary electrode and saturated calomel as a reference electrode was used for the electrochemical measurements. The solution was purged with nitrogen for 10 minutes before each experiment. 0.1 M tetrabutyl ammonium perchlorate (TBAP) was used as the supporting electrolyte for all the experiments. The cyclic voltammograms were recorded at a scan rate of 0.10 Vs⁻¹ and scanning was done from 0 to +0.8 V. The DPV were recorded from 0 to +0.8 V with increasing potential of 4 mV, pulse width of 50 ms and sampling width of 16.7 ms. Microanalyses were performed at Sophisticated Test and

Instrumentation Centre (STIC), Cochin University, Kerala.

2.1 Synthesis of (1E,5E)-1,6-bisferrocenyl-hexa-1,5-diene-3,4-dione

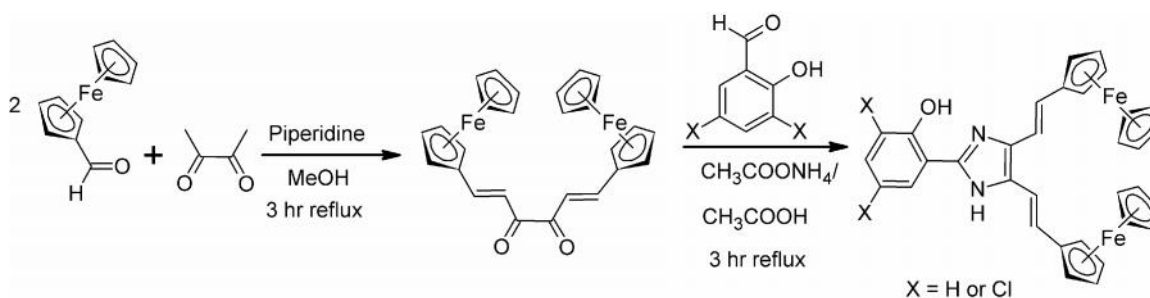
This molecule was synthesized by adopting literature procedure¹⁴. To a stirred solution of piperidine (2.0 mL, 20 mmol) in 30 mL of methanol, ferrocene aldehyde (10.7 g, 50 mmol) was added and heated to reflux. To this, hot methanolic solution of 2,3-butanedione (1.72 mL, 20 mmol) was added slowly (3 h). Refluxing was continued for another 3 h. The solution was cooled to room temperature and kept in refrigerator overnight. The dark violet precipitate was filtered washed with cold ethanol (3 X 15 mL) and dried to yield (1E,5E)-1,6-bisferrocenyl-hexa-1,5-diene-3,4-dione (Yield 7.56 g, 79 %), mp 200-202 °C; ¹H NMR (500 MHz, CDCl₃) 4.18 (s, 10H), 4.54 (s, 4H), 4.61 (s, 4H), 7.00 (d, 2H, ³J=16Hz), 7.79 (d, 2H, ³J=16Hz). ¹³C NMR (500 MHz, CDCl₃) 69.7 (CH), 70.2 (CH), 72.3 (CH), 78.7 (q), 117.4 (CH), 150.6 (CH), 189.2 (q). GCMS: m/z (relative intensity): 479 (40, M⁺). Anal. Calcd for C₂₆H₂₂Fe₂O₂: C, 65.31; H, 4.64. Found: C, 65.29; H, 4.70.

2.2 Synthesis of 2-(4,5-bis((E)-2-ferrocenyl vinyl)-1H-imidazole-2-yl)phenol, 1

(1E,5E)-1,6-bisferrocenyl-hexa-1,5-diene-3,4-dione (0.5 g, 1.04 mmol), salicylaldehyde (0.13 g, 1.04 mmol) and ammoniumacetate (2 g, 25 mmol) were dissolved in 15 mL acetic acid and heated to reflux for 3 h¹⁵. After cooling, cold water (10 mL) was added to the solution, during which orange precipitation appeared which was filtered using cold water (2 X 10 mL) and purified by column chromatography on silica using ethyl acetate:hexane (1:4) as an eluent. (Yield 0.15 g, 25 %).

2.3 Synthesis of 2-(4,5-bis((E)-2-ferrocenyl vinyl)-1H-imidazole-2-yl)-4,6-dichlorophenol, 2

An analogous synthetic procedure using 3,5-dichlorosalicylaldehyde instead of salicylaldehyde was used to prepare 2-(4,5-bis((E)-2-ferrocenylvinyl)-1H-imidazole-2-yl)-4,6-dichlorophenol (Yield 0.14 g, 24 %), ¹H NMR (500 MHz, CD₃OD) 4.15 (s, 10H), 4.30 (s, 4H), 4.57 (s, 4H), 6.82 (d, 2H, ³J=16Hz), 6.93 (d, 2H, ³J=16Hz), 6.97 (d, 1H, J=8Hz). ¹³C NMR (500MHz, CDCl₃) 66.5 (CH), 68.8 (CH), 68.9 (CH), 78.7 (q), 117.4 (CH), 150.6 (CH), 189.2 (q).

Scheme 1. Synthetic scheme of compounds 1 and 2.**2.4 Antimicrobial studies****Preparation of a Disc:**

The compound (30 μg) in DMSO (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 (1 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.

3. RESULTS AND DISCUSSION**3.1 Synthesis of the compounds 1 and 2.**

Ferrocene substituted diketone was condensed with salicylaldehyde in the presence of ammonium acetate (Scheme 1). Acetic acid served as solvent as well as cyclization catalyst for the imidazole formation¹⁶.

Table 3.1. Microanalytical data of the compounds

Compound	Colour	Empirical formula	Melting Point °C	Elemental analysis Calculated (found) (%)		
				C	H	N
1	Orange	C ₃₃ H ₂₈ Fe ₂ N ₂ O	122-124	68.30(68.23)	4.86(4.91)	4.91(4.83)
2	Orange	C ₃₃ H ₂₆ Cl ₂ Fe ₂ N ₂ O	180-183	61.06(61.02)	4.04 (4.12)	4.32(4.36)

Compounds **1** and **2** were obtained in moderate yield after column chromatography on silica gel using 1:4 ethylacetate:hexane as an eluent. Synthesized compounds which were subjected to TOF mass spectrometry showed base peaks of protonated adduct at 581 and 649 for compounds **1** and **2** respectively. Microanalytical data of the present compound **1** and **2** are given in **Table 3.1**.

3.2 Electronic Spectra

Electronic spectra of the compounds were taken in acetonitrile. The two d-d bands of ferrocene at 324.7 and 440.0 nm were considerably shifted for the synthesized compounds and the molar extinction coefficient of the transitions were low due to the forbidden nature of d-d transition of the highly symmetric ferrocene¹⁷. Ferrocenediketenone showed a maximum wavelength shift of 110.5 nm from the lowest energy d-d transition and a considerable increase in the molar extinction coefficient was also observed. Both the d-d bands of compounds **1** and **2** are found to be red shifted when compared to the parent ferrocene. (**Table 3.2**), (**Figure.1**) Molar extinction coefficient of the d-d bands of these two compounds are considerably higher than that observed for ferrocene. This is because the synthesized ferrocenediketenone and compounds **1** and **2** have low symmetry and so as expected, the d-d bands of these compounds are found to be

much intense compared to that of ferrocene. As these compounds have extended conjugation, d-p mixing which will again lead to increase in the intensity of the d-d transitions could also be expected.

3.3 Electrochemical measurements

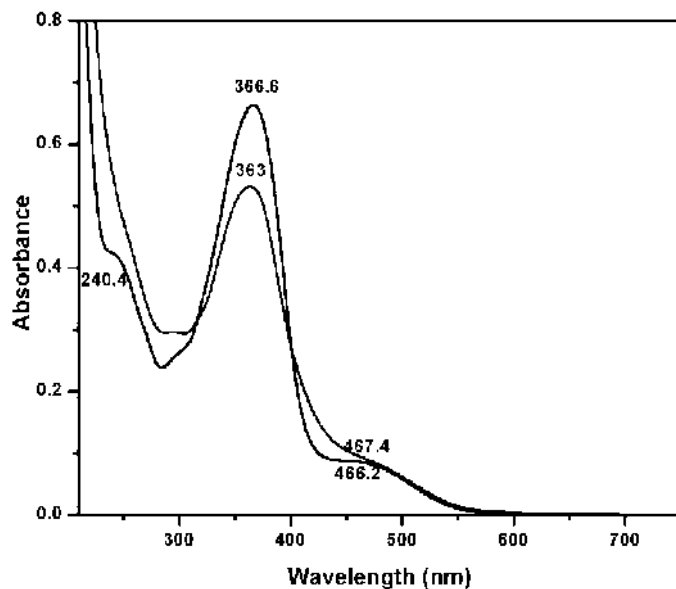
The compounds in acetonitrile showed only one oxidation peak and the corresponding reduction peak was observed when the sweep was reversed. In non-aqueous solutions, it is commonly observed that E_p values lie between 70 and 100 mV owing to the IR drop resulting from the uncompensated and relatively large solution resistance. It is more convenient to compare the measured E_p with that of a known reversible reaction measured under similar conditions than the not much reliable IR compensation techniques. The anodic and cathodic current ratio was close to unity in the case of the diketone indicating that the redox process is reversible in this case. Both the compounds on the other hand showed quasi reversible redox process in CV.

Table 3.2. Electronic Spectral data (λ_{\max} in nm; ν_{\max} in $M^{-1} cm^{-1}$ in paranthesis) for the compounds in acetonitrile.

Compounds	λ_{\max} in nm ($\nu_{\max}, M^{-1} cm^{-1}$)
1	202.0 (54000)
	366.6 (33200)
	466.2 (4200)
2	203.0 (63650)
	363.0 (26600)
	467.0 (4550)

Table 3.3. Redox potential and peak separation values for synthesized compounds

	$E_{1/2}$ (V) vs SCE	E (mV)
Ferrocene	0.450	70
Fcketenone	0.572	71
1	0.401	121
2	0.417	131

Figure.1. UV-visible spectra for compounds 1 and 2.

Under our experimental conditions, ferrocene showed the redox peak with $E_{1/2}$ of 450 V with the cathodic and anodic peak separation of 70 mV. (Table 3.3), (Figure.2) The starting material with non conjugated system showed redox peak with $E_{1/2}$ of 0.572 V, which is more positive compare to its parent compound ferrocene at the same experimental condition. Compounds **1** and **2** were easily oxidisable compared to their starting materials ferrocene and ferrocenediketenone.

This could be due to the high conjugation of ferrocene unit with imidazole phenolic group as

well as the large electron density around the ferrocene system. Looking at the oxidation potentials of the two compounds **1** and **2**, the higher electron withdrawing nature of chlorine atom in phenolic counterpart of the receptor **2** resulted in an increase in its oxidation potential, when compared to that of **1**. Separation of the cathodic and anodic peaks for the synthesized receptors are slightly more compared to the acceptable limit of 70-100 mV for non-aqueous system.

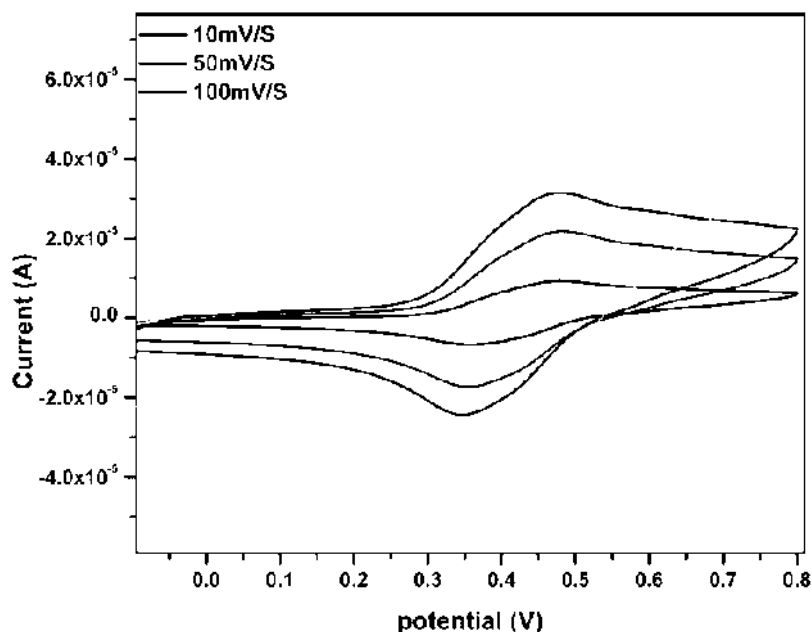
Figure.2. Cyclic Voltammogram for compound 2.

Table.3.4. Antimicrobial activity (Zone of Inhibition) of compounds 1 & 2

Test compound	Antimicrobial activity								
	<i>C. albicans</i>			<i>E. coli</i>			<i>P. aeruginosa</i>		
	0.25 %	0.5 %	1 %	0.25 %	0.5 %	1 %	0.25 %	0.5 %	1 %
1	28	32	31	17	20	20	18	22	25
2	24	27	25	13	16	17	16	21	25
Standard (Chloramphenicol)	17			16			20		

Values of zone of inhibition [mm, including the diameter of the disk (6 mm)]

3.4 Antimicrobial Studies

Anti-microbial activity of the present compounds were explored by determining zone of inhibition (Disc Diffusion Tests) using chloramphenicol as reference standard (**Table 3.4**) at 0.25 %, 0.50 % and 1 % concentration. The observed order of zone of inhibition was *Candida albicans* > *Pseudomonas aeruginosa* > *Escherichia coli*. The compounds showed variable in-vitro antimicrobial activities against test strains. Out of the two compounds **1** and **2**, the antimicrobial activity of **1** is found to be more effective than that of **2**. This may be due to presence of two electron withdrawing chlorine atoms in compound **2**.

According to the overtone concept of cell permeability, the lipid membrane that surrounds the cell favors the passage of only lipid-soluble materials in which liposolubility is an important factor that controls the antimicrobial activity. It is likely that the increased liposolubility of the present compounds may contribute to its facile transport into the bacterial cell, thereby disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of the organism. The variation in the effectiveness of different compounds against different organisms depends either on the impermeability of the cells of the microbes or on differences in ribosome of microbial cells. These studies however, provided a useful information

about the biological activity of ferrocene-containing compounds and the knowledge that this activity/potency could become more pronounced when more potent compounds are coupled with ferrocene molecule and thus introduce a new potential class of biologically active compounds.

4. CONCLUSIONS

In the present work ferrocene containing compounds **1** and **2** have been isolated and characterized. The electrochemical properties of the compounds have also been studied in acetonitrile solution using cyclic and differential pulse voltammetric techniques with a view to relate the electrochemical behavior to the structural aspects and biological reactivity of the complexes. The electronic spectral properties of the compounds have also been studied. These complexes were also subjected to find out their antimicrobial activity and the compound **1** exhibits greater activity than the compound **2** and also both the compounds show greater activity than the respective standards.

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