Synthesis and Characterization of Ferrocenyl Esters: Their Anti-Oxidant Activity and DNA-Binding Ability

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(Received on 12th May 2017, accepted in revised form 13th October 2017)

Summary: In continuation to our work on biologically active material, some new ferrocenyl esters were prepared by condensation of 4-ferrocenyl phenol (ROH) with ferrocenyl dicarboxylic acid chlorides (DCC) at low temperature. The synthesized compounds (E1-E9) were characterized by analyzing their physical properties, FT-IR, ^{1}H-NMR, UV-visible spectroscopic and cyclic voltammetric studies. The DPPH free radical scavenging assay was performed to explore their potential as antioxidant which showed that the E1 had maximum scavenging ability (77.11%) whereas E3 showed minimum (52%) compared with the standard. Cyclic voltammetric studies indicated that these compounds were electroactive in potential window of 2.0-0.0V. Binding mode found in these esters was an electrostatic interaction which is considered as strongest amongst all. Therefore, these compounds are considered to have DNA-binding capability and are potential DNA binders.

Keywords: Ferrocenyl esters, Antioxidant activity, DNA binding ability, Synthesis, Characterization.

Introduction

Since their discovery, organometallic compounds have drawn much attention from researchers all over the world [1]. Discovery of ferrocene has greatly affected the research in advanced organometallic materials. Thermal stability, resistance to the acid and bases [2], electrochemical activity, non-toxicity [3], diverse coordination number and geometries [4, 5] make ferrocene the most widely studied compound in various areas of research for their potential [6-8] applications in organic synthetic methodology, anti-cancer drugs [8, 9] and electrochemical devices [10-13].

Based on their resilient nature in aerobic and aqueous media, lower cytotoxicity in biological systems, lipophilic character and specially reversible redox behavior, these compounds have been exploited extensively for their increased bioactivities [7, 13] and DNA-binding capabilities and thus have potential to be used as drugs [13].

Flourescence [14] and UV-visible spectroscopic techniques [15- 17] have been frequently used to study these phenomena. Recently electrochemical methods [18-21] have also been developed to study DNA interaction as they are simple, rapid and sensitive in nature.

In the presented study, new ferrocenyl esters were synthesized by employing low temperature condensation reaction and studied by UV-visible spectroscopic and cyclic voltammetric methods for the DNA-binding capability of the compound. The DPPH free radical scavenging assay was used for the purpose of evaluating the anti-oxidant behavior of the prepared ferrocenyl esters.

Experimental

Materials

Ferrocene (m.p=172.5°C), hexadecyltrimethyl ammonium bromide (m.p=248-251°C), terephthaloyl chloride (m.p=81.5-83°C), triethyl amine (b.p=88.6-89.8ºC) and n-hexane (b.p= 68.5-69.1°C) were purchased from Fluka, Switzerland. Thionyl chloride (b.p=79°C), Ethanol (b.p=78.1°C), dichloromethane (b.p=39.6ºC), diethyl ether (b.p=−23°C), methanol (b.p=64.7ºC) and dimethylsulfoxide (m.p=189°C) were procured from Merck, Germany; tetrahydrofuran (b.p=66°C) was obtained from Riedel-deHaan, Germany.

Analytical Techniques

Melting point was determined using open capillary tubes on melting point apparatus, SMP10 (Stuart). TLC was carried out using pre-coated kieselgel 60-HF TLC platter. The Fourier transform infrared spectra were taken on a Perkin Elmer Spectrum One (Ver.B) FTIR Spectrophotometer. UV-Visible 1601 Shimadzu Spectrophotometer was used for UV-visible studies. ^{1}HNMR spectrum was recorded on Bruker 300 MHz ultrashield Spectrophotometer. The cyclic voltammograms were obtained using Eco Chemie Auto lab. PGSTAT 12 potentiostat/galvanostat instrument.

Synthesis

The methods used for the synthesis are given below.

Synthesis of4-ferrocenyl phenol (ROH)

4-Ferrocenyl phenol was synthesized by a method given in literature, scheme 1 [21].

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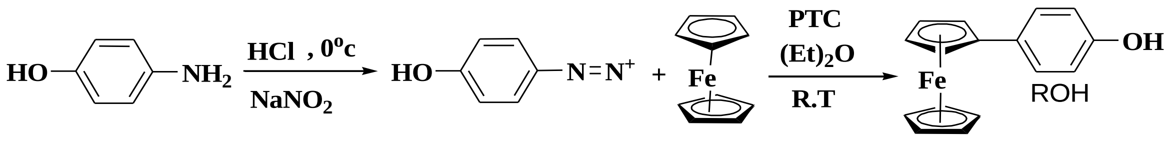


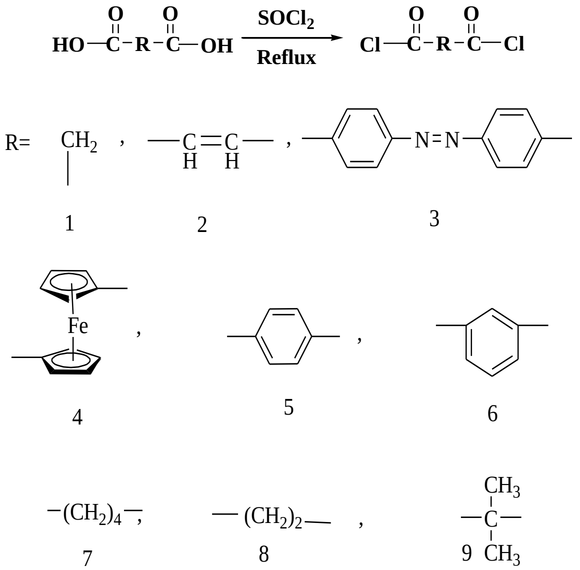
Scheme-1: Preparation of 4-ferrocenyl phenol (ROH).

Synthesis of dicarboxylic acid chloride (DCC)

Stoichiometric amount of dicarboxylic acid (DCA) and thionyl chloride were added in a 250 mL three-necked round bottom flask equipped with a condenser and a magnetic stirrer (under inert environment). Few drops of DMF were added to the flask to facilitate the reaction. The reaction mixture was stirred for an hour at room temperature and then refluxed for 24 hours. After that, thionyl chloride was evaporated under vacuum to obtain the product which was then used further in in-situ reactions.

Synthesis of ferrocenyl esters **(E1-E9)**

0.9 mmol of diacid chloride was dissolved in THF at 0-5°C in a two-necked round bottom flask fitted with reflux condenser and magnetic stirrer under inert environment. (1.8 mmol) ROH was dissolved in 10 mL of THF and then was added dropwise to the reaction flask. After complete addition, 10 mL of triethylamine was added to the reaction mixture at low temperatures and then it was allowed to stir for 24 hours. The progress of reaction was monitored by using TLC method. When the reaction was completed, the product was filtered, washed and recrystallized.



Scheme-2: Preparation of dicarboxylic acid chlorides.

2.3.4 Synthesis of bis(4-ferrocenylphenyl) malonate **(E1)**

4-Ferrocenyl phenol (0.5 g, 1.80 mmol) dissolved in 10 mL THF alongwith 10 mL triethylamine and malonoyl chloride (0.18 g, 1 mmol) were made to react according to the general procedure as mentioned above to get E1.

Yield 60 %, Mp 191-194 °C. FTIR (cm^{-1}): 1710 ($\upsilon_{C=O}$), 3010 (υ_{C-H})$_{aromatic}$, 473 (υ_{Fe-cp}), 1250 (υ_{C-O}).^{1}H NMR (DMSO-d6, ppm): 4.01 (Cp-ring, singlet), 4.38-4.29 and 4.86-4.37 (Cp-ring, triplet), 7.03-6.95(phenyl, multiplet), 3.19(CH$_2$, singlet). ^{13}C NMR (DMSO-d$_6$, ppm): 137 (Cp), 168 (C=O), 129, 121, 135, 148 (Cp-phenyl).

Synthesis of bis(4-ferocenylphenyl) but-2-enedioate **(E2)**

4-Ferrocenyl phenol (0.5 g, 1.80 mmol) in 10 mL THF, 10 mL triethylamine and fumaryl chloride (0.18 g, 1 mmol) were made to react according to the general procedure as mentioned above to get E2

Yield57 %, Mp 109-111 °C. FTIR (cm^{-1}): 1695 ($\upsilon_{C=O}$), 3040 ($\upsilon_{C-H)}$ $_{aromatic}$, 496 (υ_{Fe-cp}), 1269 (υ_{C-O}).^{1}H NMR (DMSO-d$_6$, ppm): 4.08 (Cp-ring, singlet), 4.41-4.33 and 4.89-4.41 (Cp-ring, triplet), 7.03-6.95(phenyl, multiplet), 6.49(CH=CH, singlet).^{13}C NMR (DMSO-d$_6$, ppm): 138 (Cp), 130, 122, 138, 148 (Cp-phenyl), 165(C=O), 135 (CH$_2$)

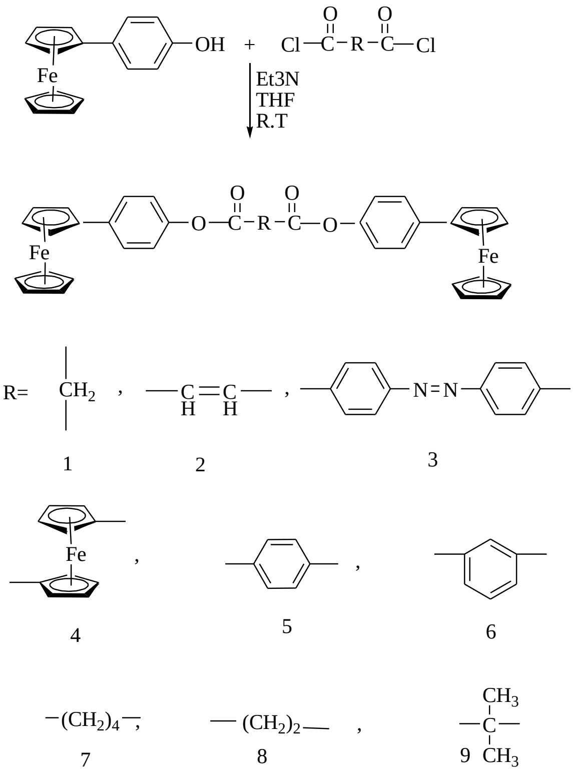
2.3.6 Synthesis of bis(4-ferrocenylphenyl) trans-azobenzene-4,4'-dicarboxylate **(E3)**

4-Ferrocenyl phenol (0.5 g, 1.80 mmol), 10 mL THF, 10 mL triethylamine and trans-azobenzene-4,4'-dicarbonyl chloride (0.18 g, 0.6 mmol) were made to react together to obtain E3.

Yield 61 %, Mp 281-283 °C. FTIR (cm^{-1}): 1725 ($\upsilon_{C=O}$), 3038 (υ_{C-H})$_{aromatic}$, 506 (υ_{Fe-cp}), 1243 (υ_{C-O}). FTIR (cm^{-1}): 1725 ($\upsilon_{C=O}$), 3028 (υ_{C-H})$_{aromatic}$, 475 (υ_{Fe-cp}), 1196 (υ_{C-O}).

^{1}H NMR (DMSO-d6, ppm): 4.18 (Cp-ring, singlet), 4.48-4.40 and 4.98-4.47 (Cp-ring, triplet), 7.33-7.15(multiplet), 8.34-8.08 (multiplet).

^{13}C NMR (DMSO-d_6, ppm): 137 (Cp), 128, 120, 138, 149, (Cp-phenyl) 163, (C=O) 131 ,129, 121, 156(phenyl with azo)



Scheme-3: Synthesis of ferrocenyl esters.

Synthesis of bis(4-ferrocenylphenyl) 1,1'-ferrocenedicarboxylate **(E4)**

4-Ferrocenyl phenol (0.5 g, 1.80 mmol), 10 mL THF, 10 mL triethylamine and ferrocene-1,1'-dicarbonyl chloride (0.18 g, 0.6 mmol) were reacted to get E4.

Yield 57 %,Mp 241-245 °C. FTIR (cm^{-1}): 1718 ($\upsilon_{C=O}$), 2976 $(\upsilon_{C-H})_{aromatic}$, 518 ($\upsilon_{Fe-cp}$), 1210 ($\upsilon_{C-O}$).^{1}H NMR (DMSO-$d_6$, ppm): 4.16 (Cp-ring, singlet), 4.46-4.32 and 4.88-4.41 (Cp-ring, triplet), 4.01 (Cp-ring, singlet), 4.30-4.22 and 4.71-4.31 (Cp-ring, triplet).

^{13}C NMR (DMSO-d_6, ppm): 138 (Cp), 132, 123, 138, 150 (Cp-phenyl), 166 (C=O) 133, 131, 123, 156 (phenyl).

Synthesis of ferroceyl ester (bis(4-ferrocenylphenyl) terephthalate) **(E5)**

4-Ferrocenyl phenol (0.5 g, 1.80 mmol), 10 mL THF, 10 mL triethylamine and terephthaloyl chloride (0.18 g, 0.9 mmol) were made to react to obtain E5.

Yield 65 %, Mp 281-283 °C. FTIR (cm^{-1}): 1725 ($\upsilon_{C=O}$), 3028 $(\upsilon_{C-H})_{aromatic}$, 506 ($\upsilon_{Fe-cp}$), 1243 ($\upsilon_{C-O}$). ^{1}H NMR (DMSO-$d_6$, δ ppm): 4.05 (Cp-ring, singlet), 4.48-4.49 and 4.96-4.47 (Cp-ring, triplet) 8.81-7.49(phenyl multiplet).

^{13}C NMR (DMSO-d_6, ppm): 138 (Cp), 131, 134, 136 (Cp-phenyl), 166 (C=O), 132, 131, 120, 156 (phenyl).

Synthesis of bis(4-ferrocenylphenyl) isophthalate **(E6)**

4-Ferrocenyl phenol (0.5 g, 1.80 mmol), 10 mL THF, 10 mL triethylamine and isophthaloyl chloride (0.18 g, 0.9 mmol) were made to react to procure E6.

Yield 67 %, Mp 203-207°C. FTIR (cm^{-1}): 1729 ($\upsilon_{C=O}$), 3095 $(\upsilon_{C-H})_{aromatic}$, 499 ($\upsilon_{Fe-cp}$), 1219 ($\upsilon_{C-O}$).4.05 (Cp-ring, singlet), 4.50-4.53 and 4.99-4.51 (Cp-ring, triplet) 8.85-7.52(phenyl multiplet).

^{13}C NMR (DMSO-d_6, ppm): 137 (Cp) 126, 128, 139, 135 (Cp-phenyl), 164 (C=O), 131, 130, 121, 157 (phenyl).

Synthesis of bis(4-ferrocenylphenyl) adipate **(E7)**

4-Ferrocenyl phenol (0.5 g, 1.80 mmol), 10 mL THF, 10 mL triethylamine and adipoyl chloride (0.18 g, 0.9 mmol) were made to react to get E7.

Yield 54 %, Mp130-134 °C. FTIR (cm^{-1}): 1739 ($\upsilon_{C=O}$), 2998 $(\upsilon_{C-H})_{aromatic}$, 518 ($\upsilon_{Fe-cp}$), 1240 ($\upsilon_{C-O}$).^{1}H NMR (DMSO-d6, ppm): 4.01 (Cp-ring, singlet), 4.37-4.27 and 4.84-4.35 (Cp-ring, triplet), 7.01-6.92(phenyl, multiplet), 3.15($(CH_2)_4$, multiplet).

^{13}C NMR (DMSO-d_6, ppm): 138 (Cp), 130, 131, 135, 128 (Cp-phenyl), 165 (C=O).

Synthesis of bis(4-ferrocenylphenyl) succinate **(E8)**

4-Ferrocenyl phenol (0.5 g, 1.80 mmol), 10 mL THF, 10 mL triethylamine and succinyl chloride (0.18 g, 1 mmol) were made to react to procure E8.

Yield 65 %, Mp 260-262 °C. FTIR (cm^{-1}): 1701 ($\upsilon_{C=O}$), 3045 (υ_{C-H})$_{aromatic}$, 518 (υ_{Fe-cp}), 1218 (υ_{C-O}).^{1}H NMR (DMSO-d6, ppm): 4.02 (Cp-ring, singlet), 4.39-4.29 and 4.86-4.38 (Cp-ring, triplet), 7.02-6.94(phenyl, multiplet), 3.20((CH_2)$_2$, singlet).

^{13}C NMR (DMSO-d$_6$, ppm): 139 (Cp), 137, 130,128, 127 (Cp-phenyl), 165 (C=O).

Synthesis of bis(4-ferrocenylphenyl) dimethylmalonate **(E9)**

4-Ferrocenyl phenol (0.5 g, 1.80 mmol), 10 mL THF, 10 mL triethylamine and dimethylmalonoyl chloride (0.18 g, 1 mmol) were made to react according to the general procedure as mentioned above to get E9.

Yield 52 %, Mp 61-63 °C. FTIR (cm^{-1}): 1683 ($\upsilon_{C=O}$), 2983 (υ_{C-H})$_{aromatic}$, 461 (υ_{Fe-cp}), 1257 (υ_{C-O}).^{1}H NMR (DMSO-d6, ppm): 4.02 (Cp-ring, singlet), 4.37-4.28 and 4.85-4.34 (Cp-ring, triplet), 7.03-6.95(phenyl, multiplet), 2.19(CH_3, singlet).

^{13}C NMR (DMSO-d$_6$, ppm): 137 (Cp), 130, 131, 135, 128 (Cp-phenyl), 163 (C=O).

DNA-Binding studies

Investigation of DNA-binding capabilities was carried out by UV-visible spectroscopy and cyclic voltammetric studies. For the former method, absorption of solution (in ethanol) was measured with and without the addition of DNA. In cyclic voltammetry, the solution (2.5 mmloes) of ferrocenyl ester in DMSO, with different concentrations of DNA, was prepared and scanned in the window of potential -2.0-0.0 V. The obtained data was used to determine the DNA-binding ability of the compound.

DPPH free radical scavenging assay

The DPPH free radical scavenging assay was performed according to the reported method [17], with slight modifications. Solutions of the synthesized compound and DPPH (0.1 mM) were made in ethanol. Final solution mixture had 3 mL of sample solution and 0.6 mL of DPPH solution, and left for half an hour in dark for the compound to scavenge. Ascorbic acid was used as a positive control. Change in color of DPPH (purple to yellow) in test sample, was observed spectrophotometrically at wavelength of 517 nm. The scavenging effect was calculated using equation given below.

$$\textbf{Scavenging Effect} = \frac{\textbf{Control absorbance/Sample absorbance} \times 100}{\textbf{Control Absorbance}}$$

Results and Discussions

The novel ferrocenyl esters were prepared by using condensation method at low temperatures. This method involved coupling of ROH and various selected diacid chlorides in the presence of a base (triethylamine), scheme 3. Esterification is highly susceptible to hydrolysis and it gives back phenol and acid, thus, to minimize chances of reversibility, reaction system was set moisture free. Triethylamine act as a catalyst by capturing HCl (evolved as a condensation product) and giving triethylammonium salt, which is a stable, consequently, minimizing reversibility during the reaction. Initially, all the products obtained were black viscous oil, which gave amorphous solid powders upon purifying. Solubility of the products were tested in a wide range of solvents and the data showed that these esters were insoluble in solvents like n-hexane, toluene, benzene, pet-ether, acetone, THF, ethyl acetate. For the other solvents, solubility behavior is given in the supporting information. In FT IR-spectra, formation of esters was confirmed by the disappearance of broad band which is characteristic of -COOH moiety in region of 3500-2200 cm^{-1} and appearance of an intense band of carbonyl group –C=O which is characteristic of esters in the region 1730-1700 cm^{-1}. The aromatic moieties showed characteristic bands in regions of 3090-3010 cm^{-1} due to the presence of aromatic –CH stretch and 1615-1600 cm^{-1} due to C=C stretch of aromatic functionality. The aliphatic moiety showed two signals in regions of 2940-2900 cm^{-1} and 2880-2850 cm^{-1} for asymmetric and symmetric –CH stretch respectively. Signal owing to C-O linkage appeared around 1200 cm^{-1}and that of Fe-cp stretch at 506 cm^{-1}. The signal appearing at the value of 830-800 cm^{-1} confirmed para-substitution of benzene ring.

^{1}H-NMR spectra of these ferrocene-based esters showed substitution at one terminal cp-rings by the presence of one singlet and two triplets between 5-4 ppm [17]. The peak due to proton of carboxylic/phenol moiety was absent which confirmed the formation of ester-linkage. The signals due to aromatic group appeared in the range of 7-8 ppm.

UV-Visible spectroscopy ferrocene showed two absorption peaks at wave length 438 nm and 330 nm due to the transitions related to d-d and π-π^* excitations, respectively. The peak due to π-π^* transition showed bathochromic shift as expected, because the attachment of aryl-moiety with cp-ring of ferrocene cause increase in conjugation which leads to a better overlap of π orbitals causing decrease in energy gap, thus λ_{max} was observed at higher values. The UV-visible spectral data for ferrocene-based esters is tabulated in Table-2.

A hypochromic shift in absorbance was observed with varying concentrations of DNA for ferrocenyl esters. The hypochromism suggested the presence of mix modes for DNA-binding including interacalation and groove binding. In Fig. 1, the UV-vis spectrum of a representative ester (E6) is presented with different concentrations of DNA, confirming the hypchromic shift.

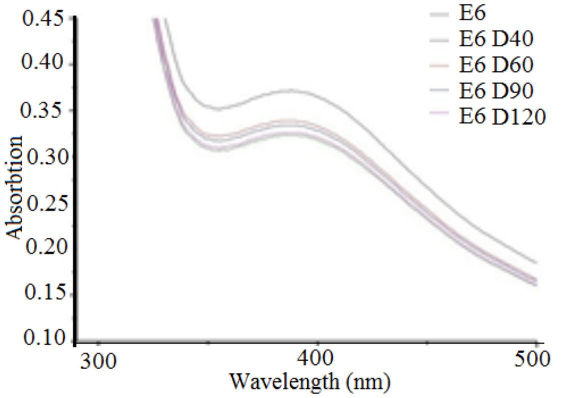


Fig. 1: UV-vis spectrum E6 at different concentrations of DNA, where D10 equals ten micro-liters of DNA solution mixed into the sample-solution.

The synthesized ferrocene-based esters were found to be electrochemically active in potential window -2.0-0.0 V. The diffusion process of their chemical activity was facilitated with the increase in scan rate. The influence of scan rate on electrochemical behavior is depicted in Fig 2. A reversible redox behavior is shown by values like E_p-$E_{p1/2}$ (difference of anodic peak potential and its half) and i_p^a / i_p^c (ratio of anodic peak current to cathodic peak current) which is common for many of ferrocene derivatives [18]. Variation in scan rates, at gradually higher values, accompanied by no change in the range of potential window confirmed the reversible redox behavior of the compounds. The redox behavior of compounds can be utilized in many purposeful applications like drug designing, evaluation of mechanism of electrode reaction and elucidation of many kinetic parameters of redox processes. The representative electrochemical data, for the synthesized ester compounds, at scan rate of 100 mV/s is given in table 1.

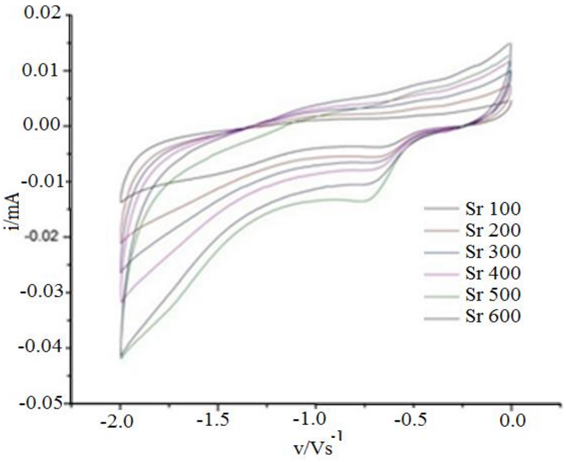


Fig. 2: Voltammogram showing effect of scan rate (Sr) variation on compound E1.

E1 were selectively titrated against dsDNA and binding was examined via cyclic voltammetry, Fig 3. The values of peak current i_p and peak potential E_p were altered by adding DNA-solution giving an idea about the interaction of nucleic acid with the given compound. This variation in peak current i_p after adding DNA can be exploited for evaluation of binding parameters e.g. binding constant (table 2), and the change in peak potential can give an idea about the type of interaction between DNA and the compound. Cyclic voltammograms of compounds in the presence of DNA showed shift in cathodic current (cathodic shift), which indicated electrostatic mode of interaction with phosphate group, present in the DNA. The simultaneous cathodic and anodic shifts indicate that the interaction is carried out via intercalation and electrostatic modes of binding [19]. It was observed that the value of i_p^a (current of oxidation peak) is reduced after each addition of DNA in solution of compounds, shift in the value of i_p^c (current of reduction peak) was also noticed. These behaviors are linked with electrostatic mode of interaction which is considered as strongest mode of bonding [18, 20, 21]. The interaction of DNA with the compounds is further confirmed by the decreased value of diffusion coefficient (table 2) after the addition of DNA, this is because of slow diffusion of DNA-ligand adduct formed after electrostatic interaction. The values of diffusion coefficients were calculated, using Randles-Sevick equation, for selected compounds in the presence and absence of DNA which gave an estimation of interactions between DNA and compound under study

Table-1: Electro chemical data for ferrocene-based esters at scan rate 100 mV/s.

Compound	E_p^a	I_p^a	$(E_p-E_{p1/2})^a$	E_p^c	I_p^c	$(E_p-E_{p1/2})^c$
E1	-0.485	1.878	0.571	-0.648	-3.687	-0.117
E3	-0.470	-0.182	0.191	-0.749	-5.253	-0.159
E4	-0.467	1.658	0.105	-0.678	-9.991	-0.122
E7	-0.491	3.614	0.801	-0.621	-6.52	-0.071
E9	-0.469	2.410	0.076	-0.652	-8.852	-0.117

E_p^a= anodic peak potential, i_p^a = anodic peak current, $(E_p-E_{p1/2})^a$ = the difference of anodic peak potential and its half, E_p^c = cathodic peak potential, I_p^c = cathodic peak current, $(E_p-E_{p1/2})^c$ = the difference of cathodic peak potential and its half

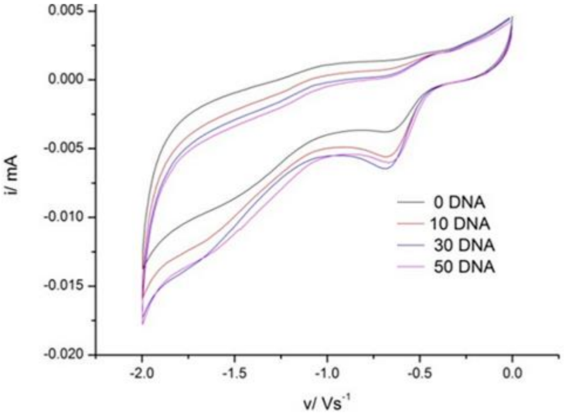


Fig. 3: Voltammogram of E1 with Different DNA concentration.

Table-2: Kinetic parameters for DNA-binding capabilities of representive compounds.

Compound	D_o ($Cm^2 s^{-1}$)	D_o (DNA) ($Cm^2 s^{-1}$)	DNA-Binding constant $K_b x10^{-3}$
E1	$44.25x10^{-2}$	$37.37x10^{-2}$	6.01
E7	$15.43x10^{-3}$	$14.57x10^{-3}$	23.23

DPPH (2,2-diphenyl-1-picrylhydrazyl), by nature, is a free radical (purple color). It has a tendency to react with antioxidants converting itself into reduced form diphenyl picrylhydrazine (yellow color). This conversion of radical into reduced form can be monitored spectrophotometrically, as free radical form of DPPH shows absorbance at 517nm and its reduced form does not absorb at this wavelength. Thus a decrease in the absorption indicates antioxidants activity of the compound. Precursor ROH showed very low antioxidant activity *i.e.* up to 20% while its corresponding esters showed increased antioxidant activity in a range of 50-77%, fig 4. Among synthesized esters lowest antioxidant (52%) activity was displayed by E3, that was derived from trans-azobenzene-4,4'-dicarbonyl chloride, that is yet a satisfying performance as compared to ROH. The maximum scavenging effect was exhibited by E1, E7, E8 and E9 *i.e.* greater than 75%.

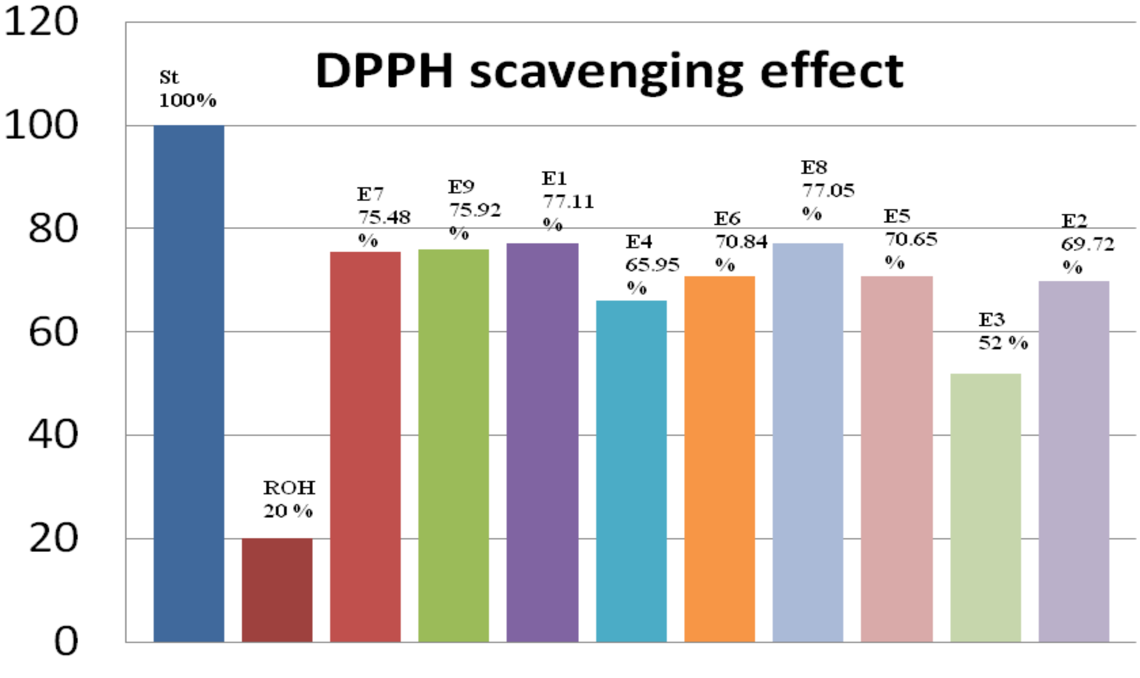


Fig. 4: Graphical representation of DPPH free radical scavenging activity of 2ET.

Conclusion

Novel ferrocenyl esters were prepared by low temperature solution polycondenstaion and characterized successfully by using standard techniques like FT-IR, ^{1}H-NMR, UV-visible spectroscopic studies. Cyclic voltammetric analysis showed that these compounds were electroactive in nature. DNA-binding capability and anti-oxidant activity was studied by UV-visible spectroscopic studies and DPPH free radical scavenging assay showed that the material is biologically active in nature, therefore, have potential to be used as DNA-binders and anti-oxidants.

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