

Synthesis and biologic activity of bis and mono nitrophenyl substituted ferrocene and their interaction with O_2/O_2^- redox couple using cyclic voltammetry

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ARTICLE INFO

Article History :

Received : 10/09/2021

Accepted : 31/05/2022

Key Words:

Ferrocene; DPPH;
phosphomolebdenum assay;
superoxide anion;
cyclic voltammetry.

ABSTRACT/RESUME

Abstract: In this work, 3-nitrophenylferrocene (3-NPF) and bis-3-nitrophenylferrocene (bis-3-NPF) were synthesized through the arylation of ferrocene by diazotating of 3-nitroaniline by sodium nitrite in the presence of H_2SO_4 to form meta-nitrobenzediazonium sulfide. The synthesized compounds were characterized by IR infrared and NMR magnetic resonance spectroscopy (1H , ^{13}C and DEPT). The superoxide scavenging effect was assessed by electrochemical assay; cyclic voltammetry, the DPPH scavenging assay and total antioxidant capacity were assessed using spectrophotometric assay. In this paper, the antioxidant capacity index (IC_{50}) towards superoxide anion radical, DPPH radical and TAC were determined, we found that the IC_{50} of compounds against superoxide ranged from 1.212 to 2.709mg/ml, the lowest value of IC_{50} was detected in bis-3-NPF, the DPPH radical inhibition capacities are in the order of ascorbic acid > α -Tocopherol > bis-3-NPF > 3-NPF > with IC_{50} respectively of 0.0063mg/ml, 0.0134mg/ml, 0.773mg/ml and 1.44mg/ml and the maximum Mo(VI) reducing activity was observed at high concentrations (0.8 mg/ml), the TAC of the compounds was found to be in the following order: Bis-3-NPF > 3-NPF. The antioxidant activity coefficients (K_a), the interaction coefficients (K_b) and the binding free energy of these two ferrocene derivatives towards the superoxide radical were determined using the values of the intensities of the anodic currents from the cyclic voltammograms of the O_2/O_2^- couple.

I. Introduction

The history of ferrocene begins a century ago, in 1951[1], with the temptation to synthesize fulvalene by Kealy and Pauson while Miller, Tebboth and Tremain try to synthesize $Fe(C_5H_5)_2$. Then, on X-ray analysis, in 1952, Fischer proposed a double cone structure[2]. Wilkinson and Woodward proposed the structure "sandwich" on the basis of infrared data and polarity measurements [3] giving rise to a new discipline: organometallic chemistry. Fischer and Wilkinson were rewarded for their

pioneering work on the complexes "sandwiches" by the Nobel Prize for Chemistry in 1973[4].

Ferrocene has particular physicochemical properties, among the properties exploited for the creation of compounds with biological activities: its structural diversity, the possibility of exchange of its ligands, its catalytic and its redox properties. A diversity of ferrocenic molecules have proved their use as antitumor agents [5-8], antiviral agents[9], antibacterial[10-12], antiparasitic[13-14], antifungals[15] and antioxidants[16-20]

Free radicals in biological systems are ubiquitous and can come from various extracellular sources (UV radiation, X-ray and gamma radiation, atmospheric pollution, cigarettes) and intracellular (mitochondria via its respiratory chain, oxidation of small molecules: cyclooxygenases and lipoxygenases, xanthine oxidase and NADPH oxidase, endoplasmic reticulum and peroxisomes). Unbalanced diet is another cause of the formation of free radicals. The reaction of these reactive oxygenated and nitrogen species with the lipid molecules and their interactions with the nucleic acids and the proteins causes certain alterations and change their functions[21]. Compounds capable of retarding or inhibiting oxidative damage to a target molecule are called antioxidants, an antioxidant molecule can react with the free radical and neutralize it by giving one of their electrons, while preventing cellular and tissue damage[22].

In this work, 3-NPF and bis-3-NPF were prepared, the antioxidant capacity towards DPPH free radical and superoxide anion radical were studied index (IC50) and some binding parameters: the antioxidant activity coefficient (Ka), the binding coefficient (Kb), and binding free energy (ΔG) of these compounds with the superoxide radical anion. The antioxidant activity of those compounds was evaluated by the scavenging activity of superoxide anion radical carried out by the cyclic voltammetry.

II. Experimental

II.1. Materials and methods

II.1.1. Chemicals and reagents

For antioxidant study, used chemicals are methanol (99%) from Biochem chemopharma, dimethylsulfoxide (DMSO) 99.9% Sigma Aldrich, 1,1-diphenyl-2-picrylhydrazyl (DPPH) (99%) from

Merck. sulfuric acid (98%), ammonium molybdate and ammonium phosphate (NaH_2PO_4) from Merck. Dimethylformamide (DMF) 99.9% from Biochem Chemopharma (Canada), tetrabutylammonium tetrafluoroborate Bu_4NBF_4 (99%) from Sigma-Aldrich, ascorbic acid (99.7%), α -tocopherol (97%) were from Alfa Aesar and used as standard antioxidants.

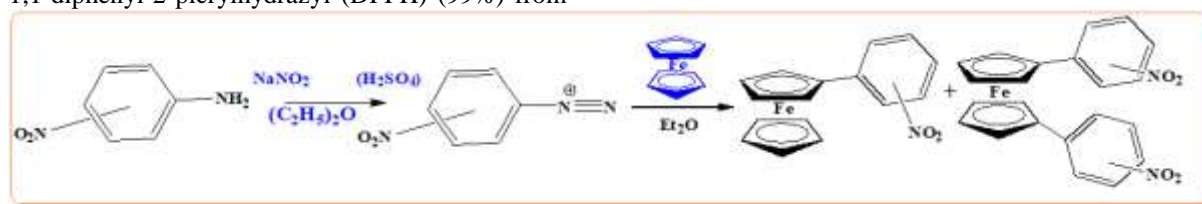
II.1.2. Instrumentation

The progress of the synthesis was monitored by thin layer chromatography. Infra-red (IR) spectra was recorded on FTIR 830 SHIMADZU and was recorded in cm^{-1} . The NMR spectra ^1H , ^{13}C and DEPT were recorded on a Bruker AM-300 spectrometer with a frequency of 300MHz for the proton and 75MHz for the carbon ^{13}C . The chemical shifts δ were given in part per million (ppm) using the CDCl_3 as internal reference which is characterized by 7.26 ppm for the proton and 77.16 for the ^{13}C and the coupling constants (J) in Hertz (Hz).

Electrochemical measurements were carried using Voltalab 40 model PGZ301 Potentiostat/Galvanostat in an electrochemical cell of volume $V=25\text{ml}$. All the measurements were made using conventional three electrode; glassy carbon (0.03 cm^2) served as the working electrode, a saturated calomel electrode (SCE) functioned as a reference and platinum wire functioned as a counter-electrode. All experimental data were recorded at $28 \pm 1^\circ\text{C}$.

II.1.3. Synthesis

3-nitrophenylferrocene (3-NPF) was prepared by the diazonium salt method [23] according to the scheme below:



Scheme (01) . mechanism of synthesis of 3-NPF and bis-3-NPF.

Preparation of 3-nitrophenylferrocene (3-NPF) and bis-3-Nitrophenylferrocene (Bis-3-NPF)

10.7g of 3-nitroaniline dissolved in 22.90 ml of water + 16.7 ml of H_2SO_4 (98%) and diazotized with 5.5 g of sodium nitrite. 13.08g of ferrocene in 316.45ml of ether is added to the filtrate (3-nitrobenzenediazonium sulfate). After 3 hours of stirring at room temperature, the organic phase was recovered, washed with water, dried and evaporated. After separation by column chromatography, two products were recovered, the first have an orange color ($R_f = 0.73$) and melting

point of 123°C and the second is an orange-red pallets of melting point of 145°C .

3-NPF: $\text{Mp} = 123^\circ\text{C}$. IR (KBr, cm^{-1}); 1652.9, 1635.5, 1521.7, 1456.2 and 1346.2 cm^{-1} . ^1H NMR (CDCl_3 , 300MHz); 4.05(5H, s, C4); 4.40 (2H, s, C2 or C3), 4.71(2H, s, C3 or C2), 7.43(1H, t $J = 7.98\text{Hz}$, C9), 7.74

(1H, d $J = 7.70\text{Hz}$, C10), 8.00(1H, d $J = 8.34\text{Hz}$, C8), 8.27(1H, s, C6). ^{13}C NMR (CDCl_3 , 75 MHz); 66.82 (2C, C2 or C3), 69.84 (5C, C4); 69.90 (2C, C3 or C2), 82.49 (1C, C1) ; 120.38 (1C, C8),

120.49 (1C, C9), 129.24 (1C, C10), 131.68 (1C, C6), 142.17 (1C, C5), 148.63 (1C, C7).

Bis-3-NPF: $Pf = 145^{\circ}\text{C}$. IR (KBr, cm^{-1}); 1625.9, 1523.7, 1342.4, 806.2, 723.3 cm^{-1} . $^1\text{H NMR}$ (CDCl₃, 300MHz);

4.03(s, 4H), 4.38 (s, 2H), 4.66 (s, 2H), 7.18(m, 2H), 7.54(m, 2H), 7.94 (s, 2H).

II.1.4. QSAR's properties study

The synthesized molecules have been pre-optimized using the Molecular Mechanics (MM+) force field included in HyperChem version 8.0.3. Hyper Chem's

QSAR property module was used to calculate some physico-chemical properties of 3-NPF and bis-3-NPF like: molar weight (MW), molar polarisability (Pol), molar refractivity (MR), octanol/water partition coefficient (logP), hydration energy (HE), molar volume (MV), surface grid (SAG) and Surface Area Approx (SAA).

II.1.5. Antioxidant activity

II.1.5.1. DPPH free radical scavenging assay

This test is based on the principle of the method described by Brand-williams with some modifications[24].

A solution of DPPH• is prepared by dissolving 0.985 mg of DPPH• in 25 ml of methanol. A stock solution was prepared for each compound by dissolving 1.5 mg in 1 ml of DMSO. The stock solution was diluted to obtain solutions of concentrations ranging from 0.1 to 0.7 mg/ml. 0.1 ml of each concentration of each product (standard or product to be tested) is added to 1.9 ml of DPPH solution [25]. The tubes were incubated in the dark for 30 minutes. Afterwards, the decrease in absorbance was recorded at 517nm at room temperature.

The inhibition percentages of the free radical DPPH• by 3-NPF and bis-3-NPF were calculated by applying the equation:

$$\text{Inhibition of DPPH} = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}} \times 100\% \quad (1)$$

II.1.5.1. Total antioxidant capacity by phosphomolybdenum assay

The phosphomolybdate (PPM) test was carried out according to the method described by Prieto and al [26]. It consists of introducing into tubes 100 μL of each product mixed with 0.1 ml of reagent composed of H₂SO₄ (0.6 M), NaH₂PO₄ (28 mM) and ammonium molybdate (4 mM). The tubes were then tightly closed and then incubated at 95°C for

90 minutes. After cooling, the absorbance was measured at 695 nm. The phosphomolybdenum reduction potential of the compound was expressed as equivalent of ascorbic acid ($\mu\text{g.l}^{-1}$ AAE) using the following equation obtained from the standard ascorbic acid graph:

$Y = 0.003X + 0.143$ where, Y is the absorbance at 695 nm and X is the equivalent of ascorbic acid.

II.1.5.2. Superoxide radical scavenging assay

The capability of 3-NPF to scavenge the superoxide anion radical was assessed according to le Bourvellec et al and it was discussed earlier [23]. The inhibitory activity of the superoxide radical anion by bis-3-NPF is determined by cyclic voltammetry, this test is based on the Bourvellec et al method with some modifications [27]. Prior to use, the working electrode is polished, rinsed with distilled water, and dried with paper towels. This cleaning procedure is always applied before each electrochemical measurement. The superoxide radical anion is generated by commercial molecular oxygen dissolved in DMF which contains 0.02 M Bu₄NBF₄ at room temperature (27-29°C). The scanning rate is maintained at 100mV/s. The applied potential range was -1.6V to 0.0 V versus SCE. The studied ferrocene derivatives are added to the superoxide radical dissolved in the DMF and the voltammograms have been recorded.

• Antioxidant capacity index (IC₅₀)

The inhibition percentage of superoxide (O₂•-) caused by the addition of the ferrocenic compounds is calculated by the following formula:

$$\text{Superoxide inhibition (\%)} = \frac{I_{p0} - I_{ps}}{I_{p0}} \times 100$$

I_{p0} , are the intensities of the anodic peak current of oxygen respectively with and without test compound

• Antioxidant Activity coefficient (Ka)

The relative capacity of 3-NPF and Bis-3-NPF to scavenge superoxide radical was determined as antioxidant activity coefficient (Ka). The constant Ka is calculated according to equation (3)

$$Ka = \frac{\Delta J}{(J_0 - J_{res}) \Delta C} \quad (3)$$

Where: ΔJ ; change in the oxygen anodic current density with the addition of substrate. J_0 ; limiting current density of oxygen without the substrate in the solution. J_{res} ; residual current density of oxygen. ΔC ; change in the concentration of substrate (mol/l).

- **Binding constant (Kb)**

parameter named kb binding constant, this constant was calculated using the following equation [28-29]

$$\text{Log} \frac{1}{[\text{AO}]} = \text{log Kb} + \text{log} \frac{I_p}{I_p - I_{p0}} \quad (4)$$

Where: I_p , I_{p0} ; anodic current densities of superoxide with and without additives, respectively. [AO]; concentration of antioxidant

- **Binding free energy (ΔG)**

The binding free energy ΔG was calculated using equation (5)[30]

$$\Delta G = -RT \ln Kb \quad (5)$$

Where: ΔG ; binding free energy, KJ.mol⁻¹. R; gas constant; 8.32 J.mol⁻¹.K⁻¹. T; absolute temperature equal to 298K

III. Results and discussion

III.1. Synthesis and characterization

The interaction strength between the superoxide radical and the antioxidant was quantified by a ¹H NMR spectrum: The ¹H NMR spectrum of Bis-3-NPF shows peaks at 4.03ppm corresponding to 4H bonded to the Cp of the ferrocene, a peak at 4.38ppm corresponding to 2H bonded to the C2 or C3 atom, a peak at 4.66ppm corresponding to 2H bonded to the C3 or C2 atom, a multiplet at 7.18ppm corresponding to 2H, a multiplet at 7.54ppm corresponding to 2H, a singlet at 7.94ppm corresponding to 2H.

Specters ¹³C and DEPT: By combining the three spectra ¹³C-NMR, DEPT and ¹H-NMR, we can deduce:

-The ¹³C spectrum shows peaks at 81.62, 130.83, 139.52, 142.76, 148.37 and 148.84ppm which have disappeared in the DEPT spectrum, so the product 3 molecule contains 6 quaternary C_s.

-According to the DEPT spectrum of compound 3, we can see that there are no negative peaks, therefore there is no CH₂.

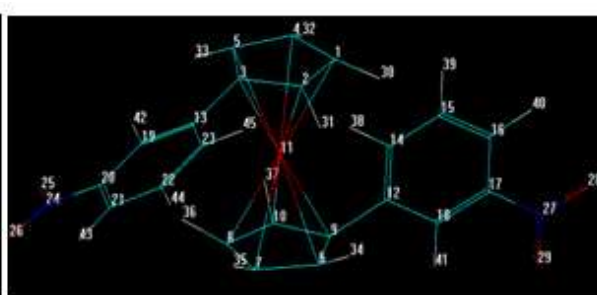
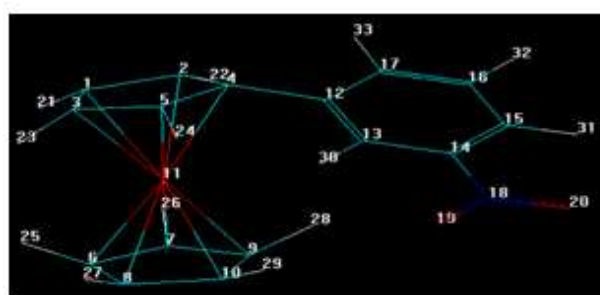
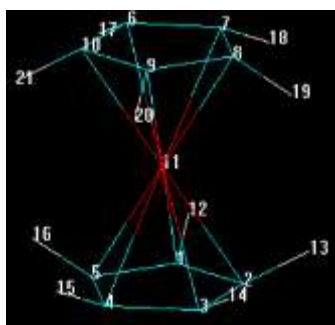


Fig.1. Geometric optimization of ferrocene (parent molecule), 3-NPF and bis-3-NPF by HyperChem 8.0.6 software

III.2. QSAR's properties study

All the calculations of ferrocenic derivatives were performed using HyperChem 8.0.6 software. The QSAR properties were obtained from the module

QSAR properties in HyperChem software. Table (1) summarize the QSAR'S properties of FC, 3-NPF and Bis-3-NPF.

Table 1. QSAR's properties of FC, 3-NPF and Bis-3-NPF.

Compounds	Stability energy	MW (amu)	POL (Å ³)	MR (Å ³)	Log P	HE (Kcal/mol)	MV (Å ³)	Area A	SAG (Å ²)
FC	686.06	186.04	14.50	33.98	-1.87	1.83	503.25	168.09	315.72
3-NPF	657.601587	307.13	26	68.84	-1.93	-4.91	745.06	290.15	433.33
Bis-3-NPF	661.909815	428.23	37.50	103.70	-2.00	-11.87	984.30	558.00	417.76

refractivity increase relatively with the size and molecular weight of the studied compounds. This result is in agreement with the Lorentz formula which gives a relationship between polarizability, molar refractivity and volume [31]

This relationship shows that molar refraction and polarizability are proportional with volume and molecular mass. Unsubstituted ferrocene has a low polarizability equal to 14.50 and a molar refractivity value equal to 33.98, on the other hand, the ferrocene compounds whose pentadienyl rings are mono- and bi-substituted by nitrobenzene radicals record values of polarizability and of Higher molar refractivity equal to 26 for 3-NPF, 37.50 for bis-3-NPF and 68.84 for 3-NPF, 103.70 for bis-3-NPF respectively (Table 1).

The presence of hydrophobic groups in the structure of 3-NPF and bis-3-NPF causes the decrease in hydration energy; as well as, the presence of hydrophilic groups' increases the energy of hydration. In table 1, the hydration energy of the ferrocene molecule (1.83 Kcal/ mol) decreased when the ferrocene is substituted, for the 3-NPF, the hydration energy in absolute value reached (-4.91 Kcal/mol) and for bis-3-NPF, the hydration energy reaches -11.87, which means that the introduction of the nitrobenzene group on the ferrocene makes the molecule more hydrophilic. Indeed, in biological media, polar molecules are surrounded by water molecules where hydrogen bonds can be established between water molecules and these molecules. Proton donor sites interact with the oxygen atom in water and proton acceptor sites interact with the hydrogen [32, 33]. Lipophilic is an important factor in the processes of solubility,

absorption, distribution, metabolism and excretion, as well as pharmacological activity.

Hansch and Leo have shown that very lipophilic molecules are distributed and preserved inside the lipid layers of cell membranes [34]. For good oral bioavailability, log P should be greater than zero and less than 3 ($0 < \log P < 3$). In fact, for a log P that is too high, the drug exhibits low aqueous solubility. With very low log P, the drug has difficulty in penetrating the lipid bilayers of cell membranes [35]. In contrast to hydration energy, lipophilicity increases in proportion to the hydrophobic character of the substituent groups. The compounds FC, 3-NPF and bis 3-NPF respectively bear the values of log P equal to -1.87, -1.93 and -2.00.

III.3. Antioxidant study

III.3.1. DPPH

The reactivity of 3-NPF and bis-3-NPF was analyzed by the DPPH. DPPH (1,1-diphenyl-2-picrylhydrazyl) is a stable free radical with a purple color ($\lambda_{\max} = 517\text{nm}$) which turns yellow when scavenged [36]. The absorbance at 517 nm decreases proportionally with the loss of a proton radical by exchanging it with the antioxidant, resulting in a color change from purple to yellow. DPPH' can be used to estimate the antioxidant capacity of natural or synthesized molecules. The DPPH radical inhibition capacities shown in table 2 are in the order of ascorbic acid > α -Tocopherol > bis-3-NPF > 3-NPF > with IC_{50} respectively of 0.0063mg/ml, 0.0134mg/ml, 0.773mg/ml and 1.44mg/ml.

Table 2. IC_{50} of DPPH scavenging in the presence of 3-NPF and bis-3-NPF compared with Ascorbic acid, and α -tocopherol

Compound	Linear regression	R ²	IC ₅₀ (mg/ml).
3-NPF	Y= 36.30x-2.481	0.983	1.44
Bis-3-NPF	Y= 58.22x+4.955	0.998	0.773
Ascorbic acid	Y=1.19x+42.45	0.960	0.0063
α -tocopherol	Y=1.56x+29.093	0.995	0.0134

From the literature [37-38], it can be concluded that the DPPH' scavenging activity by the synthesized ferrocene derivatives is due to ferrocene itself and to the substituted electron withdrawing group NO₂[36]

III.3.2. TAC

The total antioxidant capacity test is a spectroscopic method used to quantitatively determine the antioxidant capacity, by the formation of phosphomolybdenum complex. The assay is based on the reduction of Mo (III) to Mo (V) by the

sample and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH. The total antioxidant capacity is calculated by the method described by Prieto et al [26]. The phosphomolybdenum reduction potential of the compounds was expressed as equivalents of ascorbic acid (mmol/l) using the equation obtained from the standard ascorbic acid graph. The results show that the polarizability and molar.

Table 3. Total antioxidant capacity of 3-NPF and Bis-3-NPF expressed as ascorbic acid equivalents (mmol.L⁻¹ AAE).

Concentration (mg/ml)		Absorbance		TAC (mmol.L ⁻¹ AAE)	
3-NPF	Bis-3-NPF	3-NPF	Bis-3-NPF	3-NPF	Bis-3-NPF
0.2	0.2	0.201	0.208	19.33	20
0.4	0.4	0.301	0.267	52.66	41.33
0.6	0.6	0.61	0.8	155.66	219
0.8	0.8	0.899	0.962	252	273

The results are depicted in Table 3 as a function of concentration. It can be seen that the total antioxidant capacity of compounds were found to be dose-dependent, and the maximum Mo(VI) reducing activity was observed at high concentrations (0.8 mg/ml). Statistically, the TAC of the compounds was found to be in the following order: Bis-3-NPF > 3-NPF.

III.3.3. Superoxide scavenging test:

III.3.3.1. Voltammetric behaviour of superoxide radical in the presence of ferrocenic derivatives

The cyclic voltammograms of superoxide anion radical in the presence of 3-NPF and bis-3-NPF are presented in Fig. 2.

The addition of ferrocenic compounds decreases the anodic currents from 145.1 to 135.3 $\mu\text{A}/\text{cm}^2$ for 3-NPF and from 136.3 to 121.9 $\mu\text{A}/\text{cm}^2$ for bis-3-NPF (Table 5). Maximum effect was observed up to 62.58. 10⁻⁵M concentration while further additions of 3-NPF and bis-3-NPF had little effect on the peak potentials and peak currents

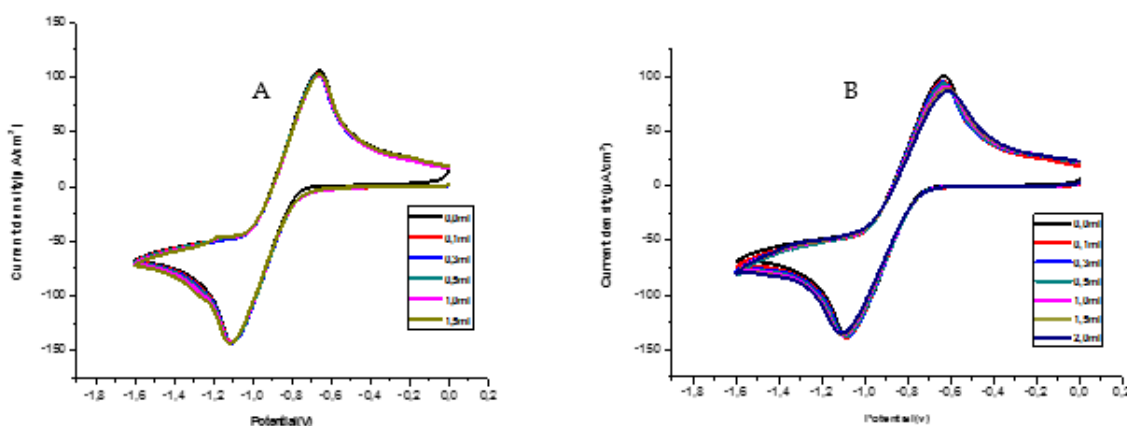


Fig. 2. Cyclic voltammograms of $\text{O}_2\bullet^-$ in the presence of different concentration of 3-NPF(A) and bis-3-NPF (B) in DMF + 0.02 M Bu_4NBF_4 on GC as working electrode vs. SCE with scan rate of 0.1 V/s

The decrease of the anodic peak current of $\text{O}_2\bullet^-$ suggests that the ferrocene derivatives reacts irreversibly with $\text{O}_2\bullet^-$. For each antioxidant compound, a series of values is determined from the CVs recorded for increasing antioxidant concentrations (Table 5).

The scavenging activity of the antioxidant is often evaluated according to its IC₅₀; it is defined by the concentration inhibiting the reaction by 50%. In this system, which were calculated from the linear regression of the percentage antioxidant activity versus compounds concentration (Fig. 3), results

shown in Table 4. The IC₅₀ values of tested compounds ranged from 1.212 to 2.709 mg/ml. The lowest value of IC₅₀ was detected in bis-3-NPF and it corresponds to the highest antioxidant activity; while the highest value of IC₅₀ was detected in 3-NPF. The antioxidant activity in the tested compounds decreases in the order bis-3-NPF > 3-NPF.

Table 4. IC50 values of different compounds.

Compound	Linear regression	IC ₅₀ (mg/ml).
3-NPF	17.29x+3.153	2.709
Bis-3-NPF	38.56x+3.258	1.212
Ascorbic acid	8.578x-0.420	0.00587
α-tocopherol	2.192x+1.692	0.02203

III.3.3.2. Binding constant (K_b)

The interaction strength between the superoxide radical and 3-NPF, Bis-3-NPF was quantified by a parameter named kb binding constant. Table.5 regroupes the anodic current densities of superoxide

in the presence of 3-NPF and bis-3-NPF at different concentrations and parameters deduced to calculate.

Table 5. Anodic current densities of 3-NPF, bis-3-NPF at different concentrations

C.10 ⁻⁵ (M)	I _p (μA/cm ²)		Log(1/[AO])		Log Ip/(Ip0-Ip)	
	3-NPF	Bis-3-NPF	3-NPF	Bis-3-NPF	3-NPF	Bis-3-NPF
0	145.1	136.3	/	/	/	/
3,35	140.6	131.25	4,47	4,47	1.495	1.41
10,03	139.2	130.77	4,00	4,00	1.373	1.37
16,59	138.9	128.87	3,78	3,78	1.350	1.24
32,59	138.7	126.21	3,48	3,48	1.336	1.10
47,91	137.1	124.2	3,31	3,31	1.234	1,01
62,58	135.3	121.9	3,20	3,20	1.14	0.93

The additive which gives high value of binding constant shows strong interaction with the radical. From table 7 it is evident that the percentage

decrease of the anodic current of superoxide anion radical and binding constant (K_b) values follows the following order: 3-NPF > bis-3-NPF

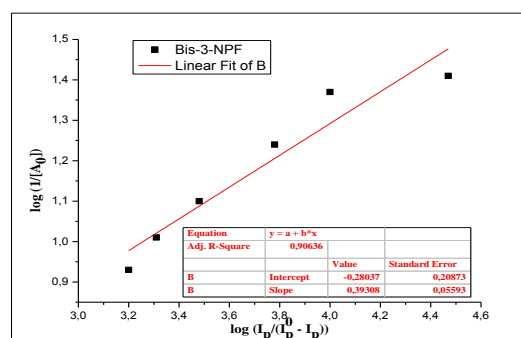
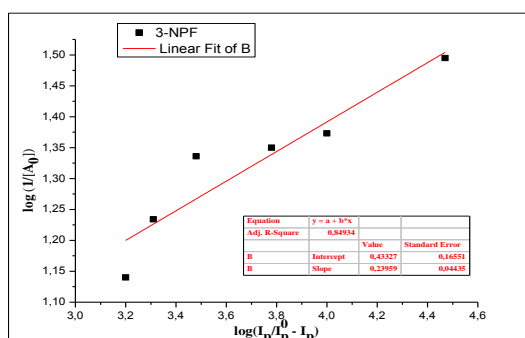


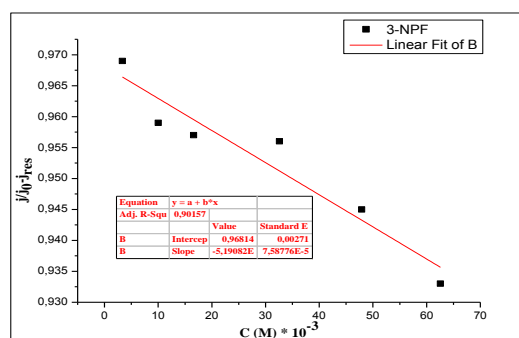
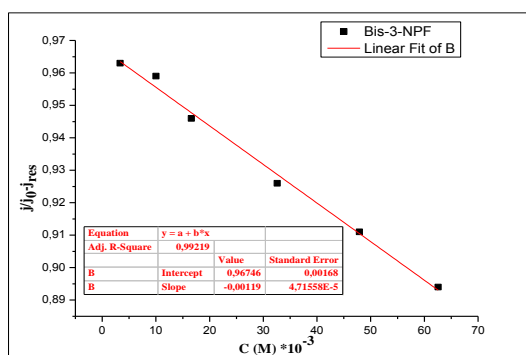
Fig. 3. Plots to determine binding constant (k_b) using equation log (1/[AO]) versus log [Ip/(Ip0-Ip)] for 3-NPF and bis-NPF.

III.3.3.3. Antioxidant activity coefficient

The relative capacity of 3-NPF and bis-3-NPF to scavenge the target radical was determined as antioxidant activity coefficient (K_a).

Table 6. parameters deduced to calculate K_a

$C(M) * 10^{-3}$		$J (\mu A/cm^2)$		$J/J_0 - J_{res}$	
3-NPF	Bis-3-NPF	3-NPF	Bis-3-NPF	3-NPF	Bis-3-NPF
0	0	145.1	136.3	/	/
3,35	3,35	140.6	131.25	0.969	0.963
10,03	10,03	139.2	130.77	0.959	0.959
16,59	16,59	138.9	128.87	0.957	0.946
32,59	32,59	138.7	126.21	0.956	0.926
47,91	47,91	137.1	124.2	0.945	0.911
62,58	62,58	135.3	121.9	0.933	0.894

Fig.4. Relative change in $O_2^{\bullet-}$ current density versus change in sample concentration for the anode peak of 3-NPF and bis-3-NPF.Table 7. K_a , K_b and ΔG values of the 3-NPF and Bis-3-NPF

Compound	K_a	K_b	$-\Delta G(KJ/mol)$
3-NPF	5.19	2.704	2.466
Bis-3-NPF	0.001	0.525	-1.597

III.3.3.4. Binding free energy (ΔG)

The negative values of free energy support that the binding of 3-NPF and bis-3-NPF to superoxide is spontaneous and exothermic [39]. The ΔG values in table 7 indicate more affinity of superoxide to bis-3-NPF followed by 3-NPF which is consistent with the above mentioned results [40-42].

IV. Conclusion

The current work was aimed to investigate the antioxidant character of two ferrocene derivatives; 3-nitrophenylferrocene (3-NPF) and bis-3-nitrophenylferrocene (bis-3-NPF), and against superoxide anion radical ($O_2^{\bullet-}$) while employing cyclic voltammetry method.

The obtained results indicate that the scavenging activity of the two studied compounds gradually increased in a concentration-dependent manner and the best one is bis-3-nitrophenyl ferrocene which has the highest potential scavenging activity against superoxide anion radical.

V. Acknowledgments: The authors would like to thank to Algerian Ministry of Higher Education and Scientific Research and General Directorate of

Scientific Research and Technological Development (DGRSDT) for their support and providing the necessary facilities to carry out this research.

VI. References

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Please cite this Article as:

Benabdesselam S., Rahim O., Synthesis and biologic activity of bis and mono nitrophenyl substituted ferrocene and their interaction with O₂/O₂⁻ redox couple using cyclic voltammetry, *Algerian J. Env. Sc. Technology*, 9:3 (2023) 3238-3247