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Der Pharma Chemica, 2015, 7(6):318-323
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

In vitro assays of the antioxidant activities of ferrocene derivatives bearing amine, amide or hydrazine groups

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ABSTRACT

The aim of this work was to synthesise a series of three ferrocene derivatives bearing either amine, amide or hydrazine groups and to evaluate their in vitro antioxidant activities. Spectrophotometrical and electrochemical techniques were used to quantify the total antioxidant activities, the former was carried out using 1,1-diphenyl-2-picrylhydrazyl and phosphomolybdenum reagents, the latter was based on measuring the oxidation peak current of superoxide anion radical electrochemically generated by reduction of commercial molecular oxygen in DMSO. The ferrocenic derivative N'-ferrocenylmethyl-N'-phenylbenzohydrazide shows the highest DPPH and $O_2^{\cdot-}$ radicals scavenging activities (0.1 ± 0.00245) and (0.92 ± 0 .04 mg/ml) respectively which was significantly closer to that of standard antioxidant ascorbic acid. The results of both spectrophotometrical and electrochemical tests indicate that the activity depends strongly upon the presence of a nitrogen atom in the molecule but is improved by the influence of the hydrazine function. In addition spectrophotometrical tests of N-(Ferrocenylmethyl)-2-nitrobenzenamine does not give any activity due to its deep purple colour, however the antioxidant activity of this derivative was easily measured using electrochemical tests.

Key words: ferrocene derivatives, cyclic voltammetry, DPPH, superoxide anion radical.

INTRODUCTION

The medicinal applications of ferrocene and its derivatives continuous to attract the attention of the scientific community, in the last decades many reports have shown that some ferrocene derivatives have highactivity in vivo and in vitroagainst many maladies such as cancer [1-3] andaids [4]. In addition, some ferrocene derivatives are known to be associated with a wide range of biological activities such as analgesic, antitumor, antioxidant, anticonvulsant and anti-HIV properties [5-14]. Ferrocene derivatives containing nitrogen atoms are known tohavein vitro free-radical scavenging capacity and antioxidant activity [15, 16].The antioxidant activity of ferrocene derivatives was first studied in the late 1950s when Acton and Silverstein report the synthesis and antioxidant evaluation of a number of N-substituted ferrocenecarboxyamides and ferrocenylamine derivatives [17].Zhang and Liu [18] also investigated the antioxidant activity of ferrocenylhydrazones and found that they exhibit an interesting antioxidant activity. Since then, a large number of ferrocenic compounds have been synthesized and evaluated in terms of antioxidantactivities.

After our contribution to the field of corrosion using ferrocene derivatives as inhibitors [19], and to the field of electrochemical study [20, 21]. Now we turn our attention to the field of antioxidant activities and in view of contributing to develop a potential antioxidant agents, in the present study we hereby report the synthesis of some ferrocenyl substituted amine, amides and hydrazines derivatives and evaluation of their in vitro antioxidant activities.

MATERIALS AND METHODS

Chemicals and reagents

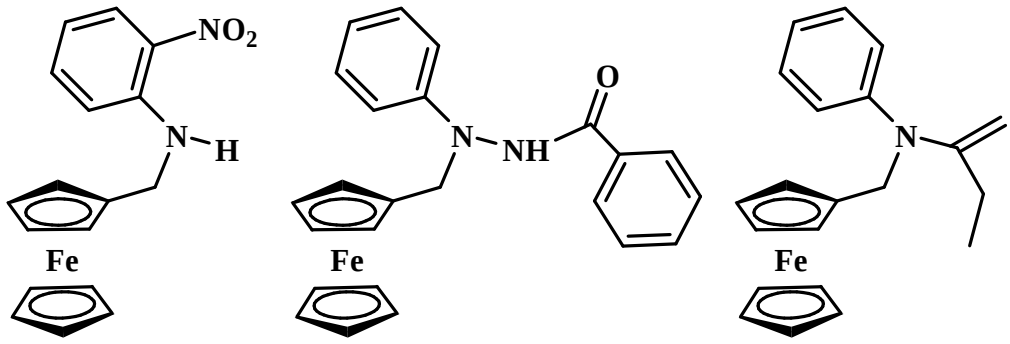
Dimethylsulfoxide (DMSO) of analytical grade purchased from Sigma-Aldrich was used as solvent without further purification, 1,1-diphenyl-2-picrylhydrazyl (DPPH) was procured from Sigma-Aldrich, tetrabutylammoniumhexa-fluorophosphate (TBFP) of electrochemical grade (99%) from Fluka Company, ammonium molybdate, sodium phosphate, sulphuric acid were purchased from Sigma-Aldrich. All other reagents used were of analytical grade.

Instrument

UV-Visible spectrophotometer (PRIM Advanced SCHOTT Instruments Gmbh), PGP301 potentiostat/galvanostat with voltamaster 4 version 7.08 software (radiometer analytical SAS), rotary evaporator (IKA Evaporator RV 06-ML).

Synthesis

N-(Ferrocenylmethyl)-2-nitrobenzenamine(1),N'-ferrocenylmethyl-N'-phenylbenzohydrazide (2), and N-ferroceny methyl-N-phenylpropionamide (3) were synthesized as described previously [22-24].



(1) (2) (3)

Scheme 1. Structures of ferrocene derivatives 1-3

Free radical scavenging activity

Free 2,2-diphenyl-1- picrylhydrazyl (DPPH) radical-scavenging ability

Free radical scavenging activity of ferrocene derivatives were measured using a stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) [25]. Ferrocenic derivative with potential antioxidant activity scavenge the initial violet colour of free DPPH radicals and turn it into yellow colour. This change in colour is proportional to the radical scavenging activity. Briefly, the assay contained 1 ml of 0.25 mM DPPH in ethanol and 0.1 ml of various concentrations of ferrocene derivatives solution and standards in the same solvent. The contents were mixed well immediately and then incubated for 30 min at room temperature (28 ± 1 °C). The degree of reduction of absorbance was recorded in UV–Vis spectrophotometer at 517 nm. All data were analysed and expressed as means ± standard deviation (in three replications, n = 3).

The ability of the test sample to quench 2,2-diphenyl-1-picrylhydrazyl radicals (DPPH) (% Inhibition of DPPH) was calculated using the following equation (1),

$$\%\ \text{DPPH radical scavenging activity} = \frac{A_c - A_s}{A_c} \times 100 \qquad (1)$$

whereA_c is the absorbance of control (without ferrocenic derivative) and A_s is the absorbance of sample.

Free superoxide anion radical ($O_2^{\cdot-}$) radical-scavenging ability

Experimentations were carried out using voltalab40 PGZ301 potentiostat/galvanostat (radiometer analytical SAS) in a double walled electrochemical cell of 25 mL and conventional three electrode system was employed. Glassy Carbon (GC) working electrode (radiometer analytical SAS), having area 0.013 cm^2, a Platinum wire counter electrode, and an Hg/Hg_2Cl reference electrode (3.0 M KCl). Data acquisitions were accomplished with a Pentium IV (CPU 3.0 GHz and RAM 1 Gb) microcomputer using VoltaMaster4 software version 7.08 (radiometer analytical SAS).

The superoxide anion radical was generated by one electron reduction of the commercial molecular oxygen (O_2) dissolved in DMSO containing 0.1 M TBFP at room temperature (28±1°C). The scan rate was kept at 100 mV/s and potential window was from -1.4 to -0.0 V. The studied ferrocene derivatives were added to the *in situ* generated superoxide anion radical and the cyclic voltammograms were recorded. The ability of the test sample to quench superoxide anion radicals ($O_2^{\cdot-}$) (% Inhibition of $O_2^{\cdot-}$) was calculated using the following equation (2),

$$\%\ O_2^{\cdot-}\ \text{radical scavenging activity} = \frac{i_0 - i_s}{i_0} \times 100 \qquad (2)$$

where i_0 and i_s are the anodic peak current densities of the superoxide anion radical in the absence and in the presence of ferrocenic derivative.

Total antioxidant capacity

Molybdate ion reduction

The antioxidant activity of different ferrocene derivatives was evaluated by the phosphomolybdenum method of Prieto et al. [26]. The experiment is based on the reduction of Mo (VI) to Mo (V) by the sample at acid pH. A 0.1 ml of sample solution (0.1 mg/ml and 0.5 mg/ml) was combined with 1 ml of reagent solution (600 mM sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. The tubes containing the reaction solution were capped and incubated in a boiling water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the solution was measured at 695 nm against a blank. The antioxidant capacity of each sample was expressed as ascorbic acid (A.A) equivalent using the following linear equation established using ascorbic acid as standard: ($y = 4.0573x + 0.1545$)($r^2 = 0.983$) where y is the absorbance at 695 nm and x the concentration as ascorbic acid equivalent (mg/ml). The values are presented as the means of triplicate analysis.

Decrease in oxidation peak current of oxygen

The antioxidant activity of the three studied ferrocene derivatives was estimated by the method of Lanez and Rebiai [27]. Briefly the decrease in the anodic peak current density of cyclic voltammograms of oxygen in the presence and in the absence of a ferrocene derivative was measured, the total antioxidant capacity was then evaluated using the following mathematical equation (3).

$$\frac{ip_0 - ip}{ip_0 - ip_{res}} \times 100 \qquad (3)$$

where, $(ip_0 - ip)$ is the change in the anodic peak current density of oxygen caused by the addition of the sample, $(ip_0 - ip_{res})$ is the difference between thelimiting anodic peak current density of oxygen without the a sample in the solution and the residual current density of the oxygen.

RESULTS AND DISCUSSION

Free radical scavenging activities study

In the present study, four antioxidant evaluation methods such as free 2,2-diphenyl-1- picrylhydrazyl (DPPH) radical scavenging activity, superoxide anion radical ($O_2^{\cdot-}$) radical scavenging activity, phosphomolybdenum and decrease in oxidation peak current of oxygen methods were used to measure the antioxidant activity of three ferrocene derivatives. In order to obtain kinetic curves and to calculate the IC_{50} values, radicalscavenging activity (DDPH and $O_2^{\cdot-}$) was plotted against different compounds concentration (0.1, 0.2, 0.3, and 0.4 mg/mL).

The antioxidant capacity of the ferrocene derivatives was expressed as IC_{50}. The IC_{50} value was defined as the concentration (mg/mL) of samples that inhibits the formation of DPPH and $O_2^{\cdot-}$radicals by 50%. All the tests were performed in triplicate and the graph was plotted with the average of three observations. The equations obtained from the linear calibration graph in the studied concentration range for ferrocene derivatives and the standard ascorbic acid are summarized in table 1 (where y represents the value of absorbance (DPPH) or the anodic peak current density ($O_2^{\cdot-}$)and x, the value of samples concentration, expressed as mg/mL.

Table 1: IC50 (mg/mL) values of three ferrocene derivatives obtained using DPPH˙ and O_2^{-} radical scavenging activity

Compound	Methods	Equation	r^2 values	IC_{50} values (mg/ml)
1	DPPH	-	-	-
	O_2^{-}	y = 0.246x + 0.002	0.96	2.02 ± 0.035
2	DPPH	y = 6.87x –0.180	0.97	0.1 ± 0.00245
	O_2^{-}	y = 0.588x - 0.0423	0.95	0.92 ± 0.04
3	DPPH	y = 0.029x + 0.0913	0.99	14.22 ± 0.354
	O_2^{-}	y = 0.113x + 0.0015	0.88	4.54 ± 0.966
Ascorbic acid	DPPH	y = 43.50x - 0.0453	0.99	0.012 ± 0.00017
	O_2^{-}	y = 1.434 + 0.1519	0.91	0.24 ± 0.006

Values are mean ± standard deviation of three separate determinations (n=3).All results are significantly at p<0.05.

The ferrocenic derivative 2 shows the highest DPPH and O_2^{-} radicals scavenging activities (0.1 ± 0.00245) and (0.92 ± 0.04 mg/ml) respectively which was significantly closer to that of standard antioxidant ascorbic acid. This has indicated that attachment of hydrazine function to the ferrocenic compound can improve the antioxidant activity of the resulting derivative.

Theparentferrocenic derivative 1 gave no DPPH free radical scavenging activity, this may due to its deep purple colour, however O_2^{-} radical scavenging activity of this derivative gave 2.02 ± 0.035 mg/ml. finally compound 3 has a significantly lower DPPH (14.22 ± 0.354) and O_2^{-} (4.54 ± 0.966 mg/ml) radicals scavenging activities.

Total antioxidant capacity

The following procedure was followed for the measurement of the antioxidant capacity: 1 ml of asolution of the corresponding ferrocene derivativein DMSO was injected into the electrochemical cell containing a solution of (DMSO + 0.1 TBFP) saturated with commercial molecular oxygen in a way to obtain a total concentration of the ferrocene derivativein the electrochemical cellequal to 0.4 mg/ml. Then, the cyclic voltammogram of ferrocene derivative was recorded in the potential window of -1.4 V to 0.0 V, the obtained voltammograms are shown in figure1.

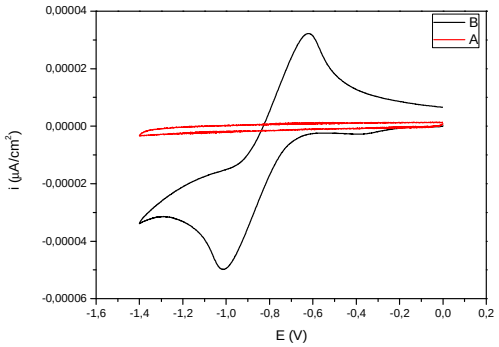


Fig. 1: Cyclic voltammograms of a solution of (DMSO + 0.1 TBFP) recorded at a scan rate of 100 mV/s on GC at 28°C (A) saturated with commercial oxygen, (B) saturated with nitrogen

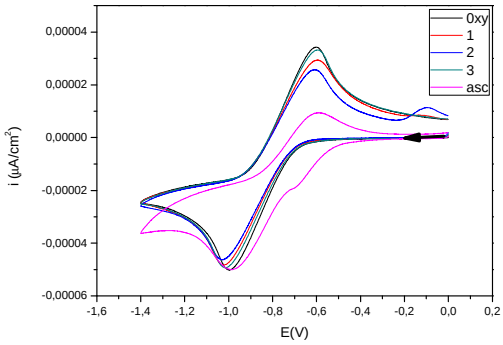


Fig. 2: Cyclic voltammograms of the O_2/O_2^{-} redox couple in oxygen-saturated DMSO/0.1 TBFP containing 0.4 mg/ml of ferrocene derivative

In generally total antioxidant capacities obtained from decrease in anodic peak current of oxygen is greater than that obtained from Molybdate ion reduction, table 2. In addition both methods show that compound 2 has the highest antioxidant capacity followed by compound 1 and 3.

Table 2: Total antioxidant capacities obtained from decrease in anodic peak current of oxygen and Molybdate ion reduction for studied ferrocene derivatives

Compound	$ip_0 - ip$ $\mu A/cm^2$	$ip_0 - i_{p_{res}}$ $\mu A/cm^2$	TAC Decrease in anodic peak current of O_2	TAC mg /mg equivalent (Molybdate ion reduction)
1	3.35	30.4	11.02 ± 0.147	0.532 ± 0.00094
2	6.38	30.4	20.98 ± 0.629	1.54 ± 0.00072
3	1.45	30.4	4.77 ± 0.927	0.418 ± 0.00058
Ascorbic acid	19.8	30.4	65.13 ± 0.779	-

The total antioxidant capacity of studied ferrocene derivatives was determined by the decrease in anodic peak current of oxygen and Molybdate ion reduction methods. The total antioxidant capacity of ferrocene derivatives ranged from (20.98 ± 0.629) to (4.77 ± 0.927) using the decrease in anodic peak current of oxygen method and from (1.54 ± 0.00072) to (0.418 ± 0.00058) using Molybdate ion reduction method. As it can be seen from table 2 both methods gave the same orders in terms of total antioxidant capacity.

CONCLUSION

In conclusion, antioxidant activities of three ferrocene derivatives bearing amine, amide or hydrazine groups were estimated using electrochemical and spectrophotometrical assays, our results clearly indicate that the three studied ferrocene derivatives possess high ability to scavenge the 1,1-diphenyl-2-picrylhydrazyl and superoxide anion radicals. We can conclude that the ethanolic solution of ferrocene derivatives showed the best antioxidant activities with electrochemical assay. Among all tested compoundN'-ferrocenylmethyl-N'-phenylbenzohydrazide (2) had the highest antioxidant activity.

Acknowledgment
The authors wish to thank Mr Ali Tliba from Laboratoire de Valorisationet et Technologie des Ressources Sahariennes, University of El Oued (VTRS) staff for his assistance.

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