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IN VITRO AND IN SILICO ANTIOXYDANT ACTIVITY, TOXICITY PREDICTION, AND MOLECULAR DOCKING STUDY OF 3- AND 3,3'-NITROPHENYLFERROCENE AND THEIR REDUCED AMINES

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ABSTRACT

The antioxidant activity of 3-nitrophenylferrocene (3NPF) and 3,3'-nitrophenylferrocene (3,3'NPF) and their reduced amines was measured using superoxide anion radical ($O^{\cdot}_2^-$). Binding parameters such as binding free energies and binding constants were also calculated. ΔG sign and values suggest respectively the spontaneity and a strong interaction between the radical $O^{\cdot}_2^-$ and all studied compounds. Molecular docking study showed that 3NPF is most inreactive compound against glutathione reductase enzyme having the the lowest docking scores of -16.96 kJ/mol. The two reduced forms were predected to be non-toxic and are not inhibitors of CYP450 2C19, 2D6 isoenzymes which suggests a decrease in their plasma concentrations and a rapid elimination route.

Keywords : Cyclic voltammetry, superoxide anion radical, binding parameters, toxicity, docking, Glutathione.

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1. INTRODUCTION

Recently a lot of work has been carried out on the antioxidant activity of ferrocenes derivatives, among these methods is the diphenyl-p-picrylhydrazyl radical method [1,2], the Trolox equivalent antioxidant capacity assay [3], ferric reducing antioxidant power [4-6], oxygen radical absorbance capacity assay [7-9]. All these methods are based on measure thing the radicals scavenging activity of the studied antioxidant compounds against the appropriate free radicals like the superoxide anion radical ($O^{\cdot -}_2$)[10-13], the hydroxyl radical ($OH^{\bullet}$) [14-15], the 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\bullet}$) radical [16-20], or the peroxyl radical ($ROO^{\bullet}$) [21-24]. Most work in the field of antioxidant activity is limited to the comparison of the antioxidant activity which are in most cases are not equal due to different applied technics. Only few reported papers dealt to the investigation of the interaction of potential antioxidant compounds with superoxide anion radical using electrochemical techniques based on cyclic voltammetry [25-27]. The reported method is based on the reaction of the electrochemical generated superoxide anion radical which the studied antioxidant compounds. The interaction parameters are determined from the voltammograms of oxygen in the presence and absence of known concentrations of the test compounds.

Glutathione plays an essential antioxidant intracellular role [28], it acts as a scavenger for oxygen radicals. The enzyme glutathione reductase (GR) reduces the oxidized form of glutathione disulfide (GSSG) to the reduced glutathione form (GSH). Elevated levels of GSSG/GSH ratio can lead to intracellular signal transduction, elimination of free radicals and reactive oxygen species, and the preservation of intracellular redox status [29]. Inhibition of glutathione reductase produces high GSSG/GSH ratio which is an important factor for choosing antioxidant compounds.

This work describes the in vitro antioxidant activity of 3-nitrophenylferrocene (3NPF) and 3,3'-nitrophenylferrocene (3,3'NPF) and their amine formula and determination of their binding parameters with superoxide anion radicals. Further, the compounds were scrutinized through toxicity study and molecular docking to predict the median lethal dose (LD50) and to afford an insight into the inhibition and binding partialities of the most potent compounds with glutathione reductase.

2. MATERIALS AND METHODS

2.1. Instrumentation and software

Cyclic voltammetry measurements were performed using PGZ301 potentiostat (radiometer analytical SAS) and a voltammetric cell with a volumetric capacity of 10 mL containing a glassy carbon electrode (GC) working electrode (radiometer analytical SAS), having an area equal to 0.013 cm^2, a Pt wire counter electrode, and an Hg/Hg_2Cl_2 reference electrode (3.0 M KCl). The solutions were saturated with high purity commercial oxygen for 10 min prior to each experiment.

2.2. Synthesis

3-Nitrophenylferrocene, 3,3'-nitrophenylferrocene and their reduced amines, figure 1 were synthesized as previously reported [30,31]

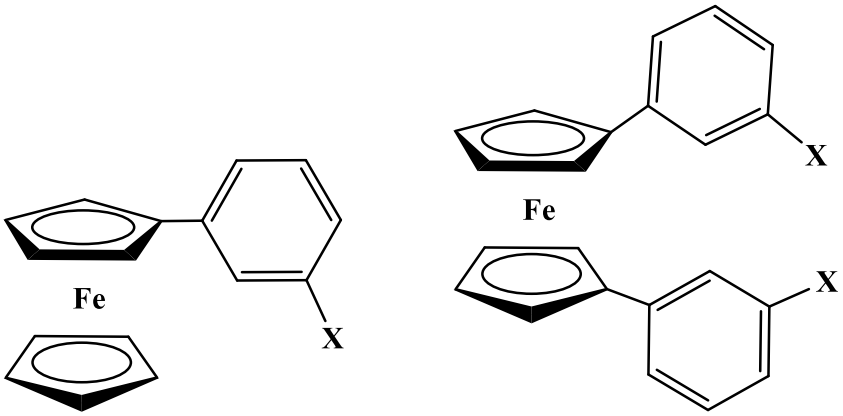


Fig.1. Structure of 3- and 3,3′-nitrophenylferrocene and reduced form X = NO_2, NH_2

3. EVALUATION OF ANTIOXIDANT ACTIVITY

Superoxide anion radical was generated by reduction of the commercial oxygen in acetonitrile containing 0.1 M tetrabutylammonium tetrafluoroborate (TBFB at) room temperature. Then each compound was added to the previous medium and the cyclic voltammograms were recorded at a scan rate of 0.1V/s in the potential window from -1.4 to 0.0 V.

The $O^{\cdot}_2^-$ scavenging activity was calculated using the following equation (1) [32],

$$\%\ O_2^{\cdot-}\ \text{radical scavenging activity} = \frac{i_0 - i_s}{i_0} \times 100 \qquad (1)$$

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 the test compounds.

4. RESULTS AND DISCUSSION

The obtained voltammograms in the absence and presence of different concentrations of the test compounds are shown in Figure 2. All voltammograms exhibits a single oxidation peak and a single reduction peak. The decrease in anodic peak current density in the presence of the test compound was used for the calculation of the binding constant [33].

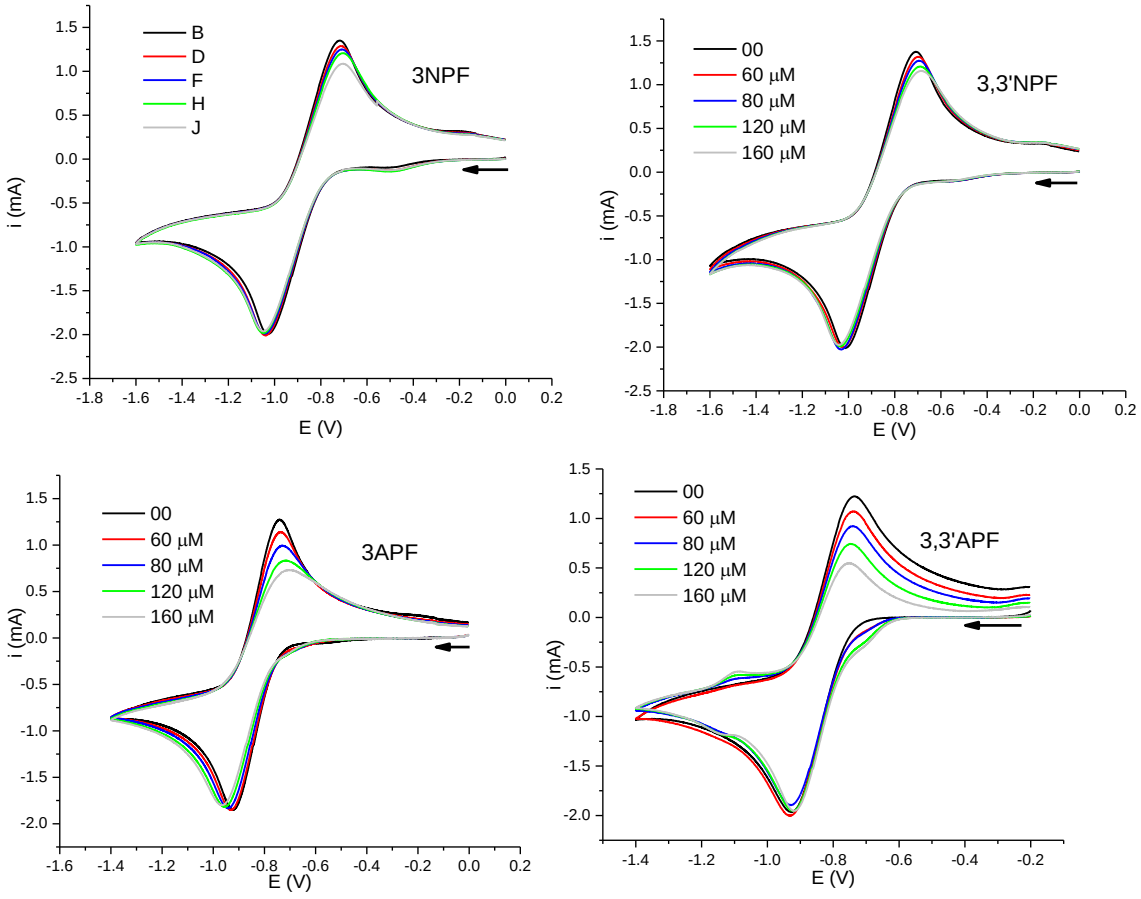


Fig. 2. Cyclic voltammograms of oxygen-saturated ACN/0.1 TBFB in the absence and presence of the test compounds at scan rate 0.1 $V.s^{-1}$

The antioxidant activity of 3-NPF, 3,3'NPF and their reduced amines was expressed as IC_{50}. The equations obtained from the linear calibration graph (data are not presented) in the studied concentration range for the test compounds and ascorbic acid are summarized in table 1.

Table 1. IC_{50} (mg/mL) values of the studied compounds

Compound	IC_{50} (mg/ml)
3NPF	0.082
3,3'NPF	0.096
3APF	0.051
3,3'APF	0.041
GA	0.011

5. BINDING CONSTANT AND BINDING FREE ENERGY

These two parameters were calculated the following equation (2) [33],

$$log\frac{1}{C} = logK_b + log\frac{i}{i_0 - i} \qquad (2)$$

C, represents the concentration of the test compound ($mol.L^{-1}$), K_b refers to the binding constant (mol^{-1}), i_0 and i are the anodic peak current densities in the absence and presence of the test compound. Obtained values of binding constant and binding free energy, obtained from the plot of equation 2 are listed in table 2.

Table 2. Binding constants and binding free energy values

Compound	K (mol^{-1})	-ΔG
$O^{\cdot-}_2$ – 3NPF	9.3×10^2	16.96
$O^{\cdot-}_2$ – 3,3'NPF	1.2×10^3	17.58
$O^{\cdot-}_2$ – AA	2.0×10^4	18.83
$O^{\cdot-}_2$ – 3APF	1.8×10^3	18.50
$O^{\cdot-}_2$ – 3,3'APF	4.2×10^3	20.67

The negative values of ΔG indicated the spontaneity of the reaction between the radical $O_2^{\cdot -}$ and the studied compounds.

6. MOLECULAR DOKING STUDY

Molecular docking study was performed in order to explore additional information about the binding affinity, conformation and orientation of the studied compounds in the active site of the receptor glutathione, this stydy also helps in chosing the appropriate antioxidant candidate and allows the simulation and visualisation of the interactions between the test compounds and the receptor glutathione.

6.1. Ligand structural optimization

The molecular structures of 3-nitrophenylferrocene, 3,3'-nitrophenylferrocene, and their reduced amines were fully optimized by employing the density functional theory, without imposing any symmetry constraints using the B3LYP level of theory [38,39] with 6-311++G(d,p) basis set usin Gaussian 09 program package [40]. Figure 3 shows the 3D optimized structure of the studied compounds.

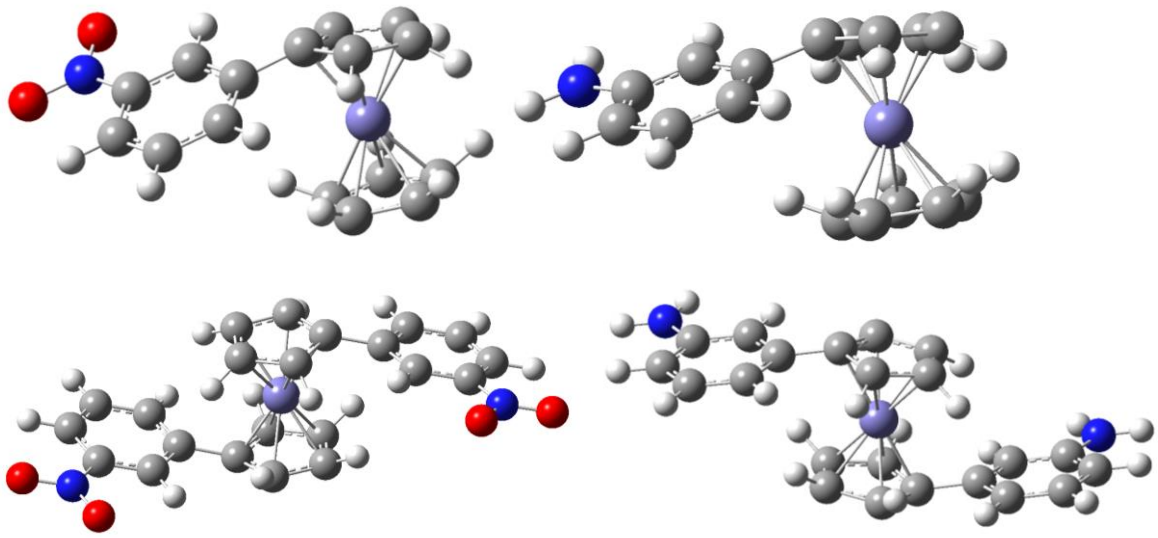


Fig.3. 3D optimized molecular structues of 3NPF, 3,3'NPF and their reduced amines; color codes are grey carbon, white hydrogen, red oxygen, and blue nitrogen

6.2. Docking simulation

Docking of the all studied compounds and the standard gallic acid into the receptor glutathione was performed using AutoDockVina software [41]. All docking studies were carried out on a Pentium 3.30 GHz and RAM 4.00 Go microcomputer MB memory with windows 10 operating system.

The crystal structure of the receptor glutathione (PDB ID: 3r3e) was obtained from protein data bank (http://www.rcsb.org./pdb) [42]. All hydrogen atoms and add kollman charges were added to the glutathione structure and non polar hydrogen atoms were merged. The grid size was set at 30×30×30 with a grid center set at x = 3.598, y = 69.809, and z = 5.406 with a spacing separation equal to 1Å. The best conformation with the lowest docking energy was selected for further docking analysis [43].

The docking results showed that all studied compounds and the standard gallic acid are placed within the active site of the glutathione recepteur. Figure 4 shows the binding mode btween the recepteur and the test compounds.

The binding energy of the docked structure of the studied compounds and the standard gallic acid with the resedues of the glutathione structure is sumurised in table 3. The magnitude of the calculated binding energy indicates a high binding affinity between the resedues of the glutathione structure and the studied compounds.

Table 3. Interaction types and binding free energy between the test compounds and glutathione reductase obtained by molecular docking

Molecule	Bond type	Amino acid (number of bonds/interactions)	Distance, Å	$-\Delta G$ / kJ mol^{-1}
3NPF	H-bonding	Trp96	2.27	30.66
		Val133	2.26	
3,3'NPF	H-bonding	His295	2.27	34.02
3APF	H-bonding	Val133	2.65	28.98
3,3'APF	H-bonding	Asn145	2.05	31.5
GA	H-bonding	Val133	2.76	24.38
		His300	2.29	
		Tyr195	2.05	
	π-sulfur	Cys63	4.85	

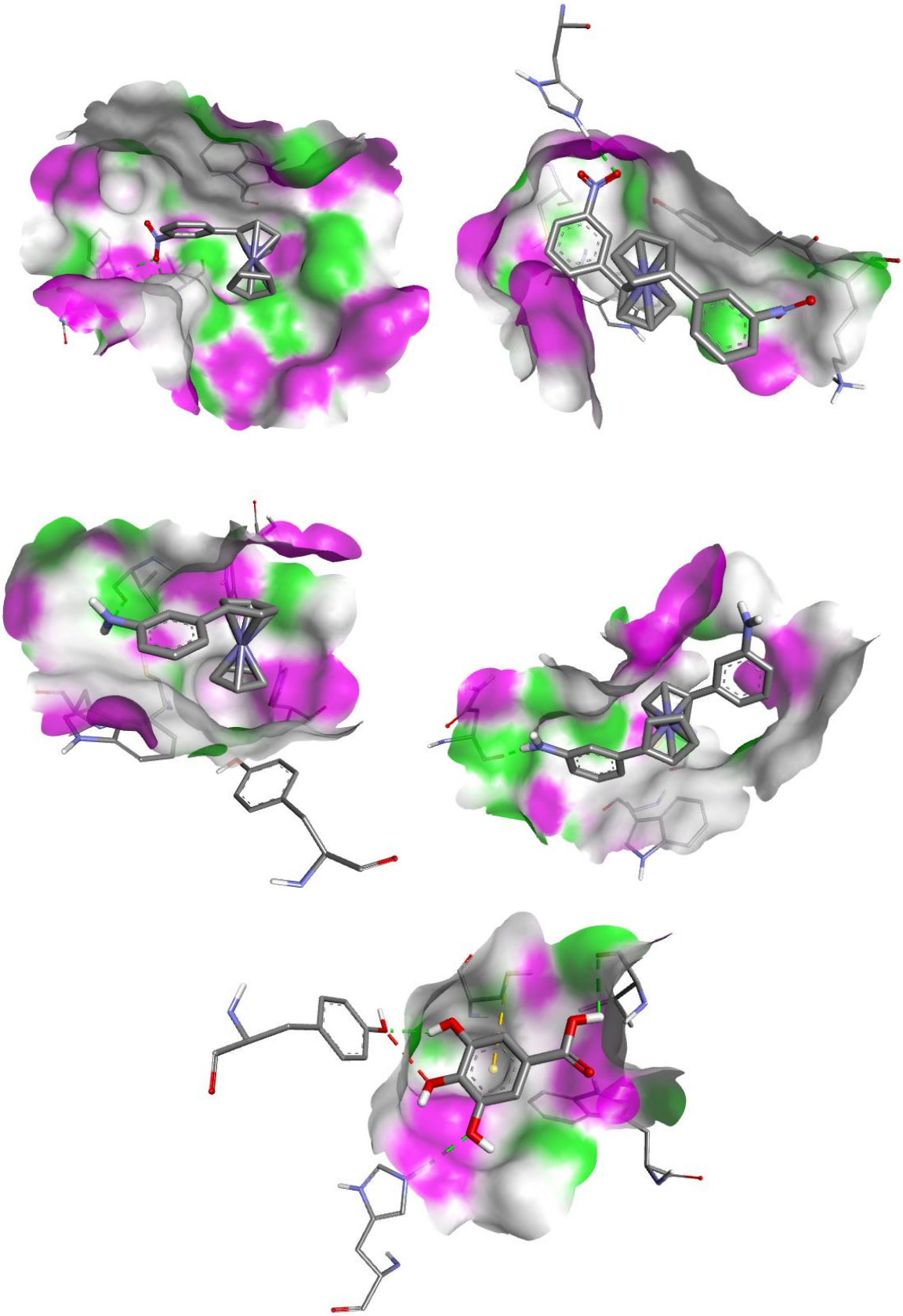


Fig.4. Best docking poses for glutathione reductase interacting with the test compounds and the control gallic acid

7. ADME STUDY

In silico ADME study was carried out to predict the adverse metabolic effects of oral administration of the studied compounds as antioxidant candidate. Cytochrome P450 isoenzymes (CYP450) are oxidases that interact with drugs in order to decrease their plasma concentration and reduce the risks of toxicity by metabolic activation, as well as making them more water soluble for elimination [34–37]. Thus, an antioxidant candidate should not inhibit cytochrome CYP450 isoenzymes because inhibition may increase the plasma concentration. Table 4 shows that all compounds are not inhibitors of CYP450 2C19, 2D6 isoenzymes which suggests a decrease in their plasma concentrations and a rapid elimination route.

Table 4. Metabolism and excretion by the CYP450 isoenzymes inhibition of the studied compounds and the control gallic acid

Compound	IA2	2C19	2C9	2D6	3A4
3NPF	Yes	No	No	No	No
3,3'NPF	Yes	No	Yes	No	No
3APF	Yes	No	No	No	No
3,3'APF	Yes	No	No	No	Yes
GA	No	No	No	No	Yes

8. TOXICITY STUDY

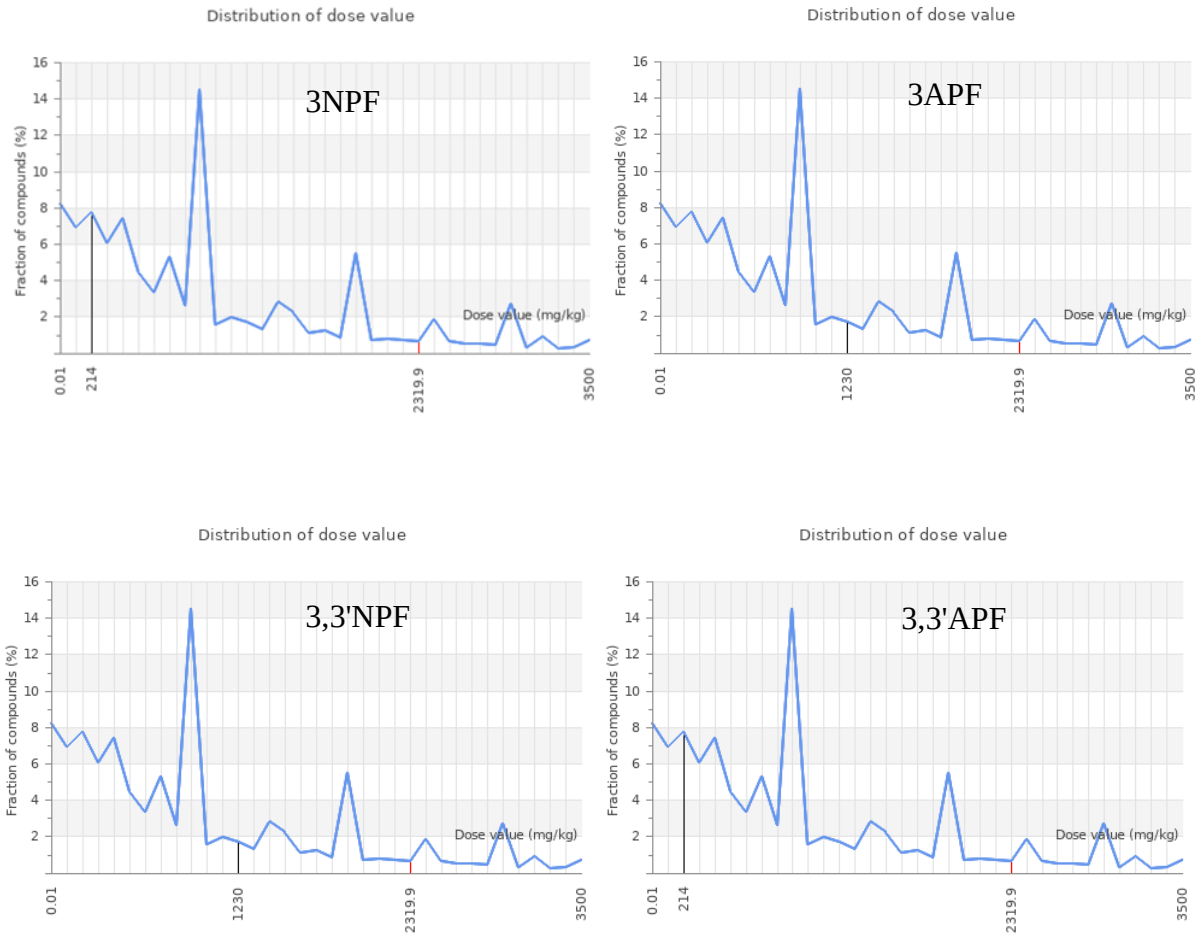
Toxicity study aims to determinate the toxicity proprieties of the studied compounds. The study aims to predict hepatotoxicity (hepato), carcinogenicity (carcino), immunotoxicity (immuno), mutagenicity (mutagen), cytotoxicity (cyto), median lethal dose (LD_{50}), and toxicity class (TC). According to in silico toxicity profiles presented in Table 5, the toxicity class was detected to be equal to 4 for both nitro derivatives and the control gallic acid, and 3 for the reduced amines. 3NPF was predicted to be very toxic, however the rest of the test compounds were less toxic.

Table 5. Toxicity end points of the test derivatives and the standard gallic acid as control

Compound	LD_{50}	Hepato	Carcino	Immuno	Mutagen	Cito	TC
3NPF	1230	+0.52	+0.64	+0.55	+0.68	-0.81	4
3,3'NPF	1230	-0.5	-0.52	-0.76	+0.90	-0.78	4
3APF	214	-0.52	+0.52	-0,84	+0.64	-0.81	3
3,3'APF	214	-0.52	+0.55	-0.95	+0.92	-0.76	3
GA	2000	-0.61	+0.56	-0.99	-0.94	-0.92	4

LD50 (mg/kg), - (Inactive toxic class (probability score)), + (Active toxic class (probability score))

Further more the distrubtion of the dose values of the studied componds and the standard gallic acid are presented in figure 5. Results indicated that the two reduced forms are better antioxidant candinated and they are comparable with the standard gallic acid.



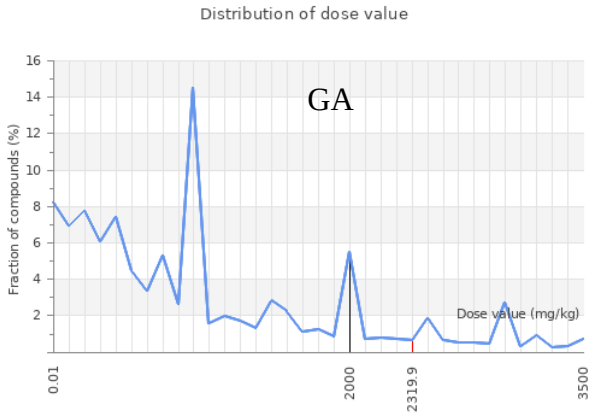


Fig. 5. Distrubtion of dose values

9. CONCLUSION

The antioxidant activity and the binding parameters of superoxide anion radical with 3-nitrophenylferrocene, 3,3'-nitrophenylferrocene and their reduced amines were successfully measured by cyclic voltammetry techniques. The obtained results indicated strong interaction of the superoxide anion radical with all studied compounds and the standard gallic acide as control. ADME analysis reavealed that the reduced forms are not inhibitors of CYP450 isoenzymes which suggests a decrease in their plasma concentrations and a rapid elimination route. Toxicity predition study demonstrated non-toxicty of the reduced forms, which is comparable with the standard gallic acid used as control.

10. ACKNOWLEDGMENT

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