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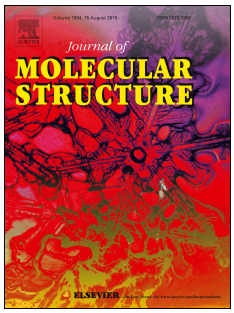

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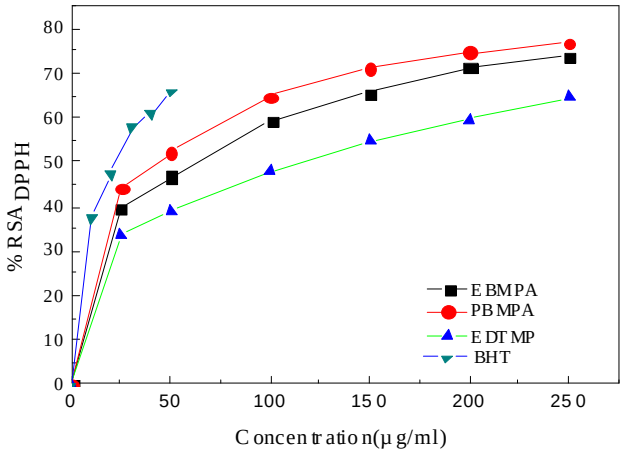

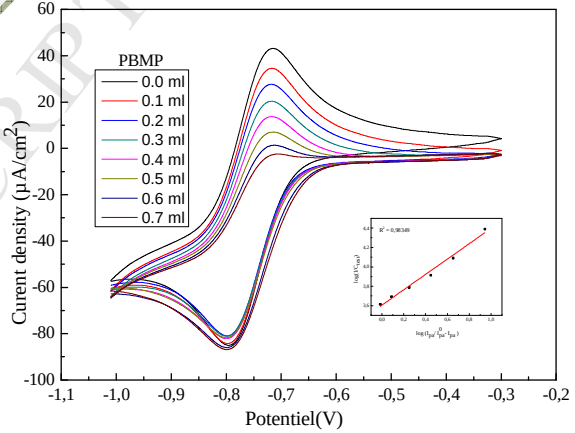

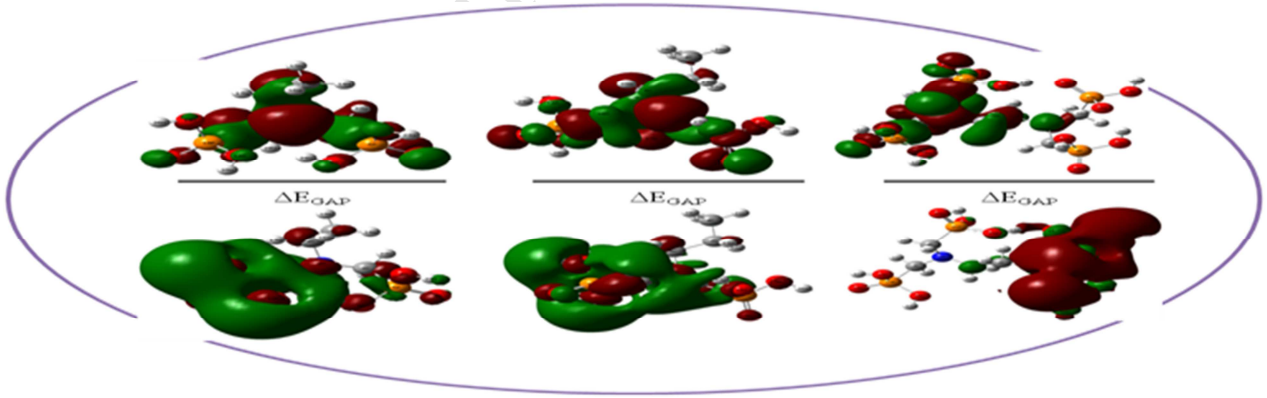

Synthesis, electrochemical and classical evaluation of the antioxidant activity of three α-aminophosphonic acids: Experimental and theoretical investigation

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Abstract

Three α-aminophosphonic acids; namely N-Ethyliminodimethylenediphosphonic acid (N-EDMPA), N-Propyliminodimethylenediphosphonic acid (N-PDMPA) and Ethylenediamine-tetrakis(methylenephosphonic acid) (EDTMP), were synthesized by the reaction of various primary amines, paraformaldehyde and phosphorous acid in acidic medium via Moedritzer-Irani reaction. The structures of the cited compounds were confirmed by UV-Vis, FT-IR, ^{1}H NMR, ^{13}C NMR and ^{31}P NMR spectra. The investigated compounds were screened for their antioxidant activity by mean of the free radical scavenging activity using 1,1-Diphenyl-2-Picryl Hydrazyl (DPPH) and Cyclic Voltammetry (CV) methods. The obtained values of the 50% inhibitory concentration (IC_{50}), show that N-PDMPA exhibit greater antioxidant activity and the order of the antioxidant activities of the studied compounds is: EDTMP < N-EDMPA < N-PDMPA. Furthermore, this biological activity is in good correlation with the density functional theory (DFT) calculations.

***Keywords*:** α-aminophosphonic acids; Antioxidant; Free radical; DPPH; Cyclic voltammetry; DFT calculations.

1. Introduction

Free radicals are highly reactive and unstable compounds. Generally, are produced in the human body during normal metabolic functions [1] or by exposure to different types of radiations: chemicals, environmental stress and the sun [2]. These reactive species are capable to oxidizing biomolecules and cause cell death and consequently cause tissue damage. Free radical oxidative processes also play a significant pathological role in causing human diseases. In medicine world, there are many illnesses are related to the oxidative tissue damage, such as cancer, emphysema, cirrhosis, atherosclerosis and arthritis [3].

Additionally, the antioxidant agents are utilized to protect human body against oxidative damage of free radicals using some complex defense systems [4]. The antioxidant behaviour of a compound is the result of its capacity to inhibit the initiation of free radical or chain breaking in the process propagating oxidation [5]. Furthermore, various antioxidants have been identified in human organism, such as endogenous enzymes (superoxide dismutase, glutathione peroxidase, catalase...) and non-enzymatic (vitamins...) [6].

Practically, there are several methods that can be used to evaluate the antioxidant activity of compounds. Mainly, these methods may differ as function of the scavenged species, the antioxidants nature, the reaction conditions and the detection methods. These techniques involve different mechanisms of the determination of the antioxidant activity [7]. However, because the results in the measurement of the antioxidant capability depend on the applied method, a single method cannot give a precise prediction of the antioxidant capacity of compounds [8, 9], it is recommended to use more than one method to estimate the in vitro antioxidant capability of a substance [10].

Nature provides a diversity of antioxidants such as ascorbic acid (vitamin C), tocopherols (vitamin E), ferritin and albumin [11]. Several works around the world have carried out that many of plant extracts and different classes of phytochemicals have been found to have quite prominent antioxidant activity [12, 13]. In some cases, the chemical synthesis makes it possible to ameliorate the activity of these natural compounds by structural modifications. Also, the organic synthesis makes it possible to obtain molecules with an antioxidant activity similar to that of the natural compounds. In particular, it has been shown that some α-aminophosphonic acids are a potential antioxidants [14-16]. The α-aminophosphonic acids are considered an important category of organophosphorus derivatives on reason of their exceptional biological activity. They have a structural analogues

of the amino acids [17], that is why the α-aminophosphonic acids are found to compete effectively with their amino acid counterparts for binding to the enzymes active centers or other cellular targets. This, together with their low toxicity makes the α-aminophosphonic acids an important class of antimetabolites and a potential source of medicinal compounds [18].

In our work, we report the synthesis of three α-aminophosphonic acids compounds employing Moedritzer-Irani reaction [19]. The antioxidant activity of the synthesized acids was evaluated by two antioxidant methodologies: cyclic voltammetry (CV) and 1,1-Diphenyl-2-Picryl Hydrazyl (DPPH) methods. In order to understand the correlation between the biological activity of the investigated compounds and their molecular structures, the different quantum chemical parameters have been determined at the optimized geometries using the density functional theory (DFT) method.

2. Experimental and computational details

All the chemical compounds utilized in this work were commercially available and were purchased from Sigma-Aldrich and Fluka. Moreover, they have used as received without any further purification.

2.1. Procedure for the synthesis of the α-aminophosphonic acids

- ***N-Ethyliminodimethylenediphosphonic acid (N-EDMPA)***: was synthesized according to the method explained by Moedritzer-Irani [19] (Fig. 1). So, 0.1 mol of ethylamine and 0.2 mol of phosphorous acid 50% were dissolved in the 100 mL of water and 50 mL of HCl 37%. The mixture was refluxed for 2 h under continuous stirring, then the paraformaldehyde (37%, 0.4 mol) was added drop by drop to the reaction mixture. Afterwards, the resulting mixture is left under reflux for 4 h. Finally, the obtained solution is cooled at room temperature and it remained clear without any formation of the precipitates. Subsequently, the solvent was removed under reduced pressure using a rotary evaporator. To the obtained concentrated solution, the ethanol has been added to obtain a white solid product, which is separated by filtration, washed with ethanol, and then dried.

- ***N-Propyliminodimethylenediphosphonic (N-PDMPA)***: was prepared by the same method as for **N-EDMPA,** using 0.1 mol of propylamine with 0.2 mol of phosphorous acid and 0.4 mol of paraformaldehyde (Fig. 1).

- ***Ethylenediamine-tetrakis(methylenephosphonic acid)*** **(*EDTMP*)**: was synthesized according to the same procedure than the previous ones, where we react 0.1 mol of ethylenediamine with 0.4 mol of phosphoric acid and 0.8 mol of paraformaldehyde.

The structures of the synthesized α-aminophosphonic acids were confirmed using IR, UV-Vis, ^{1}H, ^{13}C and ^{31}P NMR spectra.

2.2. Characterization

The melting points of the obtained α-aminophosphonic acids were measured by open capillary method in BÜCHI melting point B-540, while the UV-Vis spectra in DMSO were carried out between 200–800 nm using JASCO V-650 spectrophotometer with quartz cells of 1 cm path length. The FT-IR spectra were recorded in the region of 4000-600 cm^{-1} on a Jasco 4200 spectrometer. The ^{1}H NMR, ^{13}C NMR and ^{31}P NMR spectra of the investigated compounds were recorded on a Bruker AV III 300 MHz in D_2O with tetramethylsilane (TMS) as an internal reference.

2.3. Antioxidant activity

2.3.1. Free radical scavenging activity (DPPH)

The free radical scavenging activity of N-EDMPA, N-PDMPA, EDTMP and butylated hydroxytoluene (BHT as standard) is determined using DPPH assay. The DPPH assay is one of the most common and relatively quick methods used for estimating radical scavenging activity of a compound or a plant extract [20]. In this test, the antioxidants reduce the 1,1-Diphenyl-2-Picryl Hydrazyl ($DPPH^{\bullet}$) having a violet to a yellow color of diphenylpicryl-hydrazine (DPPH-H).

In this context, we added 1.0 mL of the α-aminophosphonic acids (or standard) dissolved in Dimethyl sulfoxide DMSO at different concentrations to 1.0 mL of the MeOH solution of DPPH, the obtained mixture is left in the dark for 30 min at room temperature. The decrease in absorbance was measured at 517 nm against blanks. The radical scavenging activity (RSA_{DPPH}) of the various tested compounds was calculated using the following equation [21]:

$$RSA_{DPPH}\,\% = \frac{A_{DPPH} - A_{sample}}{A_{DPPH}} \times 100 \qquad (1)$$

where $\boldsymbol{A_{DPPH}}$ is the absorbance of the blank (1.0 mL of DPPH–methanol solution and 1.0 mL of DMSO) and $\boldsymbol{A_{sample}}$ is the absorbance of the sample.

The 50% inhibitory concentration value (IC_{50}) expressed in µg/ml, which was defined as the effective concentration of the tested compounds, that is required to scavenge 50% of the DPPH free radicals and compared with that of the BHT [22]. All tests were carried out in three times and the IC_{50} values were reported as means SD of triplicates.

2.3.2. Scavenging towards superoxide radical using cyclic voltammetric method

Superoxide is an anionic free radical with the chemical formula $O_2^{\bullet -}$. It is formed by one-electron reduction of dioxygen (O_2) [23], which is presented abundantly in nature. Superoxide anion is the most dangerous radical among all oxygen radicals (ROS) because it has longer half-life and thus can move to a longer distance [24], also it is capable of generating other harmful radicals such as hydroxyl radical [25]. Some methods were developed to determine specifically the superoxide scavenging [26]. Recently, the cyclic voltammetry is used to determine the antioxidant activity towards this radical. This method is based on the electrochemical generation of $O_2^{\bullet -}$ by the reduction of the dissolved oxygen in aprotic solvents [27, 28].

2.3.2.1. Apparatus and accessories

The CV study was carried out employing a Voltalab 40 model PGZ301 potentiostat/galvanostat controlled by a personal computer through the voltamaster4 software. All the experiences were executed in a double walled electrochemical cell includes three electrodes engrossed in a solution including the analyte and a surplus of the supporting electrolyte. The Platinum plate having an area of 1.28 cm^2 was used as a counter electrode, a saturated calomel electrode (SCE) was used as the reference electrode and glassy carbon (GC) electrode (Ø=3.0 mm) was used as a working electrode and its surface was polished before each measurement with silicon carbide 4000 paper, then rinsed with distilled water and with the solvent used in the CV, the DMSO in our case.

2.3.2.2. Procedures

a/ Cyclic voltammetry of oxygen

In an electrochemical cell we put 20 mL of a solution containing the supporting electrolyte Bu_4NBF_4 (0.1 M) dissolved in DMSO, this solution was saturated by dry air. The solubility of oxygen in DMSO was 2.1 mM [29]. The Cyclic Voltammogram of the oxygen reduction recorded at a scan rate of 20 mV/s, the potential range was - 1.01 to - 0.3 V/SCE and at room temperature.

b/ Cyclic voltammetry of oxygen in the presence of the tested compounds

Measurement of superoxide radical scavenging activity was based on the method of Le Bourvellec *et al* [30] with slight modification in experimental conditions. The effect of various compounds was checked by the addition of the successive 0.1 mL of initial solution to 20 mL of oxygen solution and the cyclic voltammogram was recorded at the same experimental conditions used before.

The percentage of radical scavenging activity towards superoxide radicals $\left(\mathrm{RSA}_{\mathrm{O}_2^{\bullet-}}\right)$of the tested compounds was calculated using the following equation:

$$RSA_{O_2^{\bullet-}}\% = \frac{I_{pa}^0 - I_{pa}}{I_{pa}^0} \times 100 \qquad (2)$$

where $\mathrm{I_{pa}^O}$ and $\mathrm{I_{pa}}$ are the anodic peak current of $\mathrm{O_2^{\bullet-}}$ oxidation without and with the tested compounds, respectively.

2.4. Computational details

The geometric optimizations of the synthesized compounds were performed using the Gaussian program [31], based on the density functional theory (DFT), employing Becke's three parameter hybrid exchange functional with Lee-Yang-Parr correlation functionals (B3LYP) at basis sets 6-31G (d,p) [32, 33].

The quantum chemical parameters of the investigated compounds such as: energy gap ΔE_{GAP}, the ionization energy, electron affinity, electronegativity, electronic chemical potential, molecular hardness, molecular softness, and electrophilicity index which can be calculated using the following equations [34]:

Energy gap: $\Delta E_{GAP} = E_{HOMO} - E_{LUMO}$ (3)

Ionization Energy: $I = -E_{HOMO}$ (4)

Electron Affinity: $A = -E_{LUMO}$ (5)

Electronegativity: $\chi = (I + A)/2$ (6)

Chemical Potential: $\mu = -\chi$ (7)

Chemical Hardness: $\eta = (E_{LUMO} - E_{HOMO})/2$ (8)

Chemical Softness: $S = 1/\eta$ (9)

Electrophilicity Index: $\omega = \mu^2/2\eta$ (10)

These parameters are very important to understand the chemical and biological activities of the synthesized compounds.

3. Results and Discussion

3.1. Spectroscopic study

In this work, we have prepared three α-aminophosphonic acids by Moedritzer-Irani reaction, which is Mannich reaction analogous, it takes place by the nucleophilic attack of phosphorous acid on the iminium salt formed by reaction between formaldehyde and the amine in acidic medium. We obtained various aminophosphonic acids by refluxing three aliphatic amines (ethylamine, propylamine and ethylenediamine) with phosphorous acid and formaldehyde in hydrochloric media (Fig. 1). The resulting acids are very soluble in water and therefore have to be recrystallized using ethanol. No byproducts were observed and the compounds were isolated in good yields.

The structures of the obtained compounds were identified and characterized by their spectroscopic data (UV-Vis, FT-IR, and by ^{1}H NMR, ^{13}C NMR and ^{31}P NMR spectroscopy).

The obtained IR spectra of N-EDMPA, N-PDMPA and EDTMP, show bands in the region 3000−2838 cm^{-1} attributed to the stretching vibrations of C−H aliphatic. In addition, all bands occurred in the region 1400−1230 cm^{-1} are assigned to the deformation vibrations of (C−H). The new absorption bands situated in the regions, 2550–2700, 1040–910 and 769–780 cm^{-1} are ascribed to the stretching vibration of (O=)PO-H, P=O and P-C aliphatic, respectively, indicated the formation of phosphonic acid. The bands appeared at 1275, 1283 and 1207 cm^{-1} in N-PDMPA, N-PDMPA and EDTMP correspond to (C−N) linkage, respectively. The absence of the characteristic bands of primary amine (NH_2) and aldehyde (C=O) confirms the formation of the proposed α-aminophosphonic acids framework.

The electronic absorption spectra of the synthesized α-aminophosphonic acids displayed intense bands in UV region which may be assigned to $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions in their spectra. The UV-Vis spectra of these three α-aminophosphonic acids indicate that they are transparent in the visible region.

The structures of N-EDMPA, N-PDMPA and EDTMP were confirmed by analysis of NMR data. ^{1}H NMR spectrum of these compounds showed a doublet peak for methylene, due to coupling of methylene protons with the phosphorus atom. The ^{13}C NMR spectral data of the synthesized compounds are also in accordance with the proposed structures and expected

numbers of carbon were observed. The presence of phosphorus atoms in three compounds is confirmed by their ^{31}P NMR spectra. In the ^{31}P NMR spectra the signal of the phosphorus atom appeared as triplet which results from the coupling of the phosphorus atoms with the two protons of the methylene group.

3.1.1. Analytical data

N-Ethyliminodimethylenediphosphonic acid (N-EDMPA)

Mol.Wt: 233.097. Yield: 85% of white solid, mp 207 °C, UV–Vis (DMSO): λ_{max} = 282 nm (ε_{282}= 210.253 l.mol^{-1}.cm^{-1}), ^{31}P NMR (DMSO-d_6) δ (ppm) = 8.57 (t, J= 12.7 Hz), ^{1}H NMR (DMSO-d_6) δ (ppm) = 1.22; (t, 3H, J= 7.2 Hz, C**H**$_3$-CH$_2$), 3.21 (s, 4H, N-C**H**$_2$-P), 3.45 (m, CH$_3$-C**H**$_2$), 4.72 (s, 4H, P-O**H**), ^{13}C NMR (DMSO-d6), δ (ppm) = 8.32 (**C**H$_3$-CH$_2$), 49.81 (CH$_3$-**C**H$_2$), 51.21 (**C**H$_2$-P), 52.39 (**C**H$_2$-P), IR (Solid state) ν (cm^{-1}): 2999 (C-H$_{aliphatic}$), 2667 ((O=) P-OH), 1275 (C-N), 1106 (P=O), 937 (δ P-OH), 709 (P-C).

N-Propyliminodimethylenediphosphonic acid (N-PDMPA)

Mol.Wt: 247.123. Yield 83.4% of white solid, mp 184 °C, UV–Vis (DMSO): λ_{max}= 282 nm (ε_{282}= 244.157 l.mol^{-1}.cm^{-1}), ^{31}P NMR (DMSO-d_6), δ (ppm) = 8.367 (t, J= 12.7 Hz), ^{1}H NMR (D_2O), δ (ppm) = 0.71 (t, 3H, J= 7.22 Hz, C**H**$_3$-CH$_2$), 1.51 (m, 2H, CH$_3$-C**H**$_2$-CH$_2$), 3.20 (m, 2H, CH$_2$-C**H**$_2$-N), 3.38 (d, 4H, J= 12.6 Hz, N-C**H**$_2$-P), 4.76 (s, 4H, P-O**H**), ^{13}C NMR (D_2O), δ (ppm) = 9.72 (s, **C**H$_3$-CH$_2$), 16.75 (s, CH$_3$-**C**H$_2$), 49.95 (s, N-**C**H$_2$-P), 51.7 (s, N-**C**H$_2$-P), 58.79 (s, CH$_2$-**C**H$_2$-N), IR (Solid state) ν (cm^{-1}): 2999 (C-H$_{aliphatic}$), 2975 (C-H$_{aliphatic}$), 2535 ((O=)P-OH), 1283 (C-N), 1138 (P=O), 935 (δ P-OH), 715 (P-C).

Ethylenediamine-tetrakis(methylenephosphonic acid) (EDTMP)

Mol.Wt: 436.124. Yield 79% of white solid, mp 239 °C, UV–Vis (DMSO): λ_{max} = 282 nm (ε_{282}= 888.82 l.mol^{-1}.cm^{-1}), ^{31}P NMR (DMSO-d_6), δ (ppm) = 8.21(t, J= 12.4 Hz), , ^{1}H NMR (D_2O), δ (ppm) = 3.55 (d, 8H, J= 12.6 Hz -N-C**H**$_2$-P), 3.86 (s, 4H, N-C**H**$_2$-C**H**$_2$-N), 4.79 (s, 8H, P-O**H**), ^{13}C NMR (D_2O, δ ppm): 51.63 (N-**C**H$_2$-**C**H$_2$-N), 52.73 (4C, N-**C**H$_2$-**P**), IR (Solid state) ν (cm^{-1}): 2965 (C-H$_{aliphatic}$), 2593 ((O=) P-OH), 1207 (C-N), 1115 (P=O), 946 (δ P-OH), 742 (P-C).

3.2. Antioxidant activity

3.2.1. Free radical scavenging activity (DPPH)

DPPH is a very stable free radical because of its spare electron delocalization over the whole molecule. DPPH gives a maximum absorption at 517 nm with a deep violet color,

when a solution of DPPH is mixed with a substrate acting as a hydrogen atom donor, a stable non radical form of DPPH is obtained with simultaneous change of the violet to pale yellow color and decrease in absorbance [35, 36]. DPPH may be also neutralized by direct reduction via electron transfer [37].

The obtained results from DPPH radical scavenging assay are illustrated in Fig. 2, these data showed that, all the tested products show a gradual increase in activity with increase of concentration.

The DPPH results for the investigated compounds were expressed as IC_{50} and its values are given in Fig. 3. The lower IC_{50} value, proves the higher free radical scavenging activity of a sample, was indicating that a smaller amount is sufficient to decrease the concentration of DPPH by 50%. The Table 1 shows that the N-PDMPA (IC_{50} = 30.423 µg/ml) was found to be the most active DPPH radical scavenger, followed by N-EDMPA (IC_{50} = 67.408 µg/ml). The EDTMP showed the highest IC_{50} (IC_{50} = 129.236 µg/ml), which indicates that it has a moderate antioxidant capacity, the IC_{50} value of the standard in the order of 24.387 µg/ml.

The results of this study show that the α-aminophosphonic acids are free radical scavengers and primary antioxidants that reduced the DPPH to the corresponding hydrazine, which may be attributable to its proton-donating ability.

3.2.2. The capability of scavenging towards superoxide radical by cyclic voltammetric method

In the development of the procedure, the cyclic voltammogram was firstly recorded in the absence of the N-EDMPA, N-PDMPA and EDTMP to determine the anodic peak current I_{pa}^{o}, this value corresponds to the concentration of $O_2^{\bullet -}$ at the electrode surface.

3.2.2.1. Electrochemical generation and voltammetric behavior of superoxide radical

The radical $O_2^{\bullet -}$ was generated according to the reduction reaction (i) (Fig. 4, peak C). The presence of this radical is easily detected by its anodic current measured during the reverse scan according to the oxidation reaction (Fig. 4, peak A).

The cyclic voltammogram of the superoxide anion radical showed a reversible process and the obtained value of ΔEp is 70 mV which is the difference between the oxidation and reduction peaks potentials [28].

3.2.2.2. Voltammetric behavior of superoxide radical in the presence of N-EDMPA, N-PDMPA and EDTMP

In CV method, the tested compounds must be inactive in the potential range of the couple $O_2/O_2^{\bullet -}$ in order to avoid interference or a complex interpretation of the voltammogram. For this reason, before investigating the radical scavenging of our compounds towards superoxide radical, we conducted the voltammetry of the studied compounds in potential range of - 1.01 to - 0.3 V/SCE, and we found that N-EDMPA, N-PDMPA and EDTMP are inactive in this range of potential.

The cyclic voltammogram of the super oxide anion radical in the presence of N-EDMPA, N-PDMPA and EDTMP showed a proportional decrease in anodic current while the intensity of the cathodic current is not significantly modified (Fig. 5).

The decrease of the anodic peak current of superoxide anion radical suggests that the tested products react irreversibly with this radical and decrease its concentration around the electrode surface. No change in the cathodic peak current imparts that there is no interaction between the tested compounds and the molecular oxygen.

The observed changes in the shape of the cyclic voltammogram are identical in the three cases, this behavior is analogous to that reported in literature for some commercial flavonoids [30, 38]. Based upon this analogy, the proposed mechanism is thus H atom abstraction from the compound by the superoxide radical and can be represented in the followed mechanism:

$$O_2 + 1e \longrightarrow O_2^- \qquad \text{(i)}$$

$$O_2^- + ROH \longrightarrow \left(RO - HO_2\right) \longrightarrow HO_2^- + RO \qquad \text{(ii)}$$

$$HO_2 + ROH \longrightarrow H_2O_2 + RO \qquad \text{(iii)}$$

This mechanism is a reversible electron transfer followed by an irreversible chemical reaction (hydrogen atom transfer). The electrochemically formed superoxide anion radical reacts with the antioxidant and forms an electro inactive species which is a radical and a conjugate base of the hydrogen peroxide. In the other hand, the conjugate base of hydrogen peroxide is not stable and reacts with antioxidant, taking a proton to form a relatively stable product H_2O_2. The $RO^{\bullet}$ produced is assumed to be of very low toxicity because of high probability of its dimerization.

3.2.2.3. The scavenging activity and determination of IC_{50} values

The scavenging activity of the antioxidant was evaluated by its IC_{50} calculated from the linear equation (The percentage of radical scavenging activity versus compound

concentration). From Table 1, it can be seen that the lowest value of IC_{50} (51.24 µg/ml) was estimated for N-PDMPA and it corresponds to the highest antioxidant activity, while the highest value of IC_{50} (87.55 µg/ml) was detected for EDTMP.

3.2.2.4. Thermodynamic parameters

The thermodynamic feasibility of the radical scavenging by the synthesized α-aminophosphonic acids was estimated in terms of binding constant (K_b) and standard Gibbs free energy (ΔG°). The K_b values are determined using the curves of Fig. 6 plotted from the following equation [39]:

$$log\left(\frac{1}{C_{test}}\right) = log\, K_b + log\left(\frac{I_{pa}}{I_{pa}^{O} - I_{pa}}\right) \qquad (11)$$

where C_{test} is the concentration of the tested compounds.

From Table 1, it is apparent that the obtained K_b values of the tested α-aminophosphonic acids are high values which confirm the strong interaction of the investigated compounds with the radical O_2^{-}.

ΔG° can be calculated using K_b involving the following equation (12):

$$\Delta G° = -RT \ln (C_{solvent}\, K_b) \qquad (12)$$

where R is the gas constant (8.314 J K^{-1} mol^{-1}), T (K) the absolute temperature and $C_{solvent}$ is the molar concentration of a solvent, which in this case is the DMSO (C_{DMSO} = 14.08 M).

Negative values of ΔG° (Table 1) indicates the spontaneity of the antiradical reaction, but also points towards the stability of the newly formed species, which in turn is a strong evidence of the effectiveness of the tested compounds.

3.3. Computational study

In the last few years, it was shown that the quantum-chemical calculations can be a valuable tool in investigating the structure-activity relationships of various compounds and the DFT study is one of the most widely used computational methods due to its accuracy and less time consumption.

3.3.1. Structural and electronic properties

The optimized molecular structures calculated by B3LYP/6- 31G (d, p) method of the most stable form of N-EDMPA, N-PDMPA and EDTMP are shown in Fig. 7.

The electron donor distribution in the highest occupied molecular orbital (HOMO) and the electron acceptor distribution in the lowest unoccupied molecular orbital (LUMO) are calculated and the results are shown in Fig. 8.

The energy of HOMO corresponds to the ionization potential (I), whereas the energy of LUMO corresponds to the electron affinity (A). On the other hand, the energy gap is the minimum energy needed to excite an electron in a molecule and reflects the chemical activity and the biological activity of the molecule. The larger HOMO – LUMO energy gap indicates the harder and more stable/less reactive molecule. The decrease in the energy gap is responsible for the bioactivity of the molecule [40].

It can be seen from Table 2 that the energy gap of the N-EDMPA, N-PDMPA and EDTMP was found smaller, suggesting its good activity and the high antioxidant capacity has been obtained for the N-PDMPA compound (ΔE_{GAP} = -7.207 eV).

Table 2 recapitulated the obtained values of the chemical reactivity descriptors of the studied compounds determined by DFT calculations, such as total energy (E), chemical hardness (η), electronic chemical potential (μ) and electrophilicity (ω).

According to the calculated values of the total energy for the studied compounds (Table 2), we observe that the EDTMP presents the least value of E_{Tot}, which shows that the EDTMP is more stable than N-EDMPA and N-PDMPA.

The determined stable conformer of the stable compounds (EDTMP) was performed using the conformational analysis. Therefore, the potential energy surface (PES) was constructed by changing the selected dihedral angle N1−C2−C3−N4 from 0^o to 180^o with a step of 10°. On the other hand, the remaining geometrical parameters have been simultaneously relaxed. The obtained PES scan for the selected dihedral angle has presented in Fig. 9. In principle, the minimum potential energy is due to the more stable conformation, which is the geometry at equilibrium. From the results presented in Fig. 9, we observe obviously that the minimum energy conformation is attributed to an angle of 180.16°, which indicates the most stable conformation with an energy value of −71249.0587 eV.

3.3.2. Molecular electrostatic potential surfaces (MEP)

In order to find the active sites responsible for electrophilic and nucleophilic attacks, we chose to use the molecular electrostatic potential surfaces (MEP) as a useful descriptor.

MEP illustrates the charge distributions of molecules three dimensionally and correlates the total charge distribution with dipole moment, electronegativity, partial charges and site of chemical reactivity of a molecule [41]. The different values of the MEP at the surface of the studied acids appear with the different colours (Fig. 10). In general, the red and yellow colors indicate the negative regions of the MEP and correspond to nucleophilic reactivity, while the blue color indicates the positive regions corresponding to electrophilic reactivity.

The results of the MEP show that negative potentials are presented at electronegative oxygen atoms, whereas positive potentials are presented at hydrogen atoms for all compounds.

It has been proved by Aburas *et al* [42], that there are a high correlation between the antioxidant activities values of the investigated derivatives and the dipole moment and charge on oxygen atoms obtained by the natural charge distribution (NBO) method. All three compounds are polar molecules, judging by their dipole moments (Table 2) and the most polar molecule is N-PDMPA this confirms its higher antioxidant activity compared with two other acids.

3.3.3. Atomic charges for N-EDMPA, N-PDMPA and EDTMP

Recently, the use of the allocation of positive and negative charges presents an essential position in quantum chemical calculations; this is due to a several properties of molecular system which is affected by the atomic charges, such as electronic structure, molecular polarizability, dipole moment and acid-base behavior [43]. Generally, the charge allocation on the atoms indicates the contributor and acceptor pairs including the charge transport in the molecule [44, 45]. Additionally, in comparison with Mulliken charges the NBO charges are very trustworthy, because they perform on the electron density simultaneously. Moreover, the NBO examination offers a powerful technique for examining intra and intermolecular bonding and interaction between bonds, and also offers a suitable basis for examination of charge transport or conjugative interactions in molecular system. In the other hand, the NBO analysis can be employed to evaluate the delocalization of electron density among occupied Lewis-type orbitals and vacant non-Lewis NBOs, which indicates the stabilization of the donor–acceptor interactions [46].

In this context, the investigated compounds have O, N and P heteroatoms capable to interact with the reactive sites of the receptor. For the studied compounds, the natural atomic

charges of the selected atoms are listed in Table 3. We observe clearly that the highest negative charge to be found on the O7, O8, O9, O11, O12 and O13 for N-EDMPA and O7, O8, O12, O13 and O14 for N-PDMPA and O10, O11, O12, O14, O15, O16, O18, O19 and O20 for EDTMP. On the other hand, the highest positive charge to be found on the P6 of N-EDMPA, N-PDMPA and EDTMP. The positive part of the receptor is interacted more probably with the negatively charged atomic sites of the investigated compounds (O and N), while the negative part of the receptor will interact more facilely with the most positively charged atoms (P and H). These interactions maybe play an essential role in bioactivity of the investigated molecules [47]. Finally, from the NBO analysis we conclude that the acidity of the hydrogen atoms of hydroxyl group is very significant for the antioxidant activity.

4. Conclusions

In the present work, we have synthesized three bioactive α-aminophosphonic acids using various primary amines as the starting materials. The obtained compounds are stable solids characterized by high melting points and their structures were established by IR, ^{1}H, ^{13}C and ^{31}P NMR spectra. Moreover, the antioxidant activity of the target compounds was examined by both chemical DPPH radical-scavenging activity and electrochemical method. Cyclic voltammetry method developed in this study measures the scavenging effect toward superoxide anion radical and indicates the strong interaction between the $O_2^{\bullet -}$ and N-EDMPA, N-PDMPA and EDTMP, this is quantified in terms of the high obtained values of K_b. In the other hand, the negative sign of $\Delta G°$ suggests the spontaneity of the antiradical reaction. According to this method, we can also propose that the antiradical reaction mechanism is the hydrogen atom transfer.

Both methods indicate that the studied α-aminophosphonic acids may act as a moderate antioxidant. Also, they indicate that the difference in the chemical structures of the investigated α-aminophosphonic acids affect the antioxidant activity. Consequently, the highest activity of N-PDMPA is probably attributed to the increase in the number of carbon atoms. The presence of several adjacent hydroxide groups in the EDTMP molecular structure increase the intermolecular hydrogen bonds, this will decrease their antioxidant activity.

For a better understanding of the antioxidant activity, the quantum chemical parameters were calculated employing DFT method. A good correlation was visible between the experimentally reported values and the calculated quantum chemical parameters such as

ΔE_{GAP}, μ and NBO charges. It was found for N-EDMPA, N-PDMPA and EDTMP that the antioxidant activity increased with the smaller values of ΔE_{GAP}, and higher values of μ.

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Figure Captions

Fig.1. Synthesis route of α- aminophosphonic acids derivatives:

N-Ethyliminodimethylenediphosphonic acid (N-EDMPA), n=2
N-Propyliminodimethylenediphosphonic acid (N-PDMPA), n=2
Ethylenediamine tetra-methylenephosphonic acid (EDTMP), n=4

Fig.2. Radical Scavenging Activity of N-EDMPA, N-PDMPA, EDTMP and BHT with different concentration.

Fig.3. IC_{50} for the RSA_{DPPH} displayed by N-EDMPA, N-PDMPA, EDTMP and BHT, Values are Mean ± S.D (n=3 for test compounds and standards).

Fig.4. Cyclic voltammograms of (a) DMSO - Bu_4NBF_4, 0.1 M (b) $O_2^{\bullet -}$ in DMSO - Bu_4NBF_4, 0.1 M medium with scan rate = 20 mV/s:

Glassy carbon (GC) as a electrode working
Platinum Plate (1.28 cm^2) as a counter electrode
Saturated calomel electrode (SCE) as a reference

Fig.5. Evolution of the cyclic voltammograms of $O_2^{\bullet -}$ in the presence of different volumes of N-EDMPA, N-PDMPA and EDTMP, in DMSO - Bu_4NBF_4 0.1 M medium with scan rate = 20mV/s:

Glassy carbon (GC) as a working electrode
Platinum Plate (1.28 cm^2) as a counter electrode
Saturated calomel electrode (SCE) as a reference

Fig.6. $\log (I_{pa} / I_{pa}^{O} - I_{pa})$ versus $\log (1/C_{test})$ for N-EDMPA, N-PDMPA and EDTMP.

Fig.7. Optimized molecular structures of N-EDMPA, N-PDMPA and EDTMP.

Fig.8. The frontier molecular orbitals density distributions for N-EDMPA, N-PDMPA and EDTMP.

Fig.9. Scan of total energy of the more stable compounds (EDTMP) with the selected dihedral angle (N1−C2−C3−N4).

Fig.10. Molecular electrostatic potential of N-EDMPA, N-PDMPA and EDTMP.

Table Captions

Table 1

IC_{50} values of N-EDMPA, N-PDMPA and EDTMP obtained by the DPPH and superoxide anion inhibition tests.

Table 2

Global chemical reactivity descriptors for N-EDMPA, N-PDMPA and EDTMP calculated by B3LYP/6- 31G (d, p).

Table 3

Atomic charges of N-EDMPA, N-PDMPA and EDTMP calculated by B3LYP/6- 31G (d, p).

Table 1

IC_{50} values of N-EDMPA, N-PDMPA and EDTMP obtained by the DPPH and superoxide anion inhibition tests

Compound	Methods	IC_{50}(µg/ml)	K_b (l/mol)	-ΔG°(kJ/mol)
N-EDMPA	**DPPH**	67.408± 0,8		
	$O_2^{\bullet -}$	65.668	3451.437	26.736
N-PDMPA	**DPPH**	30.423± 6,5		
	$O_2^{\bullet -}$	57.635	4036,454	27.212
EDTMP	**DPPH**	129.236± 3,9		
	$O_2^{\bullet -}$	87.55	3162.27	26.519

Table 2

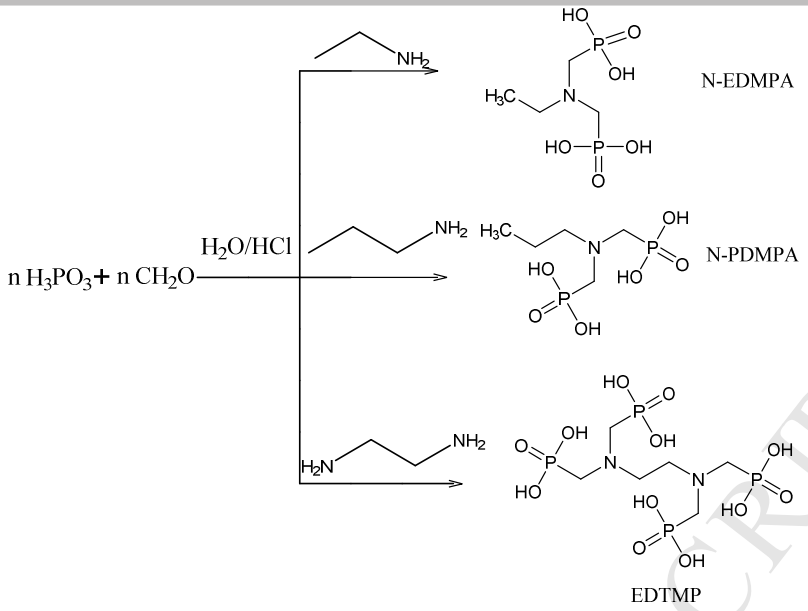
Global chemical reactivity descriptors for N-EDMPA, N-PDMPA and EDTMP calculated by B3LYP/6- 31G (d, p)

Paramètres quantiques	N-EDMPA	N-PDMPA	EDTMP
E_{Tot} (eV)	– 36713.80374	–37783.56306	− 71255.34237
E_{HOMO} (eV)	– 6.34461	– 6.59604	− 5.85671
E_{LUMO} (eV)	0.52355	0.611440	0.37552
ΔE_{GAP} (eV)	– 6.86816	– 7.20748	– 6.23223
Ionization Energy (I)	6.34461	6.59604	5.85671
Electron Affinity (A)	– 0.52355	– 0.61144	– 0.37552
Electronegativity (χ)	2.91053	2.99230	2.74059
Chemical Potential (μ)	– 2.91053	– 2.99230	–2.74059
Chemical Hardness (η)	3.43408	3.60374	3.11611
Chemical Softness (S)	0.29112	0.27749	0.32091
Electrophilicity Index (ω)	1.2334	1.24230	1.20516
Dipole Moment (Debye)	4.7193	4.9175	2.9445

Table 3

Atomic charges of N-EDMPA, N-PDMPA and EDTMP calculated by B3LYP/6- 31G (d, p)

N-EDMPA		N-PDMPA		EDTMP	
Atom	NBO Charge	Atom	NBO Charge	Atom	NBO Charge
C1	-0.6948400	N1	-0.5433800	N1	-0.5172700
C2	-0.2637600	C2	-0.6647600	C2	-0.2801700
N3	-0.5351900	C3	-0.6764000	C3	-0.2702500
C4	-0.6658700	C4	-0.6887400	N4	-0.5460600
C5	-0.6705400	C5	-0.4804200	C5	-0.6624900
P6	2.3891700	C6	-0.2595500	C6	-0.6636600
O7	-1.0715200	P7	2.3593500	C7	-0.6481700
O8	-1.0310900	O8	-1.0672900	C8	-0.6604000
O9	-1.0419400	O9	-1.0282700	P9	2.3830400
P10	2.3742500	O10	-1.0272300	O10	-1.0561700
O11	-1.0697000	P11	2.3486100	O11	-1.0526900
O12	-1.0357400	O12	-1.0687200	O12	-1.0370900
O13	-1.0201000	O13	-1.0298700	P13	2.4109200
H14	0.2332600	O14	-1.0230800	O14	-1.0990500
H15	0.2374100	H15	0.2753800	O15	-1.0146400
H16	0.2451500	H16	0.2753900	O16	-1.0469400
H17	0.2496500	H17	0.2761200	P17	2.3353700
H18	0.2065300	H18	0.2678800	O18	-1.0730700
H19	0.2520900	H19	0.2312600	O19	-1.0288200
H20	0.2773400	H20	0.2300500	O20	-1.0268300
H21	0.2492100	H21	0.2440400	P21	2.3854000
H22	0.2672000	H22	0.2448200	O22	-1.0784500
H23	0.5239400	H23	0.2360500	O23	-1.0646500
H24	0.5303700	H24	0.2266100	O24	-1.0304600
H25	0.5355900	H25	0.2386700	H25	0.2564300
H26	0.5291100	H26	0.5242600	H26	0.2298600
		H27	0.5216000	H27	0.2086900
		H28	0.5312500	H28	0.2582100
		H29	0.5263800	H29	0.2760200
				H30	0.2591500
				H31	0.2748800
				H32	0.2490500
				H33	0.2688900
				H34	0.2668400
				H35	0.2632800
				H36	0.2539400
				H37	0.5361800
				H38	0.5323700
				H39	0.5350100
				H40	0.5505900
				H41	0.5275300
				H42	0.5236300
				H43	0.5475900
				H44	0.5244700



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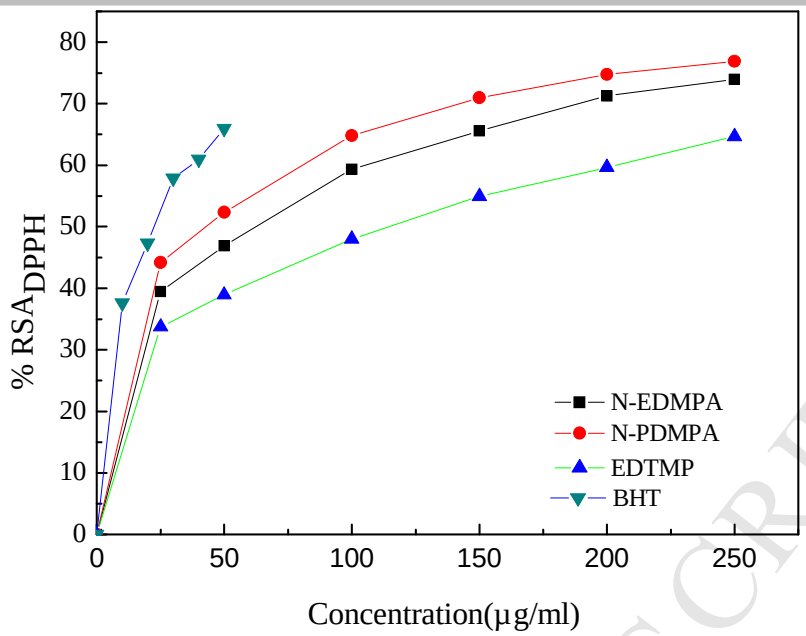
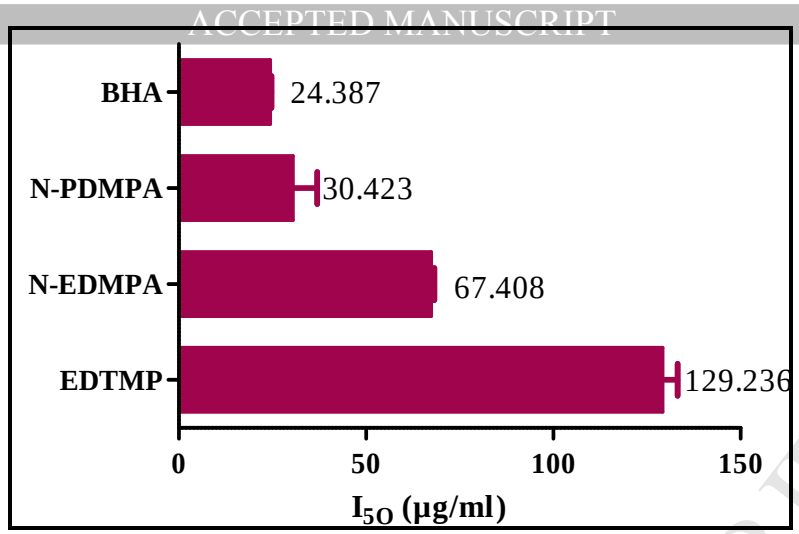


Fig.2. Radical Scavenging Activity of N-EDMPA, N-PDMPA, EDTMP and BHT with different concentration



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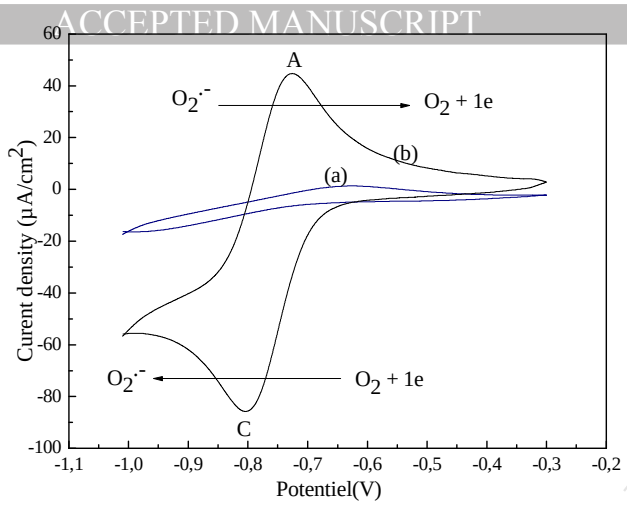

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- Glassy carbon (GC) as a electrode working
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- Saturated calomel electrode (SCE) as a reference

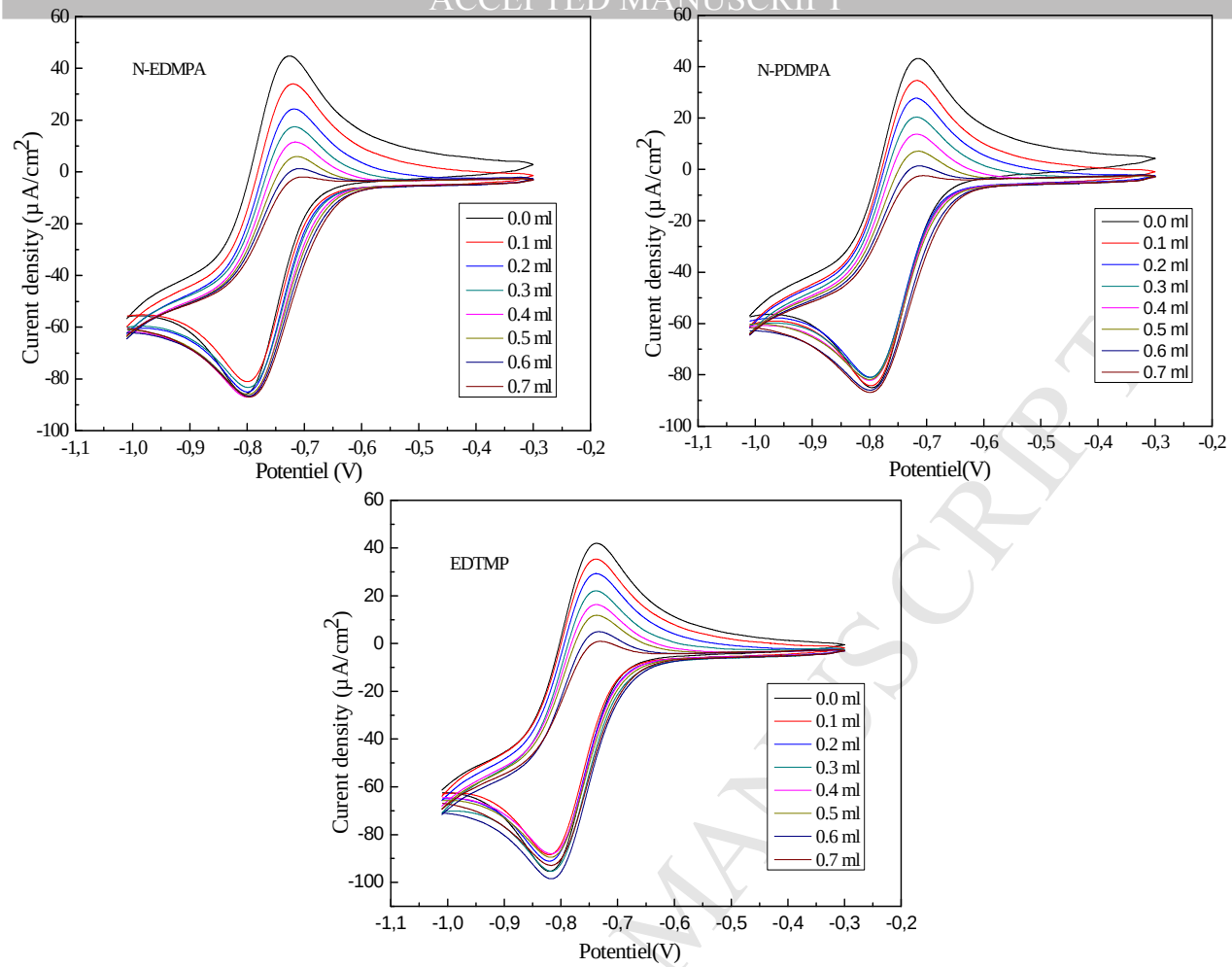
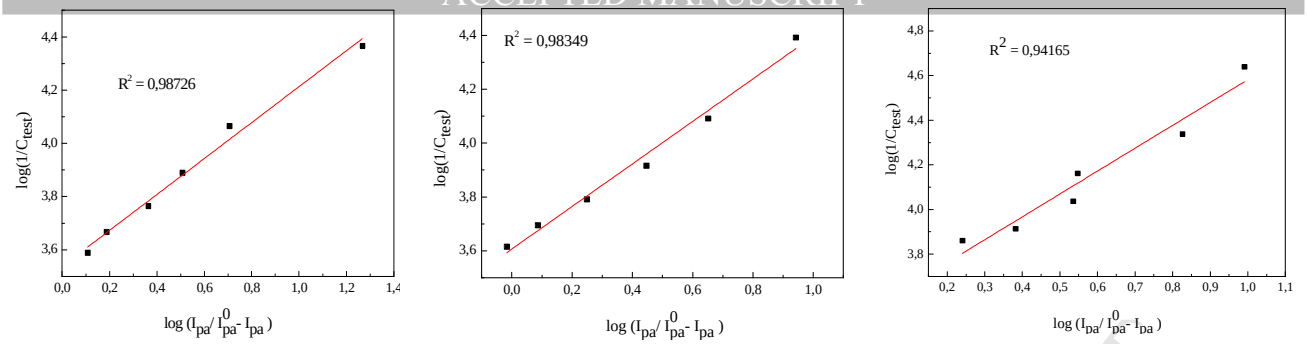


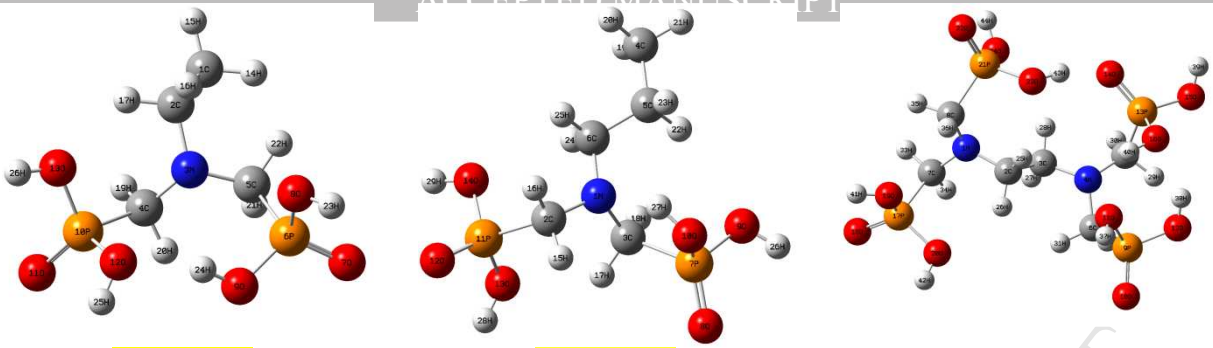
Fig.5. Evolution of the cyclic voltammograms of $O_2^{\bullet -}$ in the presence of different volumes of N-EDMPA, N-PDMPA and EDTMP, in DMSO - Bu_4NBF_4 0.1 M medium with scan rate = 20mV/s:

- Glassy carbon (GC) as a working electrode
- Platinum Plate (1.28 cm^2) as a counter electrode
- Saturated calomel electrode (SCE) as a reference



N-EDMPA *N-PDMPA* *EDTMP*

Fig.6. log **(**I_{pa} /I^{O}_{pa} − I_{pa} **)** versus log ($1/C_{test}$) for N-EDMPA, N-PDMPA and EDTMP



N-EDMPA *N-PDMPA* *EDTMP*

Fig.7. Optimized molecular structures of N-EDMPA, N-PDMPA and EDTMP

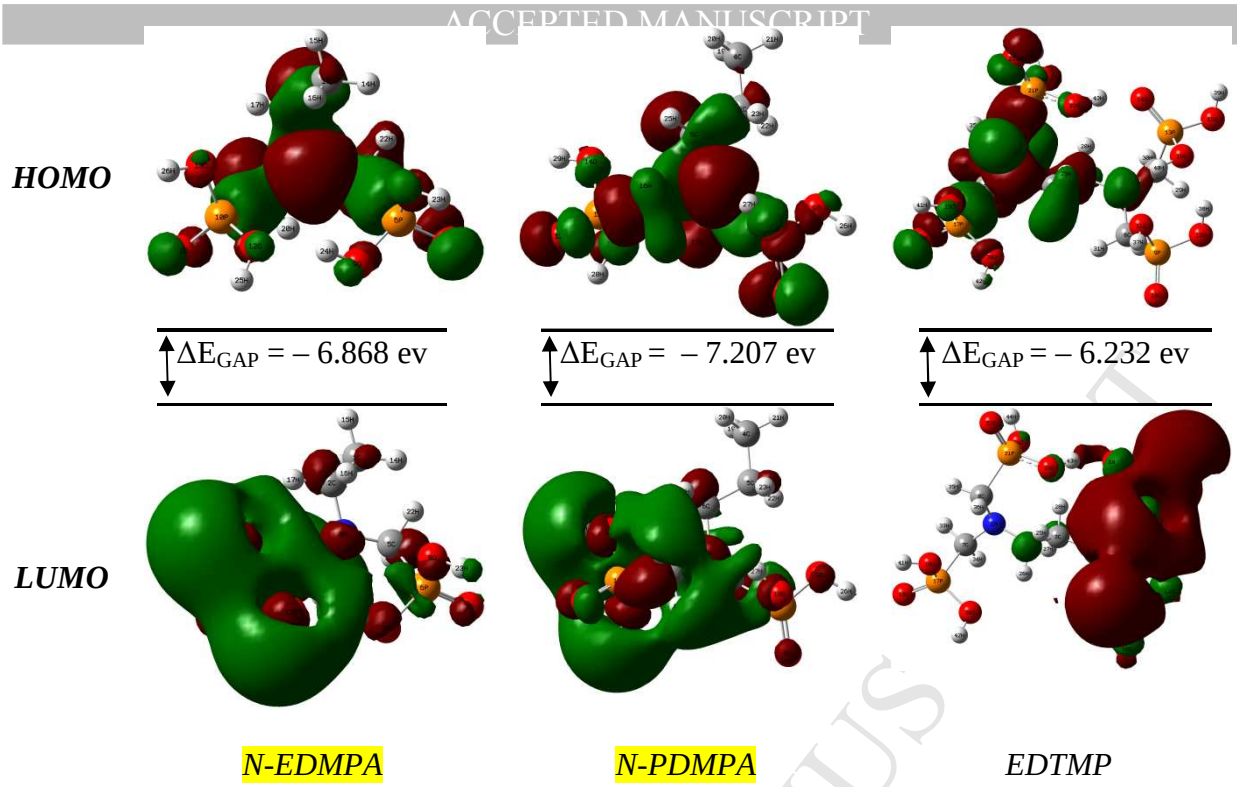


Fig.8. The frontier molecular orbitals density distributions for N-EDMPA, N-PDMPA and EDTMP

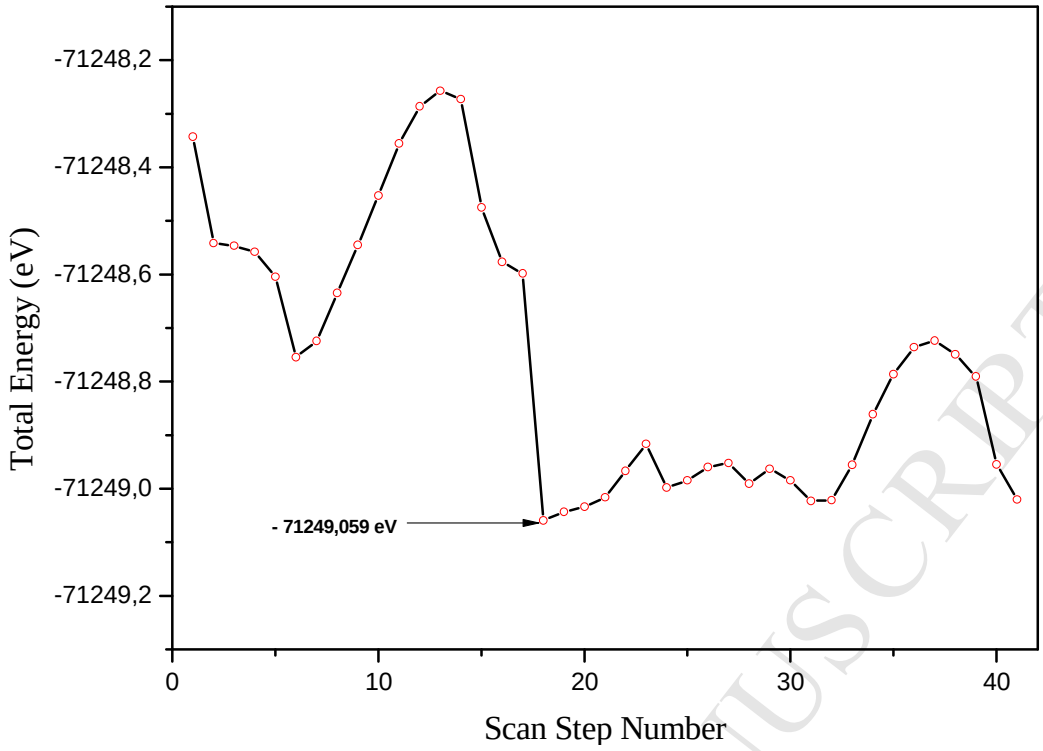
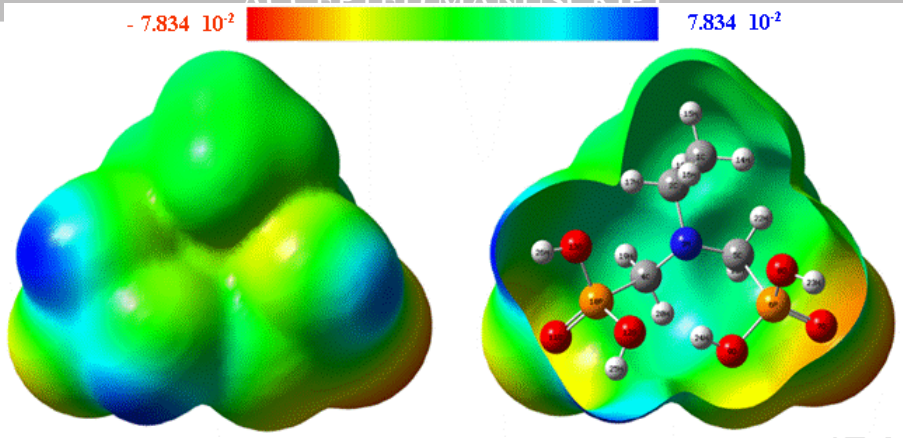
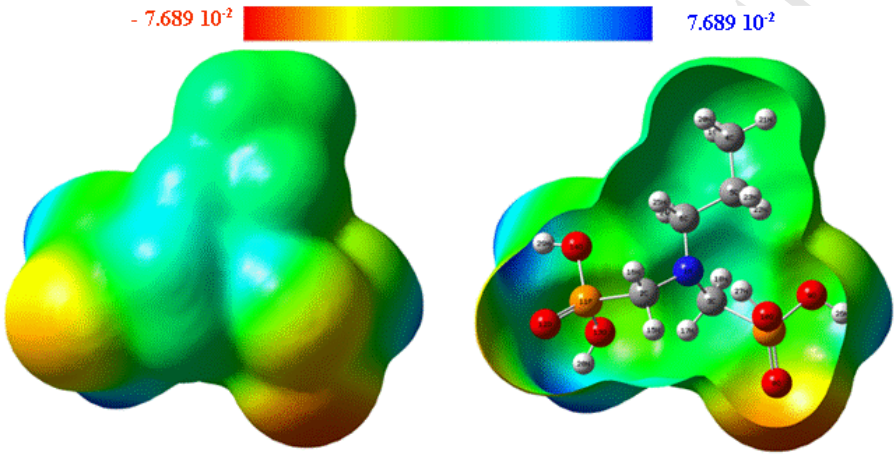


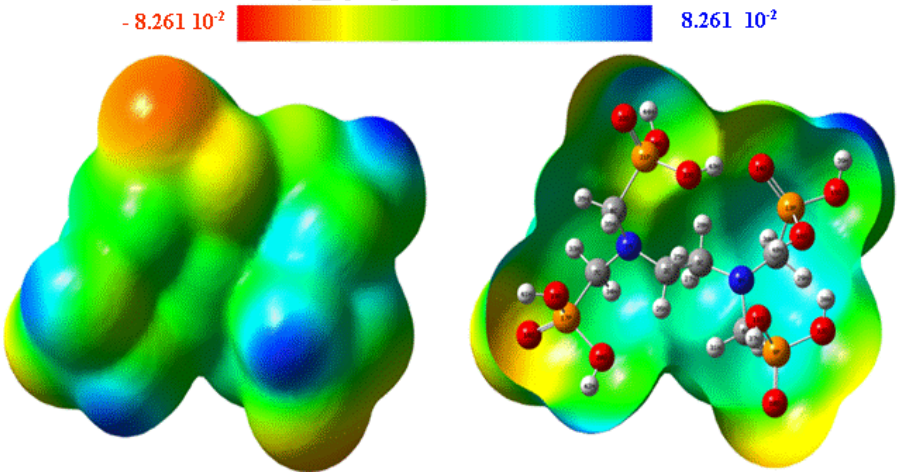
Fig.9. Scan of total energy of the more stable compounds (EDTMP) with the selected dihedral angle (N1−C2−C3−N4).



N-EDMPA



N-PDMPA



EDTMP

Fig.10. Molecular electrostatic potential of N-EDMPA, N-PDMPA and EDTMP

Highlights:

- Three bioactive α-aminophosphonates have been synthesized and theoretically studied
- The antioxidant activity was evaluated by electrochemical and classical methods
- The N-Propyliminodimethylenediphosphonic acid has a better antioxidant activity
- The electrochemical study allowed to establish the antiradical reaction mechanism
- The experimental results are in very good agreement with the theoretical studies