Structural effects on the reactivity 1,4-dihydropyridines with alkylperoxyl radicals and ABTS radical cation

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Abstract—A series of eight commercial C-4 substituted 1,4-dihydropyridines and other synthesized related compounds were tested for direct potential scavenger effect towards alkylperoxyl radicals and ABTS radical cation in aqueous Britton–Robinson buffer pH 7.4. A direct quenching radical species was established.The tested 1,4-dihydropyridines were 8.3-fold more reactive towards alkylperoxyl radicals than ABTS cation radical, expressed by their corresponding kinetic rate constants. Furthermore, NPD a photolyte of nifedipine and the C-4 unsubstituted 1,4-DHP were the most reactive derivatives towards alkylperoxyl radicals.The pyridine derivative was confirmed by GC/MS technique as the final product of reaction. In consequence, the reduction of alkylperoxyl and ABTS radicals by 1,4-dihydropyridines involved an electron transfer process. Also, the participation of the hydrogen of the 1-position appears as relevant on the reactivity. Results of reactivity were compared with Trolox.

1. Introduction

1,4-Dihydropyridine (1,4-DHP) calcium channel antagonists have been used for many years in the treatment of angina pectoris, hypertension and other cardiovascular diseases. According to the common view, their mechanism of action is based on an inhibition of the smooth muscle L-type calcium current, thus decreasing intracellular calcium concentration and inducing smooth muscular relaxation.[1] However, in recent years evidence has accumulated that besides the smooth muscle effects of these agents, their antioxidant effects also to be taken into account.[2–7]

Several Ca^{2+}-antagonists, possess antioxidant activities on phospholipid liposomes, isolated microsomal and mitochondrial membranes, LDL, in isolated rat hearts[8–10] and so their usefulness is recognized in several ischaemic processes of different aethiology in heart, blood vessels, brain, liver, kidney and muscle.[11–13]

Keywords: Commercial C-4 substituted 1,4-dihydropyridines; Alkylperoxyl; ABTS; Radicals; GC/MS; Voltammetry; UV–vis spectroscopy; Scavenging.

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The peroxidation step in lipid transformation is considered to be essential for the pathogenesis of atherosclerosis. Although data concerning the mechanism by which lipid peroxidation occurs in vivo are scarce, several lines of evidence suggest that some endogenous and exogenous compounds with antioxidant activity could have beneficial effects in the prevention of atherosclerosis. Potentially useful antiatherosclerotic drugs would be those that not only have a direct effect on the arterial wall, but also demonstrate antioxidant and free radical scavenging properties. Calcium antagonists appear to have at least in vitro antioxidant effects in addition to their potent vasorelaxant properties. Thus, calcium antagonists inhibit LDL oxidation by oxygen radicals.[14,15]

Considering that the antioxidant mechanism of 1,4-dihydropyridine calcium channel antagonists have not been elucidated yet, in this paper a systematic study on the direct reactivity of a series of eight commercial 1,4-DHP compounds and other structural-related derivatives towards ABAP-derived alkylperoxyl radicals and ABTS radical cation in aqueous media at pH 7.4 is presented. Some additional studies to determine the mechanism, by using GC–MS and electrochemical techniques are also reported.

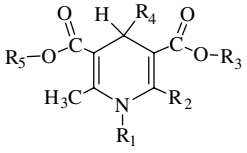
2. Experimental

2.1. Chemicals

All solvents were of high-pressure liquid chromatography (HPLC) grade and all reagents were of analytical grade.

2.2. Compounds

2.2.1. Drugs. 1,4-Dihydropyridine derivatives (Fig. 1) were supplied for different laboratories and used without previous purification. *Nisoldipine*: 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridine-dicarboxylic acid, methyl 2-methyl-propyl ester; *nimodipine*: 1,4-dihydro-



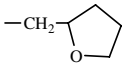
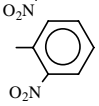
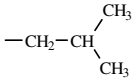
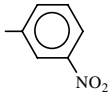
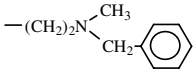
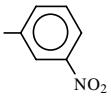
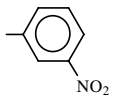
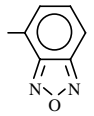
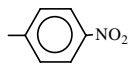
Compounds	R_1	R_2	R_3	R_4	R_5
Nifedipine	—H	—CH_3	—CH_3		—CH_3
Furnidipine	—H	—CH_3			—CH_3
Nisoldipine	—H	—CH_3			—CH_3
Amlodipine	—H	—$CH_2OCH_2CH_2NH_2$	—CH_2CH_3		—CH_3
Nitrendipine	—H	—CH_3	—CH_2CH_3		—CH_3
Nicardipine	—H	—CH_3			—CH_3
Nimodipine	—H	—CH_3	—$(CH_2)_2OCH_3$		
Isradipine	—H	—CH_3	—CH_3		
4-phenyl-1,4-DHP	—H	—CH_3	—CH_3		—CH_3
4-p-Nitrophenyl-N-Ethyl-1,4-DHP	—CH_2CH_3	—CH_3	—CH_3		—CH_3
1,4-DHP	—H	—CH_3	—CH_2CH_3	—H	—CH_2CH_3
4-methyl-1,4-DHP	—H	—CH_3	—CH_3	—CH_3	—CH_3
4,4-di-methyl-1,4-DHP	—H	—CH_3	—CN	—(CH_3) x 2	—CN

Figure 1. Chemical structures of 1,4-DHP derivatives and related compounds.

2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine-dicarboxylic acid, isopropyl 2-methoxyl ester; *nicardipine*: 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine-dicarboxylic acid, 2′-(*N*-benzyl-*N*-methylamino)ethyl methyl ester; *nifedipine*: 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridine-dicarboxylic acid, dimethyl ester; and *nitrendipine*: 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine-dicarboxylic acid, ethyl methyl ester were supplied by Sanitas Laboratories (Santiago, Chile). *Amlodipine*: 1,4-dihydro-2-(aminoethoxy-methyl)6-methyl-4-(2-chlorophenyl)-3,5-pyridine-dicarboxylic acid, ethyl methyl ester; *isradipine*: 1,4-dihydro-2,6-dimethyl-4-(2,1,3-benzozoxadiazol-4-yl)-3,5-pyridine-dicarboxylic acid, isopropyl methyl ester were supplied by Chile Laboratories (Santiago, Chile). *Furnidipine*: 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridine-dicarboxylic acid, methyl 2-tetrafurylmethyl ester was supplied by Alter Laboratories (Madrid, Spain).

The following 1,4-dihydropyridine derivatives were synthesized in our laboratory according to a previously described procedure:[16] *4-phenyl-1,4-DHP*: 1,4-dihydro-2,6-dimethyl-4-phenyl-3,5-pyridine-dicarboxylic acid, dimethyl ester, *1,4-DHP*: 1,4-dihydro-2,6-dimethyl-3,5-pyridine-dicarboxylic acid, diethyl ester, *4-methyl-1,4-DHP*: 1,4-dihydro-2,6-dimethyl-4-methyl-3,5-pyridine-dicarboxylic acid, dimethyl ester, *4-para-nitro-N-ethyl-1,4-DHP*: 1,4-dihydro-2,6-dimethyl-4(4-nitrophenyl)-*N*-ethyl-3,5-pyridine-dicarboxylic acid, dimethyl ester, and 4,4-dimethyl-1,4-DHP: 1,4-dihydro-2,6-dimethyl-4,4-dimethyl-3,5-pyridine-dicyano (Fig. 1).

Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) (Fig. 2) was purchased at Aldrich Chemical Co.

2.2.2. Preparation of nitrosopyridine derivative (NPD: 2,6-dimethyl-4-(2-nitrosophenyl)-3,5-pyridine-carboxylic acid dimethyl ester, Fig. 2). Nifedipine (0.067 M) solution in acetone was irradiated with an UV-lamp (UV-Black-Ray Lamp model B 100 AP, 366 nm filter) for 21 h with constant stirring and refrigeration by air circulation, and keeping constant temperature at 20 ± 2 °C. Photolysis products were separated by preparative thin layer chromatography (Merck silica gel 60 F_{254s} with a concentrating zone of 20×20 cm and thickness of 1 mm) using a developing solvent consisting of: petroleum ether/chloroform/acetone 50/30/20 v/v. The development was performed 3–4 times on a plate to separate photodegradation products. The separated band corresponding to NPD ($R_f = 0.59$) was scraped and collected from plates. Then it was extracted with two fractions of 25 mL chloroform. Each chloroformic extract of the adsorbent was filtered and evaporated nearly to dryness and then was dissolved in 5 mL methanol by heating and left to stand in a refrigerator overnight. Pale needles precipitated, which were collected and recrystallized several times from methanol. The isolated product (pale greenish needles) exhibited the following characteristics:

Mp 93 °C. IR (KBr): ν_{max} 30,450, 3000, 2950, 2850, 1725, 1555, 1495, 1435, 1420, 1290, 1240, 1155, 1110, 1042, 960, 945, 850, 820, 780, 770 and 690 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 2.66 (s, 6H, CH_3), 3.37 (s, 6H, OCH_3) and 6.48–7.68 (m, 4H, aromatic-H). GC–MS m/z (relative intensity): 328 (27, M^+), 311 (2, M^+-OH), 298 (7, M^+-NO), 284 (22), 269 (100, $M^+-COOCH_3$).

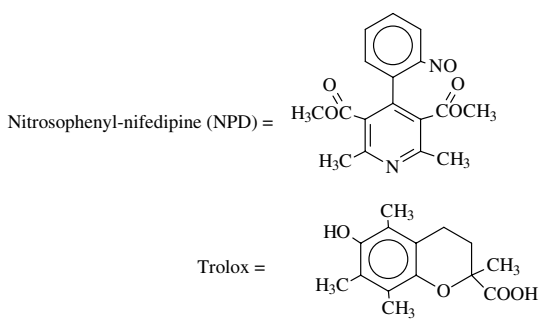


Figure 2. Chemical structures of miscellaneous compounds.

2.3. UV–vis

The progress of the reactivity with alkylperoxyl radicals was followed by UV–vis spectroscopy using an UNICAM UV-3 spectrophotometer. UV–vis spectra were recorded in the 220–700 nm range at different intervals. The reactivity with ABTS radical cation, the absorbance at $\lambda = 734$ nm was followed.

Acquisition and data treatment were carried out with a Vision 2.11 software.

2.4. Reactivity towards alkylperoxyl radicals

ABAP (2,2′-azobis(2-amidinopropane)dihydrochloride, Aldrich Chemical Company) was used as radical generator.

Different series of 20 mM ABAP solutions in 0.04 M Britton–Robinson buffer/DMF 70/30 pH 7.4 were incubated with 100 μM solutions of each 1,4-dihydropyridine or Trolox at 37 °C for 120 min with constant bubbling of oxygen. The rate alkylperoxyl radicals formation from ABAP will not be constant as it depends upon the concentration of ABAP (rate = k[ABAP]). It appears that, over 120 min at 37 °C, only a small amount of the ABAP will decay, therefore the rate will be approximately constant, that is, at 37 °C in neutral aqueous solutions. Furthermore, the half-life of ABAP is about 175 h, and the generation rate of radicals is constant for the first few hours.[17,18]

Control solutions containing either 1,4-dihydropyridines or Trolox solutions were run in the same conditions as the above mixtures. Time-course of the reactivity of 1,4-DHP derivatives with the generated alkylperoxyl radicals was followed by UV–vis spectroscopy and GC–MS technique.

The concentration change of 1,4-DHP compounds was followed by UV–vis spectroscopy using the absorption bands at $\lambda = 330$–360 nm and $\lambda = 300$ nm for Trolox. The concentration of the drugs in the aqueous buffer (0.04 M Britton–Robinson buffer/DMF 70/30 pH 7.4) was determined from the respective calibration curves (10–100 μM). For this type of radicals the kinetic rate constants were calculated by using the kinetic rate constant of Trolox with alkylperoxyl radicals reported in the literature, $k = 4.92 \times 10^3\ M^{-1}\ s^{-1}$.[19]

The reactivity towards alkylperoxyl and ABTS radicals was comparatively expressed with Trolox using the following ratio:

Relative reactivity: kinetic rate constant 1,4-DHP tested/kinetic rate constant Trolox. Control solutions (in the absence of ABAP-derived radicals) revealed no changes either in their original UV–vis absorption bands or GC–MS mass fragmentation.

Also, a possible photodecomposition of 1,4-dihydropyridines was assessed, but in the time-scale of the experiments this was negligible.

2.5. Reactivity towards ABTS radical cation

ABTS (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)diammonium salt was purchased from Aldrich Chemical Co.

Bleaching capacity assay. A solution of ABAP (2 mM) and ABTS (75 μM) prepared in the 0.04 M Britton–Robinson buffer/ethanol 70/30 at pH 7.4 was incubated at 45 °C for 1 h (Fig. 3), a period that generally proved adequate to produce an increase in the absorbance at 734 nm near to 0.24 AU (in the absence of ABAP, incubated ABTS generated no colour). By using an extinction coefficient of $1.6 \times 10^4\ mol^{-1}\ L\ cm^{-1}$ for ABTS radical cation at 734 nm,[20] we calculated the ABTS radical cation concentration formed in our experiments. The calculated value was 15 μM; indicating that a 20% ABTS was oxidized.

Afterward, the resulting coloured solution was rapidly cooled down on ice, nitrogen bubbled during 5 min, and kept at 4 °C until use. When aliquots (2 mL) of the coloured solution were placed into a cuvette and kept at 15 °C, they displayed an absorbance (at 734 nm), which remained constant for at least 4 h. The addition of aliquots of 1,4-DHP derivatives or Trolox to these cuvettes resulted in changes in the AU, which were monitored for 30 min using an UNICAM UV-3 spectrophotometer.

2.6. Voltammetry

Differential pulse voltammetry (DPV) was performed with a BAS CV50 assembly. A glassy carbon stationary electrode was employed as working electrode. A platinum wire was used as a counter electrode and all potentials were measured against an Ag/AgCl electrode.

Operating conditions: pulse amplitude, 40 mV; potential scan, $4\ mV\ s^{-1}$; voltage range, 0–1000 mV; current range, 5–25 μA; temperature, 25 °C. All the solutions were purged with pure nitrogen for 10 min before the voltammetric runs.

2.7. Controlled potential electrolysis (CPE) of nifedipine

CPE were carried out on a reticulate glassy carbon electrode in 0.04 M Britton–Robinson buffer/DMF 70/30 pH 7.4 at +1000 mV. Oxygen was removed with pure and dry pre-saturated nitrogen. A three-electrode circuit with an Ag/AgCl electrode was used as reference and a platinum wire as a counter electrode. A BAS-CV 50 assembly was used to electrolyze the different derivatives.

2.8. GC–MS

A gas chromatograph/mass selective Hewlett Packard 5890/5972 detector (Palo Alto, California, USA) and Hewlett Packard 7673 Autosampler were used for the

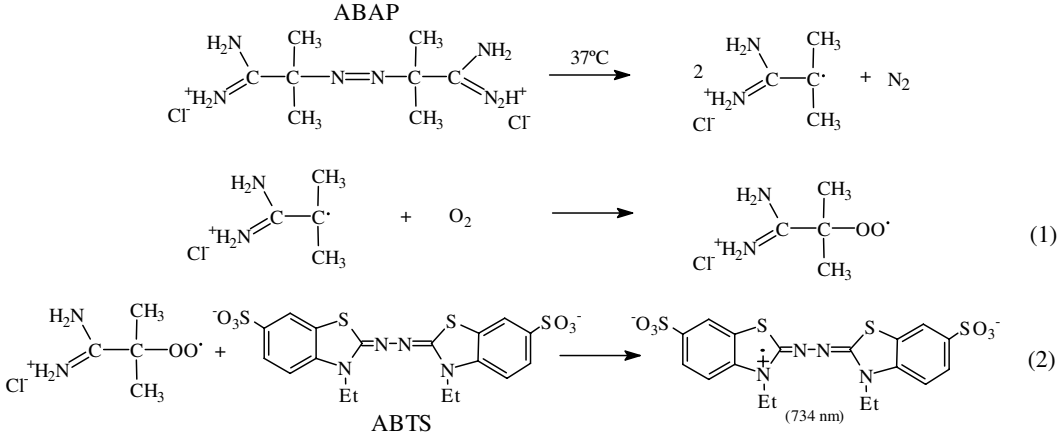


Figure 3. Chemical generation of free radicals: Eq. 1, alkylperoxyl radicals. Eq. 2, $ABTS^{+\cdot}$ radical cation.

measurements. A Hewlett Packard Pentium II Data System-Laser Jet 4000 printer, controlled instrumentation and data handling was also used.

Chromatography column: Hewlett-Packard Ultra-1 column, 25 m × 0.2 mm i.d. × 0.11 film thickness (Little Falls, Wilmington, Delaware, USA).

Chromatographic conditions: detector temperature, 300 °C; injector temperature, 250 °C; split ratio, 1/10; pressure, 13 psi; purge flow, 40 mL min^{-1}; purge time, 0.5 mL min^{-1}.

Temperature program: The oven temperature was programmed from 130 to 305 °C (hold for 5 min) at 15 °C min^{-1}; run time was 16.67 min. Helium was used as carrier gas with an inlet pressure of 35 kPa. Peak identification relies upon full spectral comparison of the test samples with reference standard material analyzed within the same batch. There must be a complete agreement with both acceptable chromatography and mass spectrometry.

Before, during and after the reaction, the final solutions were diluted and injected without any class of previous treatment. Consequently, no extraction or filtration procedures were necessary.

Acquisition of ion chromatograms was in scan mode with electron impact between 50 and 450 amu, with threshold of 20 and 1.9 scan/s.

3. Results and discussion

3.1. Reactivity towards alkylperoxyl radicals

To follow the reactivity of 1,4-DHP derivatives UV–vis spectroscopy, GC–MS and electrochemical techniques were used.

For the UV–vis spectroscopic studies, the original UV absorption bands corresponding to the 1,4-DHP derivatives between $\lambda = 330$ and 360 nm were followed to assess the kinetic rate constants. The addition of 1,4-DHP derivative solutions to an aqueous mixture containing alkylperoxyl radicals produced a significant decrease in these absorption bands with time, parallel with the appearance of a new band between 270 and 280 nm (Fig. 4). This latter signal could correspond to the oxidized derivative, that is, the pyridine derivative, consistent with previous observations.[21,22] The rate of reaction had a linear dependence with 1,4-DHP concentration within a range varying between 20 and 120 μM for all the tested compounds.

In Figure 5, the time-course of absorptivity corresponding to the formed pyridine derivative at 280 nm and the parent 1,4-dihydropyridine derivative at 364 nm is presented. From this figure it can be concluded that at 6 h, practically all the parent 1,4-DHP (100 μM) were consumed remaining stable the formed pyridine derivative at least during the following 3–4 h of the experiment. In this time-scale, the concentration of alkylperoxyl radicals practically is constant, indicating that the pyridine derivative was unable to react with alkylperoxyl radicals.

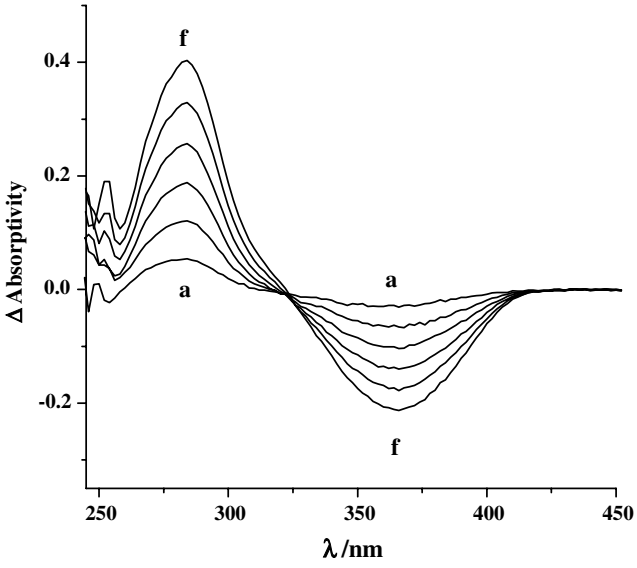


Figure 4. UV–vis differential spectra corresponding to reaction between a 100 μM isradipine solution and 20 mM ABAP-derived alkylperoxyl radicals solution in 0.04 M Britton–Robinson buffer/DMF 70/30 at pH 7.4; a–f 120 min.

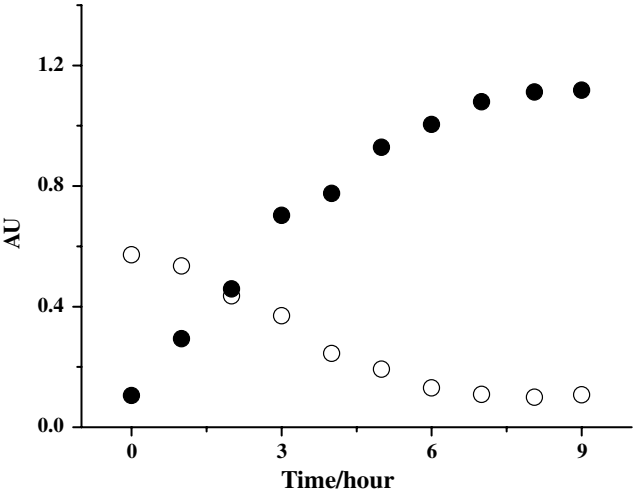


Figure 5. Time-course of the absorptivity at $\lambda = 364$ nm (○) and $\lambda = 280$ nm (●) for the reaction of 100 μM amlodipine and 20 mM alkylperoxyl radical in 0.04 M buffer Britton–Robinson/DMF 70/30 at pH 7.4 and 37 °C.

In order to identify the products obtained after the reaction between 1,4-DHP derivatives and the alkylperoxyl radicals, the GC–MS technique was used.

Typical ion chromatograms and mass fragmentation corresponding to isradipine before, during and after its reaction with radicals is shown in Figure 6A–C. As can

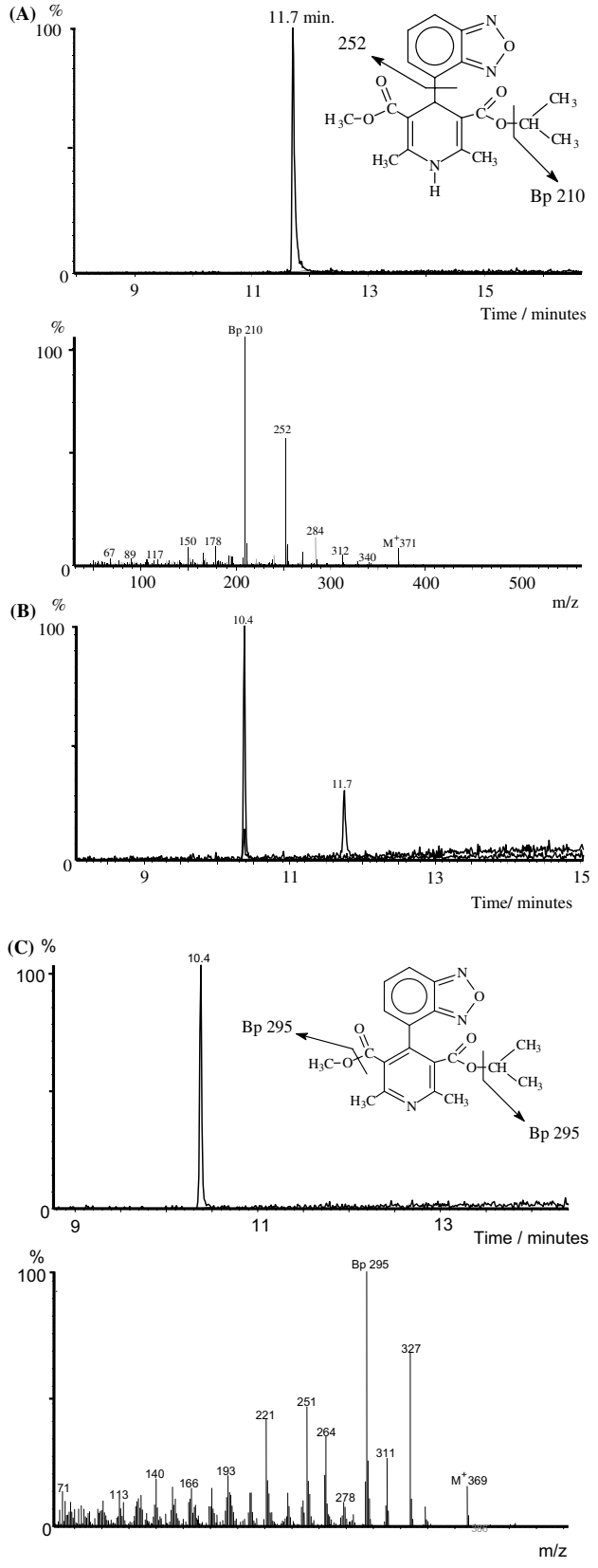


Figure 6. Extracted ion chromatograms and mass spectrum of 200 μM solution of isradipine (A). Chromatographic peaks of isradipine for the reaction with ABAP-derived alkylperoxyl radicals after 2 h. Extracted ions were m/z: 210, 295 and 369 (B). Chromatographic peak corresponding to the pyridine derivative after the reaction of isradipine with ABAP-derived alkylperoxyl radicals after 4 h (C).

be seen from this figure, the GC–MS technique made possible to follow the reaction and the characterization of the pyridine formation as a consequence of the reduction of the radicals by isradipine under our experimental conditions. This result confirms our previous results found by the UV–vis spectroscopic studies about the pyridine formation during the reaction between 1,4-DHP and the radicals.

For parent isradipine, the base peak corresponded to the loss of the isopropyl group in 3-position, also the loss of the substituent in 4-position was found. After the reaction with radicals, the base peak corresponded to the loss of the isopropyl group in 3-position and the loss of the methoxy group in 5-position. Retention time of the pyridine derivative was shorter than that corresponding to isradipine (10.4 min vs 11.7 min, Fig. 6B).

A typical time-course of the reaction between isradipine and alkylperoxyl radicals is shown in Figure 7. From this figure, it is clear that as a consequence of the reaction a significant effect on the abundances of the corresponding ions of the parent isradipine (m/z 210) and its oxidized derivative (m/z 295) was obtained. Both ions significantly changed until 60 min of reaction.

Some general conclusions on these studies can be summarized as follows: (a) The GC–MS procedure permitted the characterization of the parent 1,4-dihydropyridines and its subsequent reactivity with alkylperoxyl radicals, not requiring a derivatization process. An exception was amlodipine, the GC–MS characterization of which was not possible. (b) 1,4-Dihydropyridines after the reaction with alkylperoxyl suffered a dehydrogenation process yielding the pyridine-derived metabolite (22–80% in 2 h). This conclusion is supported by the respective retention times and mass fragmentation pattern of the compounds. Clearly, in the scavenging effect of the radicals, an electron transfer reaction is involved. (c) Retention times corresponding to the pyridine derivatives were lower than the parent compounds. (d) The mass fragmentation pattern of the

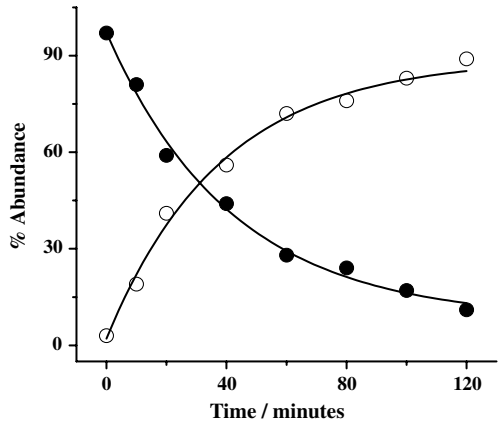


Figure 7. Time-course of intensity of m/z 210 (●) corresponding to the parent drug (isradipine), and of m/z 295 (○) corresponding to its pyridine analogue, after the reaction with ABAP-derived alkylperoxyl radicals in 0.04 M Britton–Robinson buffer/ethanol 70/30 at pH 7.4.

aromatized derivatives show a different base peak depending on 4-substitution on the 1,4-dihydropyridine ring. (e) The tested 1,4-DHP derivatives reacted with alkylperoxyl radicals, forming as a final product the pyridine derivative. The above-described results for parent commercial 1,4-DHP concerning with their mass fragmentation patterns are in accord with a previous report.[23]

The time-course of the reaction between *para*-nitrophenyl-*N*-ethyl-1,4-DHP derivative with alkylperoxyl radicals followed by GC–MS technique did not reveal any new signal. The lack of new signal observed by GC–MS technique could be explained based on the previous knowledge that the electrochemical oxidation of N-substituted-1,4-DHP give rise a pyridinium salt as a final product.[24–26] This salt did not show signals by the GC–MS technique, probably because the ionization fountain of the chromatograph could repel this positive pyridinium salt ion avoiding its detection.

In Table 1, the calculated kinetic rate reaction constants (k) with alkylperoxyl radical for all the tested 1,4-DHP (commercial and other related dihydropyridines) are shown. As can be seen from this table, all commercial 1,4-DHPs were weaker than Trolox, a well-known antioxidant. However, by comparison of the kinetic rate constants, the most reactive compound was the C-4 unsubstituted 1,4-DHP, which is between 11.8 times and 9.0 times more reactive than the commercial 1,4-DHP derivatives. Also, was 2.3 times more reactive than Trolox.

Table 1. Kinetic rate constants for the reaction between 1,4-DHP derivates with ABAP-derived alkylperoxyl radicals in 0.04 M Britton–Robinson buffer/DMF 70/30 at pH 7.4 and 37 °C

Compounds	k, $M^{-1} s^{-1}$	R^a	E_p, mV[b]
Amlodipine	1700 ± 30	0.35	725
Isradipine	1760 ± 35	0.36	729
Nitrendipine	1260 ± 20	0.26	757
Nifedipine	1170 ± 25	0.24	766
Nisoldipine	960 ± 50	0.20	681
Furnidipine	1130 ± 20	0.23	741
Nimodipine	1840 ± 70	0.37	736
Nicardipine	1000 ± 20	0.20	770
4-Phenyl-1,4-DHP	2165 ± 57	0.4	715
4-*p*-Nitrophenyl-*N*-ethyl-1,4-DHP	340 ± 20	0.07	824
1,4-DHP	11,410 ± 390	2.3	352
4-Methyl-1,4-DHP	3350 ± 80	0.68	640
4,4-Dimethyl-1,4-DHP	n.d.r.	—	861
NPD	11,097	2.3	—
Trolox[c]	4920	1	236

n.d.r. non-detected reaction.

[a] Ratio between kinetic rate constant of the tested DHP derivative/kinetic rate constant of Trolox in the presence of ABAP-derived alkylperoxyl radicals.

[b] Oxidation peak potential values obtained from differential pulse voltammetry.

[c] Kinetic rate constant value for Trolox was taken from literature,[19] $k = 4.92 \times 10^3\ M^{-1} s^{-1}$.

From results of Table 1, some general conclusions can be summarized: (a) Commercial 1,4-DHP derivatives were less reactive than the synthesized 1,4-DHP having substituents with either electron-donating properties or lacking of a substituent in C-4 position. (b) The substitution of the hydrogen of the 1-position by an ethyl group significantly decreased the kinetic rate constant value. (c) The disubstitution of the C-4 position turned the 1,4-DHP derivative into an inactive compound. (d) The position of the nitro group in the aromatic ring did not significantly affected the kinetic rate constant value between commercial 1,4-DHP derivatives.

3.2. Reactivity of 2,6-dimethyl-4-(2-nitrosophenyl)-3,5-pyridine-carboxylic acid dimethyl ester (NPD) towards alkylperoxyl radicals, a photolyte of nifedipine

Taking into account that nifedipine, which is extremely light sensitive converting under daylight into 2,6-dimethyl-4-(2-nitrosophenyl)-3,5-pyridine-carboxylic acid dimethyl ester, it seemed interesting to test the reactivity between this photolyte and alkylperoxyl radicals. For this purpose, NPD was prepared and characterized as was previously described in Section 2.

To follow the reactivity of NPD and the radicals, its UV–vis absorption band at $\lambda = 314$ nm was used. Figure 8 shows the time-course for the reaction between 100 μM NPD and 20 mM ABAP. Concomitantly with the increase of time reaction, a significant decrease in the absorption at 314 nm is observed. From the concentration/time plots (Fig. 8, inset), the kinetic rate constant for this reaction was assessed, giving a k value of 11,097 $M^{-1} s^{-1}$ (Table 1). This value becomes to be 2.3 times higher than that of Trolox, but similar to the

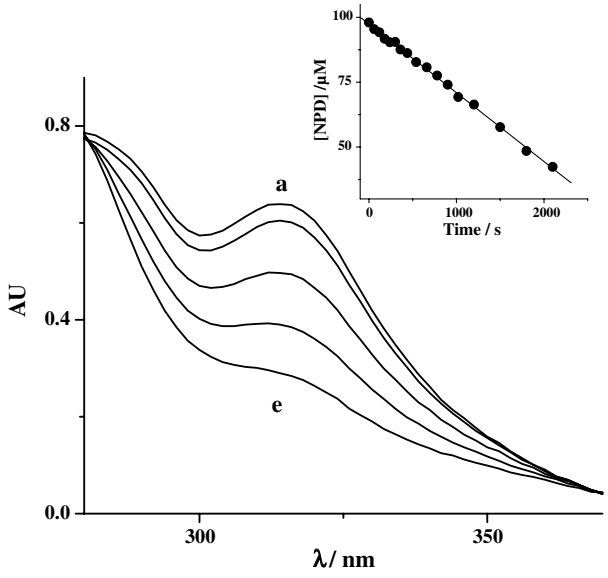


Figure 8. Time-course of the absorbance of NPD derivative in the presence of 20 mM ABAP-derived alkylperoxyl radical solution in 0.04 M Britton–Robinson buffer/DMF 70/30 at pH 7.4. a–e 30 min. Inset: concentration/time plot of NPD in the presence of alkylperoxyl radicals.

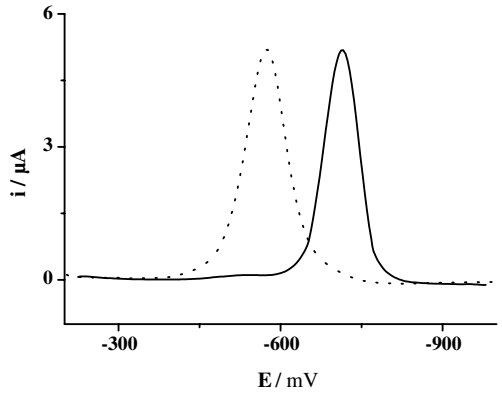


Figure 9. Differential pulse voltammograms of (—) 1 mM nifedipine and (···) after the CPE at 1000 mV. Medium: 0.04 M Britton–Robinson buffer/DMF 70/30 at pH 7.4.

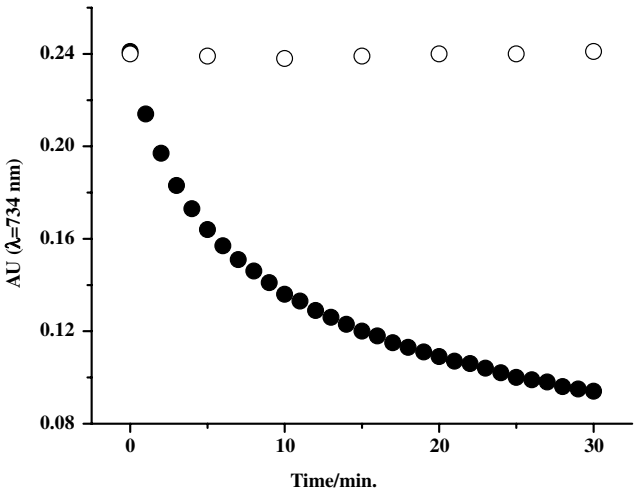


Figure 10. Time-course of the absorptivity at 734 nm of (○) 15 μM ABTS radical cation solution and (●) in the presence of 5 μM amlodipine in 0.04 M Britton–Robinson buffer/ethanol 70/30 at pH 7.4 and 15 °C.

calculated one for the C-4 unsubstituted 1,4-DHP derivative. However, a comparison of the kinetic rate constants corresponding to nifedipine and NPD, its photodegradation product, the latter was 9.5 times more reactive than the parent drug, nifedipine. This result accounts for the significant reactivity of the nitroso aromatic group compared with the dihydropyridine moiety. In order to assess the impact of either the nitroso group or the dihydropyridine moiety on the reactivity against alkylperoxyl radicals, controlled potential electrolysis experiments were conducted. Nifedipine (1 mM) solutions were electrolyzed at +1.0 V during 1.5 h and followed by differential pulse voltammetry. Results from these experiments showed that the anodic current decreased (data not shown) parallel with a shift of the reduction potential towards positive potentials (Fig. 9). After the complete electro-oxidation of the original nifedipine solutions, they were incubated for 2 h with ABAP-derived alkylperoxyl radicals. Results from these experiments demonstrated that the original spectra did not show any change, indicating that both the pyridine ring and the nitro group have no ability to reduce these type of radicals. In addition, the ability of NPD to reduce alkylperoxyl resides only on its nitroso aromatic group. This finding is consistent with previous works describing the oxidation of nitrosobenzenes to corresponding nitro compounds after the reactions with different compounds[27] and with *tert*-butylperoxide radicals.[28] On the other hand, the spin-trap properties of the nitroso group have been also well documented.[29,30]

3.3. Reactivity towards ABTS radical cation

ABTS radical cation, was generated by oxidation of ABTS with alkylperoxyl radical. All the tested 1,4-DHP compounds reacted with ABTS radical cation at different degrees.

The addition of different 1,4-DHP concentrations on solutions of ABTS radical cation produced a diminution of the absorptivity at 734 nm corresponding to the radical cation. Figure 10 shows the time-course of the absorbance at 734 nm in the presence of amlodipine derivative. To assess the kinetic rate constants of the 1,4-DHP compounds, the slopes of time-course of the absorbance at 734 nm were used. In Table 2, the reactivity of the commercial 1,4-DHP and other structural-related compounds towards ABTS radical cation is shown. Both, amlodipine and isradipine resulted to be the most reactive commercial 1,4-DHP compounds. Likewise, the *N*-ethyl substituted 1,4-DHP was one of the most weak compounds, but exhibiting a similar reactivity as nicardipine (Table 2).

In general terms, by analysis of the reactivity towards alkylperoxyl radicals and ABTS radical cation and the character in C-4 substituents of 1,4-DHP compounds, the following conclusions can be summarized: (a) 1,4-DHP having electron-donating substituents (4-methyl-1,4-DHP, 4-phenyl-1,4-DHP) had a great reactivity towards ABTS radical cation. An exception for this

Table 2. Kinetic rate constants for the reaction between 1,4-DHP derivates with ABTS radical cation in 0.04 M Britton–Robinson buffer/ethanol 70/30 at pH 7.4 and 15 °C

Compounds	k, $M^{-1}\,s^{-1}$ [a]	R [b]
Amlodipine	204 ± 4	0.05
Isradipine	66.2 ± 1.3	0.016
Nitrendipine	57.9 ± 1.6	0.014
Nifedipine	51.7 ± 1	0.013
Nisoldipine	51 ± 2	0.012
Furnidipine	48.9 ± 1	0.012
Nimodipine	43 ± 2	0.01
Nicardipine	20.9 ± 0.5	0.05
4-Phenyl-1,4-DHP	194 ± 10	0.05
4-*p*-Nitrophenyl-*N*-ethyl-1,4-DHP	22 ± 1	0.005
1,4-DHP	3213 ± 165	0.8
4-Methyl-1,4-DHP	201 ± 3	0.05
Trolox	4100 ± 10	1

[a] Kinetic rate constants calculated from the $ABTS^{\bullet+}$ concentration/time plots.

[b] Ratio between kinetic rate constant of the tested DHP derivative/kinetic rate constant of Trolox.

behaviour is the case of amlodipine, having an electron-withdrawing group in C-4 position, which exhibits a comparable reactivity with the former. (b) 1,4-DHP compounds with electron-withdrawing substituents had a weak reactivity. (c) The C-4 unsubstituted 1,4-DHP (Fig. 1) was the most reactive of the tested dihydropyridines. A feasible explanation for this result could be based on the knowledge of the planar structure of its dihydropyridine ring, displaying most easiness for double-bond conjugation.[31] (d) The relevance of the hydrogen in 4-position is confirmed by the inactivity of the 4,4-disubstituted derivative towards alkylperoxyl radicals. (e) 4-*para*-Nitrophenyl-*N*-alkyl-1,4-DHP compound had a more weak reactivity than the 1,4-DHP isomer, nifedipine.

In conclusion, these results could indicate that the reactivity towards alkylperoxyl and ABTS cation radical significantly depends on the presence of both the hydrogen in 4- and 1-position. Also, the character of the substituents on 4- and 1-positions appears playing an important role in the reactivity with this type of radicals.

Despite intensive efforts, the dehydrogenation mechanism of 1,4-dihydropyridines still remain puzzling. The debate has mainly focused on (1) whether the reaction occurs by a one-step hydride transfer or by an electron-transfer-initiated multistep hydride transfer mechanism (i.e., e^-–H^+ or e^-–$H^\cdot$); and (2) whether the reaction centre is on the carbon at the 4-position or on the nitrogen at the 1-position of the dihydropyridine ring. Experimental evidence in support of the direct hydride transfer mechanism and the electron-transfer-initiated multistep hydride transfer mechanism[32] or the hydrogen-atom-transfer mechanism for the 1,4-DHP oxidations[33] has been diversely reported. On the other hand, while the initial dehydrogenation is often found to take place at the 4-carbon position of 1,4-DHP,[34] arguments in favour of the 1-nitrogen as the initial reaction site in some 1,4-DHP oxidations also seem to be quite convincing.[35] In the present work, clearly we have found that either N-substitutions or C-4 disubstitutions affected the reactivity towards the tested radicals.

3.4. Correlations between kinetic rate constants and oxidation peak potential values

Oxidation of Hantzsch 1,4-DHP is generally the key step in their numerous reactions of biological importance and they are being used in modeling the NADH coenzyme in its biological redox processes. Because it is demonstrated that the metabolism of 1,4-DHP utilized in the treatment of hypertension, also proceeds through oxidation (i.e., aromatization) of 1,4-DHP, significant research has been carried out to study the features of these oxidations. Therefore, in this section attempts to correlate oxidation peak potential values determined in aqueous media pH 7.4 with kinetic rate constants for the reaction with the radicals are discussed.

The electrochemical oxidation of the 1,4-dihydropyridines was studied in the same experimental conditions used for the reactivity studies (aqueous media). Under these conditions all compounds exhibited only a single oxidation peak, which is consistent with previous reports.[24–26] In Figures 11 and 12, the relationship between the kinetic rate constants and the oxidation peak potentials is displayed. From these studies it can be concluded that:

(a) Kinetic rate constants for the reaction between 1,4-DHP compounds and alkylperoxyl radicals exhibited a fairly good linear correlation with oxidation peak potential (kinetic rate constant = 19,185–23.95E_p mV; c.c. = 0.97). Thus, the compounds being more easily oxidized reacted more rapidly with this radical (Fig. 11).
(b) Kinetic rate constant towards the ABTS radical cation behave independently of oxidation peak potential values. As can be seen from Figure 12, two different groups were found, that is, a first group

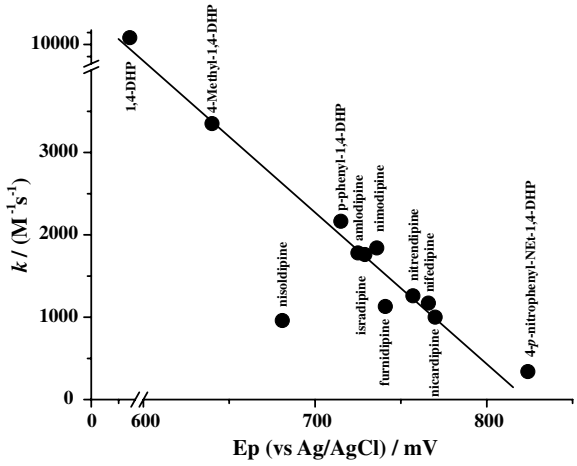


Figure 11. Relationship of kinetic rate constants (k) with oxidation peak potentials (E_p) corresponding to the reaction between tested 1,4-DHP towards ABAP-derived alkylperoxyl radicals in 0.04 M Britton–Robinson buffer/ethanol 70/30 at pH 7.4.

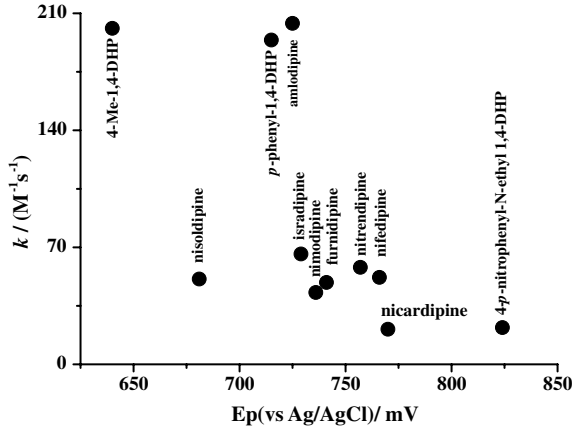


Figure 12. Relationship of kinetic rate constants (k) with oxidation peak potentials (E_p) corresponding to the reaction between tested 1,4-DHP towards ABTS radical cation in 0.04 M Britton–Robinson buffer/ethanol 70/30 at pH 7.4.

with the most potent scavenger drugs, including 1,4-DHP derivatives (amlodipine, 4-methyl-1,4-DHP and 4-phenyl-1,4-DHP) lacking of a nitro group in their molecules and a second one with the resting of the compounds.

4. Concluding remarks

1. In the present paper, a direct quenching radical species (alkylperoxyl radicals and ABTS radical cation) by a number of commercial 1,4-DHP and other structural-related compounds was determined.
2. The tested 1,4-DHP were more reactive towards alkylperoxyl radicals than ABTS radical cation.
3. NPD, a photodegradation product of nifedipine, was significantly more reactive than parent 1,4-DHP, indicating that nitroso group significant contributing to the reduction of alkylperoxyl radicals.
4. The C-4 unsubstituted 1,4-DHP was the most reactive compound towards alkylperoxyl radicals, being 2.3-fold more reactive than Trolox.
5. After the reaction between 1,4-DHP and the different free radicals, the pyridine derivative was formed as a final product at varied grades (22–80%), which was confirmed by GC–MS techniques.
6. Kinetic rate constant values for the reactivity of tested 1,4-DHP with alkylperoxyl radicals showed a direct relationship with the oxidation peak potential values ($r = 0.97$), that is, the most reactive compounds was more easily oxidized.
7. Reactivity of tested 1,4-DHP towards ABTS radical cation show an independence between kinetic rate constants and oxidation peak potentials.
8. Our results strongly support that the reduction of alkylperoxyl radicals by the tested 1,4-DHP involves an electron transfer reaction, which is documented by the presence of pyridine derivative as the product of the reaction. Also, the participation of the hydrogen of the 1-position on reduction of the radicals appears to be relevant.

Acknowledgements

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